Temperature, length of cold storage and maturity influence the ripening rate of ethylene-preconditioned kiwifruit

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Abstract

The effect of temperature, length of cold storage and maturity on the ripening of ethylene-preconditioned (100 µl l^{-1} for 12 or 24 h) kiwifruit was investigated. Low (0°C) temperatures at any point prior to, during or after ethylene preconditioning significantly delayed softening and soluble solids concentration (SSC) accumulation compared to higher temperatures (i.e. 20°C). Freshly-harvested kiwifruit responded to ethylene-preconditioning (100 µl l^{-1} at 0°C for 24 h) by softening faster than control fruit even if harvested 5 weeks after commercial maturity. In contrast, kiwifruit harvested at commercial maturity and stored at 0°C softened faster than the control only if preconditioned with ethylene during the first 2 weeks of storage. Kiwifruit had high respiration rates 1 day after being transferred from 0 to 20°C, but respiration dropped to near base-line levels by day 2. Fruit stored at 0°C always respired faster upon transfer to 20°C than did freshly-harvested fruit and preconditioning with ethylene increased the initial rate of respiration of freshly-harvested fruit but had less of an effect on stored fruit. Ethylene preconditioning did not significantly hasten the climacteric rise in respiration or ethylene production of either freshly-harvested or stored kiwifruit. The climacteric rise of individual kiwifruit began only after fruit softened to ≤ 7 N. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Kiwifruit are climacteric fruit which can be successfully stored at 0°C for 4 to 6 months but which soften markedly when exposed to even minute (i.e. 0.01 µl l^{-1}) concentrations of ethylene (Arpaia et al., 1987; Mitchell, 1990). Kiwifruit handlers and researchers have focused on ways of maintaining firmness during storage primarily through rigorous low temperature (0°C) management, ethylene exclusion and use of controlled atmospheres (Mitchell et al., 1981; Arpaia et al., 1985; Ben-Arie and Sonego, 1985).

During cold storage and ripening, kiwifruit undergo biochemical changes including conversion of starch to sugar, changes in cell wall constituents and production of characteristic volatiles which lead to the taste, texture and aroma desired by consumers. However, kiwifruit stored at 0°C for less than 1 month may reach consumers while still not "ready to eat" (soluble solids concentration (SSC) < 14% and firmness > 13 N). Such fruit lack the quality characteristics consumers demand and if eaten, may discourage consumers from purchasing kiwifruit until later in the season. Recently, Crisosto et al. (1997) developed a ripening protocol for firm, early-season kiwifruit using short exposures (6–12 h) to 100 µl l^{-1} ethylene at low (0°C) temperatures. Because application of exogenous ethylene requires special facilities, kiwifruit ripening at terminal markets can compete with commodities such as banana and tomato for ripening equipment. However, Crisosto et al. (1997) also found that the ripening rate of kiwifruit preconditioned with ethylene at the shipping point can be managed by subsequent storage temperature. Thus, kiwifruit can be treated by packers or shippers and timed, through temperature management, to arrive at consumers "ready to eat" without competing for terminal market ripening facilities.

Previous research has focused on kiwifruit responses to long (i.e. continuous) ethylene exposures (Mitchell, 1990), with little being reported on short ($\leq$ 24 h) ethylene treatments, especially at low temperatures (0°C). Thus, no data exists to show how kiwifruit responses to short, low-temperature ethylene exposure might change with fruit maturity and length in storage. Further, kiwifruit temperature prior to, during and after ethylene preconditioning is likely to have profound impacts on the subsequent ripening rate. The present study investigated how ripening of kiwifruit is affected by maturity, length of time in storage, and fruit temperature prior to, during and after ethylene preconditioning.

2. Materials and methods

2.1. Plant material

Medium sized ($\sim$ 90–130 g) kiwifruit (*Actinidia deliciosa* (A Chev) Liang et Ferguson, cv. 'Hayward') without visible defects or decay were used for all experiments. To investigate the role of fruit temperature prior to, during and after ethylene preconditioning on subsequent ripening, highly mature (about 8.5% SSC and 55 N firmness) kiwifruit were harvested in 1993 and 1994 from commercial vineyards in the San Joaquin valley and transported to the F. Gordon Mitchell Postharvest Laboratory at the University of California, Kearney Agricultural Center, Parlier. California has a kiwifruit minimum maturity standard of 6.5% SSC while New Zealand has a 6.2% SSC standard. After arrival, the fruit were either held at 20°C or forced-air cooled and held at 0°C, both in ethylene-free rooms, until ethylene-preconditioned the following day.

To study cold storage and maturity affects on ripening of preconditioned kiwifruit, harvests were taken during the 1993, 1994 and 1995 seasons from commercial vineyards in Visalia, Reedley, and McFarland, CA, respectively. Materials, methods and results for the three years were similar and thus this report will focus on the 1995 experiments and results. In 1995, kiwifruit were harvested with about 7% SSC and 60 N firmness, packed into boxes with 0.0127 mm (0.5 mil) high density polypropylene, ethylene-permeable solid liners and placed at 0°C. A potassium permanganate ethylene scrubber (Ethylene Control, Selma, CA) was placed in the storage room and ethylene levels were monitored 6 days a week. Ethylene never rose to detectable levels (< 0.005

μl 1^{-1}) in the storage room. Kiwifruit samples were withdrawn weekly from storage for preconditioning. In the same vineyard, ten healthy vines were left initially unharvested from which 350 fruit were harvested weekly between 10 Nov. and 8 Dec. 1995 and held in an ethylene-free room at 0°C for 24 h before being preconditioned.

2.2. Treatments

To study the effects of different temperatures prior to, during and after ethylene preconditioning on fruit ripening, kiwifruit were held at either 0 or 20°C for 24 h after harvest and then treated with 100 μl 1^{-1} ethylene at 0 or 20°C for 24 h in 1993 and 12 h in 1994. Subsequent ripening was monitored over 50 or 7 days at 0 or 20°C, respectively. To study the effects of cold storage and maturity on ethylene-preconditioned kiwifruit ripening, both freshly-harvested and stored fruit were treated with 100 μl 1^{-1} ethylene for 24 h at 0°C. Control fruit were treated with ethylene-free air passed through potassium permanganate pellets. Following treatment, fruit were transferred to 20°C and placed in jars with sufficient flows of ethylene-free air to keep CO_2 in the jars below 0.2 kPa. Each fruit was treated as a replicate and there were 25 fruit per treatment evaluation.

2.3. Quality evaluations

Quality evaluations included measurements of flesh firmness and soluble solids concentration (SSC) at harvest and during ripening. Kiwifruit were evaluated on the day of harvest (initial evaluation) and up to 10 or 49 days after preconditioning when ripened at 20 or 0°C, respectively. When fruit were cold, sufficient time was allowed for the fruit to warm to room temperature before being evaluated. Flesh firmness was measured using a U.C. firmness tester with an 8-mm tip (Western Industrial Supply, San Francisco, CA). Skin from opposite cheeks of each fruit was removed and flesh firmness calculated as the average of two measurements per each of 25 fruit and expressed in Newtons (N). Next, an approximately 5-mm thick transverse section was removed from each fruit and combined with

sections from four other fruit to give five composite samples per treatment. From these composite samples, juice was extracted with a hand press, filtered through cheesecloth, and SSC was measured using a temperature compensated refractometer (Atago, Japan). Analysis of variance (ANOVA), standard error and regression analysis were performed using SPSS 6.1 for Windows (SPSS Inc., Chicago, Ill.).

2.4. Respiration and ethylene production rates

For each treatment, five jars containing five fruit each were used to measure CO_2 and ethylene production. After preconditioning, kiwifruit were weighed, placed in 2.2-l jars at 20°C and allowed to equilibrate for 24 h. The jars were flushed with flows of ethylene-free air at sufficient rates to maintain CO_2 levels below 0.2 kPa. In addition, eight fruit were sealed in individual, modified, 750-ml plastic containers with flows of ethylene-free air to follow individual fruit respiration and ethylene production. Carbon dioxide and ethylene were measured 6 days a week for the next 10 to 12 days by withdrawing gas samples from each jar and injecting them into an infrared CO_2 analyzer (Horiba, Irvine, CA) and a gas chromatograph with a flame ionization detector (EG&G Chandler, Tulsa, OK), respectively. Respiration and ethylene production rates were calculated from the gas concentrations, fruit mass and air flow rates.

3. Results

3.1. Ethylene-preconditioning effects on softening and SSC

As expected, kiwifruit preconditioned with ethylene at either 0 or 20°C and ripened at either 0 or 20°C softened and accumulated SSC faster than their corresponding control fruit exposed to the same temperature regimes (Fig. 1). Fruit treated with ethylene and ripened at 0°C lost between 35 and 80% of their firmness after 1 week compared to less than a 20% loss in control fruit. Kiwifruit ripened at 20°C lost between 60 and

80% of their firmness within the first 3 days compared to less than a 25% loss in the controls. In 1994, ethylene-preconditioned fruit accumulated SSC significantly faster than the control when ripened at 20°C, but not when ripened at 0°C. However, in 1993, with 12 h longer ethylene-exposure than in 1994, fruit ripened at 0°C did accumulate SSC significantly faster than the control (data not shown).

3.2. Effects of temperature prior to, during and after preconditioning

3.2.1. Precooling temperature (prior to treatment)

Kiwifruit held at 20°C prior to ethylene-preconditioning softened and accumulated SSC markedly faster than fruit precooled to 0°C (Fig. 1). For example, kiwifruit held at 20°C before preconditioning and then ripened at 0°C softened as much as 40% more and accumulated as much as 7% more SSC after 49 days than fruit precooled to 0°C (Fig. 1, C).
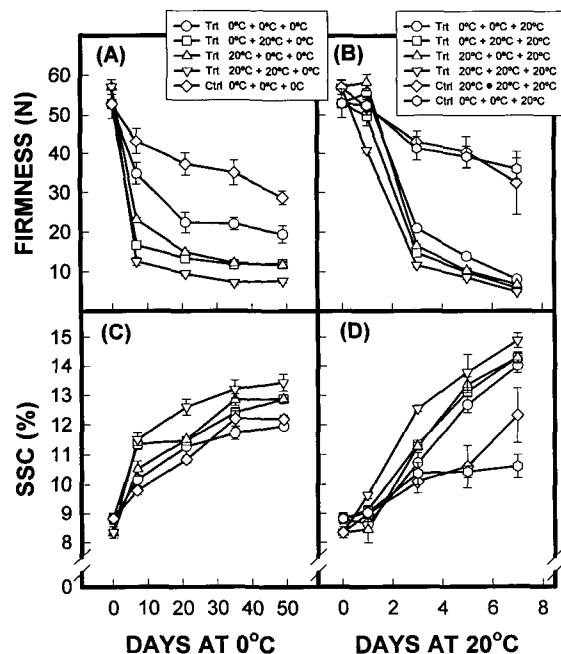

Fig. 1. Firmess (A, B) and SSC (C, D) of kiwifruit. Legends indicate the different temperatures that fruit were exposed to prior, during and after ethylene preconditioning (100 μl l⁻¹ ethylene for 12 h), respectively. Control fruit received only ethylene-free air. The vertical bars represent standard error

3.2.2. Treatment temperature

Kiwifruit preconditioned at 0°C remained firmer during ripening and accumulated SSC more slowly than fruit preconditioned at 20°C (Fig. 1). For example, fruit treated at 20°C softened about 35% more and had about 7% more SSC after 49 days of ripening at 0°C than did fruit treated at 0°C (Fig. 1A, C). The time required for the fruit to reach the treatment temperature (i.e. if the treatment temperatures were higher or lower than the precooling temperatures) likely also influenced the subsequent ripening rate.

3.2.3. Ripening temperature (after treatment)

Kiwifruit softened and accumulated SSC much faster when ripened at 20 than at 0°C. This had the greatest impact on fruit softening and SSC accumulation compared to either precooling or treatment temperatures. The time scale for ripening at 20°C (Fig. 1B, D) and 0°C (Fig. 1A, C) was in days.

3.3. Cold storage and maturity affects

3.3.1. Softening and SSC of freshly-harvested kiwifruit vs fruit from 0°C storage

During the 1995 harvest season, freshly-harvested kiwifruit tended to be slightly firmer than fruit from 0°C storage during the first 20 days after commercial (day 0) harvest (Fig. 2A). Correspondingly, SSC accumulation of freshly-harvested kiwifruit lagged behind that of fruit from storage (Fig. 2B). Field harvests were discontinued 33 days after commercial harvest because night temperatures dropped to below 0°C. After 60 d in storage, fruit had softened to 13.8 N and had lost 77% of their initial firmness. Delaying harvest for 5 weeks increased harvest SSC from 6.9 to 12.3% but resulted in no significant increase in ripe SSC compared to ripe SSC of stored fruit (about 14% for both) (data not shown). Similar responses for both firmness and SSC were observed during the 1994 season. However, in 1993, there were no significant differences in firmness between freshly-harvested and stored fruit during the 20 days after commercial harvest and freshly-harvested kiwifruit had significantly higher SSC than stored fruit (data not shown).
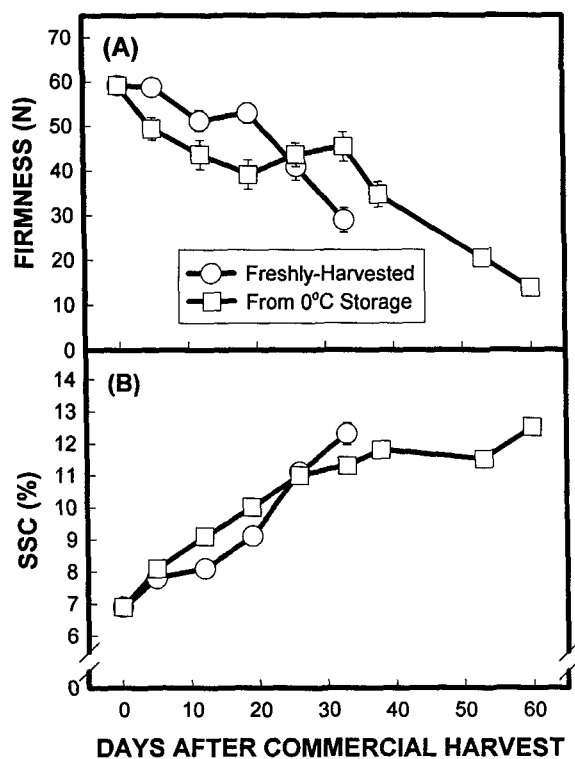
Fig. 2. Firmness (A) and SSC (B) of freshly-harvested kiwifruit or fruit from 0°C storage. Fruit from storage were held at 0°C in an ethylene-free room. The vertical bars represent standard error.

3.3.2. Softening and SSC accumulation during ripening

Average daily softening and SSC accumulation rates were calculated from the slope of the regression equation for each treatment, forced through the initial value (Fig. 3). The rate of fruit softening was greatest in ethylene-preconditioned fruit from the commercial (week 0) harvest (5.03 N day^{-1}) and lowest in week 5 freshly-harvested control fruit (0.76 N day^{-1}) which corresponds to the firmest and softest fruits, respectively, at the beginning of the ripening period. The softening rate of freshly-harvested fruit tended to decline with each week following harvest while fruit from cold storage tended to soften at a more constant rate (Fig. 3A, B). Except during week 3, all freshly-harvested, ethylene-preconditioned fruit softened significantly faster ($P \leq 0.05$) than control fruit. Compared to the control, ethylene-pre-

conditioned kiwifruit from storage tended to have faster rates of softening only if treated during the first 2 weeks of storage. As storage time progressed to 3 weeks or longer (firmness < 35–45 N), preconditioning no longer affected the softening rate. Similar responses were observed during the 1993 and 1994 harvest seasons (data not shown).

SSC accumulation rates decreased over time probably because the differences between initial and ripe SSC decreased with each passing week due to natural starch conversion over time (Fig. 3C, D). As with the rate of softening, the rate of SSC accumulation was greatest in ethylene-preconditioned fruit from the commercial (week 0) harvest (0.56% day^{-1}) and slowest in week 5, freshly-harvested control fruit (0.11% day^{-1}). Again, these correspond with the fruit with the least and most SSC at the beginning of the ripening period. Preconditioning with ethylene tended to increase the rate of SSC accumulation in
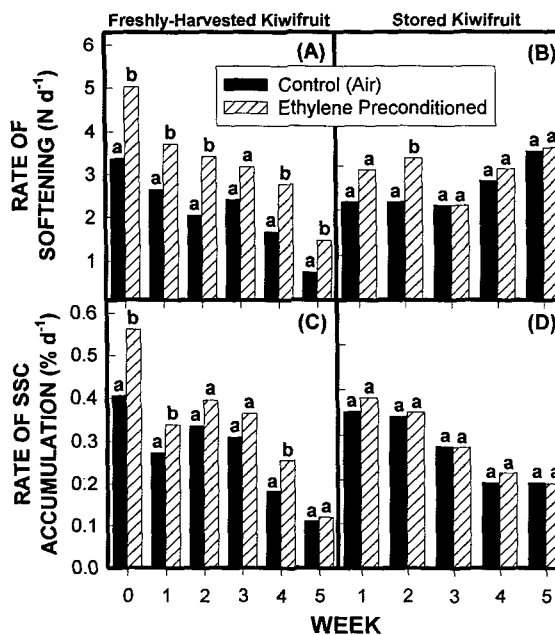

Fig. 3. Rate of softening (A, B) and SSC accumulation (C, D) during ripening at 20°C of freshly-harvested or stored kiwifruit. Preconditioned fruit were exposed to 100 μl l^{-1} ethylene for 24 h at 0°C while the control received only ethylene-free air. Letters above each bar represents significant differences ($P \leq 0.05$) within each week

freshly-harvested fruit through week 4, but had no significant effect on the rate of SSC accumulation in kiwifruit stored for 1 week or longer. Kiwifruit from the 1993 and 1994 seasons showed similar results (data not shown).

Although 24 h of ethylene exposure at 0°C did not increase the softening rate of kiwifruit stored for 3 weeks or longer, continuous 100 µl 1^{-1} ethylene exposure at either 0 or 20°C did hasten softening of kiwifruit stored for up to 9 weeks (data not shown). Continuous ethylene exposure did not significantly increase SSC accumulation in fruit at either of the two temperatures, probably because starch to sugar conversion was already nearing completion after 9 weeks of cold storage (data not shown).

3.3.3. Respiration and ethylene production during ripening

There was little difference in the patterns of respiration and ethylene production between freshly-harvested and stored kiwifruit during the 10-day ripening period (data not shown). Fig. 4 shows a representative pattern of respiration and ethylene production of control and ethylene-preconditioned kiwifruit during the ripening period. After initially high respiration rates 1 day following transfer from 0 to 20°C (on day 0), kiwifruit respiration quickly declined to base-line levels by day 2. Respiration usually remained near base-line levels until about day 5–8 at which time it began to increase. The day on which respiration began to increase was not significantly hastened by preconditioning with ethylene. The large standard error bars indicate the large variability in respiration rates as ripening progressed. In all experiments using composite samples, the climacteric peak was not observed during the 10-day ripening period.

Kiwifruit ethylene production was very low ($\sim$ 0.004 nmol g^{-1} h^{-1}) at the beginning of the ripening period; there was no initial burst in production upon transfer to 20°C as with respiration (Fig. 4). Ethylene production rates began increasing anywhere between 3 and 8 days after transfer to 20°C and preconditioning the fruit with ethylene did not significantly increase ethylene production or hasten its rise.
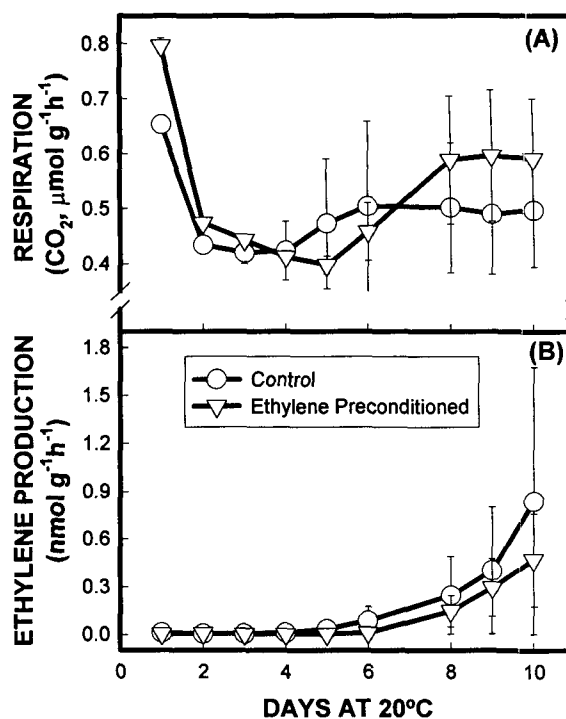

Fig. 4. Respiration (A) and ethylene production (B) during ripening at 20°C of freshly-harvested kiwifruit. Preconditioned fruit were treated with 100 µl 1^{-1} ethylene for 24 h at 0°C while the control received only ethylene-free air. Fruit were transferred to 20°C on day 0 The vertical bars represent standard error.

3.3.4. Kiwifruit respiration after transfer from 0 to 20°C

Kiwifruit, held at 0°C for as little as 48 h, had relatively high respiration rates after transfer from 0 to 20°C (Table 1 and Fig. 4). Fruit stored for more than 1 week at 0°C always had greater initial respiration than freshly-harvested fruit and ethylene preconditioning increased the initial respiration of freshly-harvested fruit held at 0°C for 48 h, but had less effect on stored fruit. Similar results were observed in 1994. In 1993, respiration was also high after transfer from 0 to 20°C, but there were no significant differences between freshly-harvested and stored fruit or ethylene-treated and control fruit (data not shown).

3.3.5. The climacteric in individual kiwifruit

In 1995, individual kiwifruit were monitored for respiration and ethylene production over 10 or 12 days. Although climacteric peaks were not identified from composite samples, individual fruit had distinct respiratory climacteric and ethylene peaks (data not shown). Only 13–50% of the individual fruit began their climacteric rise during the 10-day ripening period and this percentage was not significantly affected by ethylene treatments or by whether the fruit was freshly-harvested or from storage. However, by comparing each fruit's average respiration or ethylene production during the ripening period with its firmness at the end of the ripening period, we found that respiration and ethylene production increased dramatically only after fruit firmness dropped below about 7 N (Fig. 5). There was no clear point where respiration or ethylene production increased when plotted against SSC accumulation (data not shown).
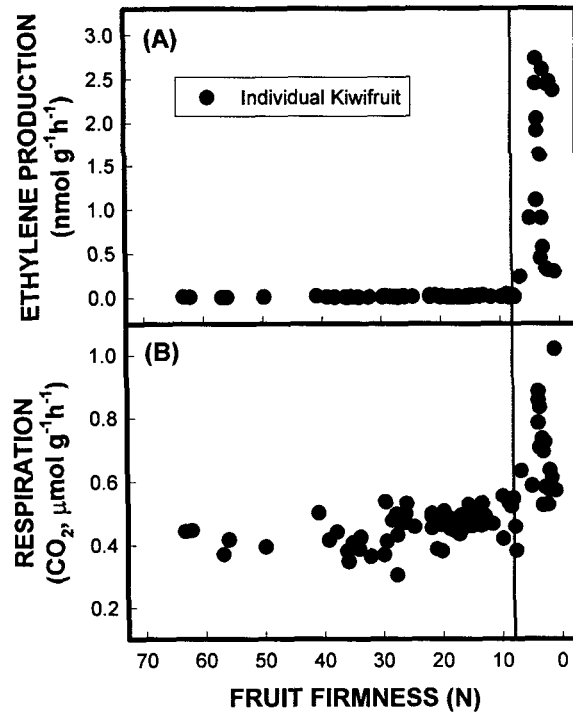

Fig. 5. Average ethylene production (A) and respiration (B) of individual kiwifruit during ripening over 10 or 12 days at 20°C with respect to fruit firmness at the end of the ripening period. Both control and ethylene-preconditioned fruit are included.

Table 1
Respiration (CO_2, μmol g^{-1} h^{-1}) of control or ethylene-preconditioned kiwifruit 24 h after transfer from 0 to 20°C

Week	Respiration (CO_2, μmol g^{-1} h^{-1})			
	'Freshly-harvested'		Stored	
	Control	24 h ethylene	Control	24 h ethylene
0	0.54a	0.75b	–	–
1	0.65a	0.82b	1.02c	1.01c
2	0.56a	0.81b	0.98c	1.06d
3	0.67a	0.75b	0.89c	0.97d
4	0.59a	0.68b	0.85c	0.86c
5	0.61a	0.75b	0.93c	0.99c

'Stored' kiwifruit were held at 0°C for 1–5 weeks while freshly-harvested fruit were held at 0°C for 48 h during cooling and preconditioning before being transferred to 20°C. Preconditioned fruit were exposed to a flow of air + 100 μl l^{-1} ethylene at 0°C for 24 h while control fruit received only ethylene-free air. Letters indicate significant differences within each week based on LSD at the 0.05 level.

4. Discussion

The rate of early season, ethylene-preconditioned kiwifruit ripening can be altered dramatically through the use of temperature management (Fig. 1). Further, preconditioning at 0°C offers greater flexibility in managing kiwifruit ripening than does preconditioning at 20°C. For example, fruit cooled rapidly to 0°C and ethylene-preconditioned at 0°C can be ripened within about a week if held at 20°C (Fig. 1B) or can be held for over 50 days before softening to 13 N if stored at 0°C (Fig. 1A). Holding the fruit at intermediate temperatures after ethylene treatment results in intermediate softening rates (Crisosto et al., 1997).

Our results agree with previous researchers that kiwifruit in cold storage become less responsive to ethylene preconditioning over time (Fig. 3). Di-Renzo and Mitchell (1985) found that cold-stored kiwifruit were most responsive to ethylene precon-

ditioning during the first weeks of storage and that responsiveness to preconditioning declined over 6 weeks. However, their ethylene treatments were administered at 20°C while ours were at 0°C. Preconditioning at higher temperatures stimulates more ripening (Fig. 1) and may explain why both they and Lallu et al. (1989) observed enhanced softening of ethylene-preconditioned (20°C) fruit even after 6 weeks of cold storage while we observed no difference between preconditioned (0°C) and control fruit after 3 weeks of cold storage. Therefore, kiwifruit held in cold storage for 3 weeks or more will not benefit from ethylene preconditioning at 0°C, but preconditioning at higher temperatures will accelerate softening (DiRenzo and Mitchell, 1985; Lallu et al., 1989). After 6 weeks or more of cold storage, kiwifruit will generally soften rapidly after transfer to 20°C without additional ethylene (DiRenzo and Mitchell, 1985; Crisosto et al., 1997).

Ethylene production did not increase after transferring ethylene-preconditioned or control kiwifruit from 0 to 20°C, but respiration did increase (Table 1 and Fig. 4). It is unclear what caused this burst of respiration upon warming. Chilling sensitive fruit often exhibit a characteristic burst of respiration upon warming to non-chilling temperatures (Lyons and Breidenbach, 1990). However, kiwifruit have not been found to be chilling sensitive (Mitchell, 1979) and even plant species that are not chilling sensitive can have increased respiration upon transfer from cold to warm temperatures (Appleman and Smith, 1936). Further, it is unlikely that changing CO_2 solubility (degassing) resulted in any significant increase in measured respiration. Using the Bunsen absorption coefficient for CO_2, at 0°C and in an atmosphere of 0.2 kPa CO_2, about 3.4 ml of CO_2 per 1 of water would be dissolved in the kiwifruit. Of this, only about half, or $1.7 \ ml \ l^{-1}$, would be expected to degas upon warming to 20°C. If the fruit normally produces about 0.42 $\mu mol \ g^{-1}h^{-1}$ at 20°C (Fig. 4), an addition of 0.08 $\mu mol \ g^{-1}h^{-1}$ of CO_2 dissipated over the 24 h equilibration period would not result in a significant increase in measured respiration. Rather, there may be an imbalance or shift in the predominant metabolites during storage at 0°C which

does not result in cellular damage but is rapidly metabolized upon warming. Further work is needed to determine the physiological event(s) which give rise to this burst of respiration.

While ethylene exposure hastens the ripening of climacteric fruits, exposing kiwifruit to 100 $\mu l \ l^{-1}$ ethylene for 24 h at 0°C did not significantly hasten the climacteric of either composite or individual fruit (data not shown). Further, neither DiRenzo and Mitchell (1985) nor Lallu et al. (1989) detected the climacteric of composite samples of ripening kiwifruit preconditioned with ethylene at 20°C. However, they both observed accelerated softening of ethylene-preconditioned fruit just as we did. Arpaia et al., (1994) reported that the kiwifruit climacteric occurred late in the ripening process when the fruit softened below about 13 N. We found that the climacteric rise of both ethylene-preconditioned and control kiwifruit began only when fruit soften to below 7 N, well after the fruit were soft enough to eat (Fig. 5). Therefore, one would expect that an observed increase in the rate of softening would be accompanied by a hastening of the climacteric. This suggests that with the large variability in kiwifruit ripening (Pratt and Reid, 1974), it is likely that a hastening of the climacteric would be detected if a greater number of individual fruit were monitored and the fruit were monitored for a longer period of time. Although ethylene has profound effects on kiwifruit softening, the function of the late-occurring climacteric in kiwifruit remains unclear.

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