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"Stepped Down" Storage Temperature Regimes for Fuerte Fruit Grown in the Kwazulu/Natal Midlands: Do they Reduce the Incidence of Physiological Disorders?

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ABSTRACT

Avocados grown in the Kwazulu/Natal Midlands mature approximately 2 months later than the same cultivars grown in the Northern and Eastern Transvaal, and the stepped down temperature regimes used for cold storing Transvaal fruit during export are not as effective for Kwazulu/Natal fruit. Nine different temperature regimes were tested on Fuerte fruit from Everdon Estate in the Kwazulu/Natal Midlands during the 1993 and 1994 seasons. The 1993 fruit showed a lower incidence of internal physiological disorders than the 1994 fruit, associated with a heavier crop and lower tree vegetative vigour. Stepped down temperature regimes (beginning at around 7.5°C and ending at around 4.5°C) appeared to be no more effective than continual storage at 5.5°C and generally did not reduce the incidence of physiological disorders associated with cold storage. There were no significant fluctuations in fruit ethylene evolution during 4 weeks of cold storage at 5.5°C or 7.5°C and the rise in ethylene production associated with the respiratory climacteric occurred after removal from cold storage. Total fruit phenolic levels did not change significantly during the 1994 harvesting season at Everdon Estate.

INTRODUCTION

Considerable progress in temperature management during shipping of South African avocados has been made in recent years. Toerien (1986) proposed a system of reducing storage temperatures throughout the season as the fruit became more mature and less sensitive to low storage temperatures. Vorster *et al.* (1990) found that higher temperatures during the earlier stages and lower temperatures during the later stages of cold storage tended to decrease the incidence of internal physiological disorders. Temperature management of avocados grown in the Eastern and Northern Transvaal is currently carried out according to these principles. The development of an export based avocado industry in the Kwazulu/Natal Midlands is fairly recent. The 1991 avocado census showed 162 405 trees in Kwazulu/Natal with a production potential of 6 % of the South African avocado industry (Finnemore, 1991). The main feature of this industry is the relatively later maturity of each cultivar due to the greater latitude (±30°S as opposed to 23 to 25°S) and the generally cooler growing conditions. Kaiser and Wolstenholme (1994) also noted differences in fatty acid composition and lipid content

of Kwazulu/Natal as compared to Northern Transvaal fruit. There is evidence therefore, that Kwazulu/Natal fruit, cultivar for cultivar is different to Transvaal fruit, and that norms drawn up for the latter may not be strictly applicable to the Kwazulu/Natal Midlands, and it has been found that temperature regimes which are effective in storing Transvaal avocados of similar maturity are not effective for Kwazulu/Natal fruit. Nine different temperature regimes were tested at Everdon Estate, Howick, throughout the 1993 and 1994 Fuerte harvesting seasons in an attempt to determine which regimes will produce firm fruit with minimal internal and external disorders after a 4 week storage period.

Mesocarp discolouration in the form of grey pulp and/or pulp spot is as a result of the oxidation of o-diphenols by the enzyme polyphenol oxidase to o-quinones which are in turn oxidised to brown melanin pigments (Bower & Cutting, 1988). Levels of total phenolics and polyphenol oxidase activity were found to be higher in avocado fruit with mesocarp discolouration than healthy fruit (van Lelyveld *et al.,* 1984). Cutting *et al.* (1992) found that total phenols in Fuerte avocado fruit increased with increasing maturity, as did mesocarp discolouration. The concentration of total phenolics in Fuerte mesocarp was determined throughout the harvesting season at Everdon Estate to determine whether an increase in physiological disorders with increasing maturity could be explained by an increase in total phenolic concentration.

Because stepped down temperature regimes are reported to reduce internal physiological disorders (Vorster *et al,* 1990), it was hypothesised that at some point during cold storage, there is a decrease in sensitivity to cold storage, and that this may be marked by a change in ethylene evolution associated with the climacteric rise in respiration. Consequently, ethylene evolution was measured during cold storage to see if a change in sensitivity to chilling temperatures was marked by a fluctuation in ethylene production.

MATERIALS AND METHODS

Temperature regime trials

Standard 4 kg export cartons of count 14 and 16 fruit (236 g to 305 g) were taken from the pack line at Everdon Estate every week during the 1993 and 1994 Fuerte harvesting seasons (Table 2). Ten Cartons were used for each treatment (Table 1). Fruit moisture content was determined for each trial as an index of maturity (Swarts, 1976). After the designated period (three to five weeks) in cold storage (Table 1), fruit firmness was determined using a firmometer (Swarts, 1981) where after the fruit was placed in a room at approximately 20°C and allowed to ripen. When eating ripe, as determined by a firmometer (Swarts, 1981), the fruit was rated internally and externally on a "presence or absence" basis for the following disorders: Cold damage (also known as black cold), pulp spot, grey pulp, and vascular browning (Swarts, 1984); a general browning of the distal end of the fruit known in the industry as "bolverkleuring" or "dusky cold", anthracnose rot, and stem end rot. The occurrence of lenticel damage was also recorded.

Table 1
Temperature regimes used for Fuerte fruit

Regime	*Temperature (C)*				
	Week 1	*Week 2*	*Week 3*	*Week 4*	*Week 4*
1	8,5	8,5	7,5	6,5	—
2	8,5	7,5	6,5	5,5	—
3	8,5	7,5	5,5	4,5	—
4	7,5	7,5	6,5	5,5	—
5	7,5	7,5	5,5	4,5	—
6	5,5	5,5	5,5	5,5	—
7	4,5	5,5	7,5	7,5	—
8	7,5	7,5	6,5	—	—
9	7,5	7,5	6,5	5,5	5.5

Table 2
Dates on which temperature regime trials were commenced

Trial	*1993*	*1994*
A	19 May	17 May
B	26 May	24 May
C	2 June	1 June
D	9 June	6 June
E	16 June	15 June
F	23 June	21 June
G	30 June	29 June
H	8 July	6 July
I	15 July	12 July
J		19 July

The data collected was analysed using a logistic regression model for a binomial distribution (Gujarati, 1988). The incidence of each disorder was analysed separately for each week that a trial was run.

Phenolic quantification

Eight Fuerte avocado fruit of mass 236 g to 265 g (count 16) were taken from the pack line at Everdon Estate once a week for the duration of the 1994 Fuerte harvesting season, which lasted from the first week in June to third week in July. A 1.5 cm ring of mesocarp tissue from the widest point of each fruit was freeze dried and stored at -20°C until analysis. A modification of the method used by Osborne (1992) to quantify total phenols in *Pinus patula* cuttings, which is a modification of the method of Torres *et al*

(1987) to determine total phenols in avocado mesocarp, was used.

Ethylene determination

Mature Fuerte fruit of mass 236 g to 265 g were harvested at Everdon Estate on 15 July 1993. Twenty fruits were stored in open glass jars either at 5.5°C or 7.5°C (ten at each temperature) for 28 days and then ripened at room temperature. Ethylene evolution was determined daily by sealing the jars for 2 h and drawing off a 1 ml sample of head gas in a gas tight syringe, which was injected into the megabore column of a Varian® 3400 gas Chromatograph equipped with a flame Ionisation detector.

RESULTS AND DISCUSSION

Temperature regime trials

In most cases in 1993 and 1994, there were significant differences in the incidence of disorders between treatments in any one trial. This was often due to differences in the incidence of disorders between treatments 8 and 9 which were of 3 and 5 weeks duration respectively, instead of the standard 4 week duration of the other treatments. Increased duration of storage is known to increase the incidence of physiological storage disorders. Because it was not possible to see any trends in the incidence of disorders in relation to storage temperature from these analyses, further analyses of the incidence of disorders in treatments one to six (Table 1) were carried out. Treatment 6 (5.5°C throughout the storage period) was used as a control against which the other treatments were compared. Significant differences between treatment 6 and the other five treatments are shown in Table 3.

Although treatments 1 to 5 showed significantly less lenticel damage than treatment 6 in a number of trials in both the 1993 and 1994 seasons (Tables 3 and 4), this disorder was not used as a measure of the effect of temperature regime on cold storage disorders as treatment 9 often had a lower incidence of lenticel damage than treatment 4, even though the first 4 weeks of treatment 9 were identical to treatment 4 (Table 1). Lenticel damage is probably as a result of preharvest factors or rough handling rather than low storage temperatures.

Table 3

Summary of analyses of treatments 1 to 6 of 1993 temperature regime trials showing those treatments which were different from treatment 6 (control) for the various disorders.

Trial	*Black cold*	*Pulp spot*	*Grey pulp*	*Vasc. brown*	*Anth.*	*Stem end rot*	*Lenticel damage*
A	$1^{h}3^{h}$	—	—	—	—	—	$1^{l}2^{l}$
B	$(1–5)^{l}$	—	—	—	—	—	$2^{h}(3–5)^{l}$
C	—	—	—	—	—	4^{l}	—
D	$2^{h}3^{h}5^{h}$	—	—	—	—	—	—
E	—	—	—	—	—	—	—
F	—	4^{h}	—	—	—	—	—
G	—	$2^{h}4^{h}$	—	$1^{h}4^{h}$	—	—	$1^{l}3^{l}4^{l}5^{l}$
H	—	—	—	—	—	—	$1^{l}5^{l}$
I	—	—	—	3^{h}	—	—	$2^{l}4^{l}$

^{h}Significantly higher incidence of disorder at 95 % level.
^{l}Significantly lower incidence of disorder at 95 % level.

Table 4

Summary of analyses of treatments 1 to 6 of 1994 temperature regime trials showing those treatments which were different from treatment 6 (control) for the various disorders.

Trial	*Black cold*	*Distal end brown*	*Pulp spot*	*Grey pulp*	*Vasc. brown*	*Anth.*	*Stem end rot*	*Lenticel damage*
A	—	—	—	—	—	—	2^{h}	$1^{l}4^{h}$
B	—	—	—	—	—	—	$3^{l}4^{l}5^{l}$	$1^{l}3^{l}4^{l}5^{l}$
C	—	—	—	—	1^{l}	—	—	$2^{l}3^{h}5^{h}$
D	—	2^{h}	—	—	4^{l}	—	—	5^{l}
E	—	—	—	—	4^{h}	—	—	$1^{l}2^{l}3^{l}5^{l}$
F	$(1–5)^{l}$	$4^{l}5^{l}$	—	3^{l}	3^{l}	—	$1^{h}3^{l}$	$1^{l}2^{l}3^{l}5^{l}$
G	—	—	—	$2^{l}3^{l}$	—	—	2^{l}	3^{h}
H	—	5^{h}	$2^{l}3^{l}$	5^{h}	$2^{l}5^{l}$	$1^{l}5^{l}$	$1^{l}4^{l}5^{l}$	$1^{l}4^{h}$
I	—	—	$2^{h}5^{l}$	2^{h}	$2^{h}5^{h}$	4^{h}	$1^{h}3^{h}$	1^{l}
J	1^{h}	2^{h}	—	—	—	—	—	$3^{l}4^{l}$

^{h}Significantly higher incidence of disorder at 95 % level.
^{l}Significantly lower incidence of disorder at 95 % level.

In 1993, treatments 1 to 5 of trial B and treatment 4 of trial E had a lower incidence of black cold and stem end rot than treatment 6 (Table 3). In all the other cases where there were significant differences between treatment 6 and the other five treatments the incidence of those disorders was higher than those in treatment 6. The incidence of black cold in 1993 trial B was significantly higher in treatment 6, but in trial D of the same year, treatments 2, 3 and 5 had a higher incidence of this disorder then treatment 6 (Table 3). From this, it is obvious that one cannot conclude that the stepped down temperature regimes of treatments 2, 3 and 5 reduced the incidence of black cold. However one can more easily conclude that stepped down temperature regimes were no more effective than 5.5°C throughout the storage period for Fuerte fruit from Everdon Estate in 1993. In some cases, e.g. 1994 trail G (Table 3), the stepped down regimes produced fruit with a higher incidence of vascular browning and grey pulp.

For the first 5 weeks of the Fuerte harvesting season (trials A to E), treatments 1 to 5 seldom produced a lower incidence of internal physiological disorders than treatment 6 (Table 3), which is contrary to the findings of Vorster *et al.* (1990) where higher temperatures during the earlier stages of the season reduced the incidence of internal physiological disorders in Transvaal fruit. It must be borne in mind however, that fruit from different geographic locations may react differently to cold storage (Rowell, 1988).

During the 6th to 8th weeks of the 1994 season (trials F to H), some of the stepped down temperature regimes showed a lower incidence of pathological and physiological disorders than treatment 6, although trials I and J proved the stepped down temperature regimes in some cases to be worse (Table 4). As was the case with the 1993 trials, higher storage temperatures during the earlier part of the 1994 season did not reduce the incidence of internal physiological disorders. The lower incidence of disorders in some of the stepped down temperature regimes in trials F-H cannot be explained by fruit maturity as there was very little difference in fruit maturity between trials H and I based on fruit moisture content (Table 5).

Table 5
Moisture content (% by mass) of Fuerte fruit used in the 1993 and 1994 temperature regime trials.

Trial	*1993*	*1994*
A	76,6	77.8
B	74.2	75.7
C	73.8	76.3
D	73.8	73.7
E	72.9	71.5
F	69.8	72.4
G	69.3	74.1
H	65.4	68.1
I	64.5	67.2
J	—	66.8

Fruit firmness on arrival in Europe was identified by Bezuidenhout and Eksteen (1994) as an important quality determining factor. Fruit with firmometer readings of < 35 are classified as acceptably firm by the South African Avocado Growers' Association and fruit with a firmometer reading of < 30 are classified as "hard". With the exception of treatment 7 in 1993 trial B (Data not shown), all treatments in the 1993 and 1994 trials yielded fruit with a firmometer reading of < 30.

In order to observe seasonal trends in the occurrence of physiological disorders, the occurrence of each disorder in treatment 6 was plotted for both the 1993 and 1994 seasons (Figures 1-5). The incidence of black cold was generally low in both seasons except in trial B of 1993 (Figure 1), however the incidence of black cold in treatments 1-5 for that trial was very low or non-existent. As it seems that fruit moisture loss plays a role in causing black cold (Donkin & Cutting, 1994), the higher incidence of this disorder may have been due a higher percentage of water-stressed fruit in that treatment.

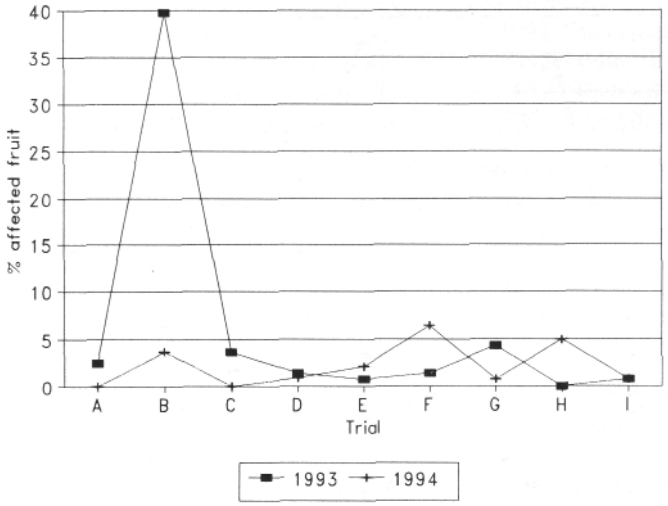

Figure 1
Incidence of black cold after 4 weeks at 5.5°C, during the 1993 and 1994 Fuerte harvesting seasons.

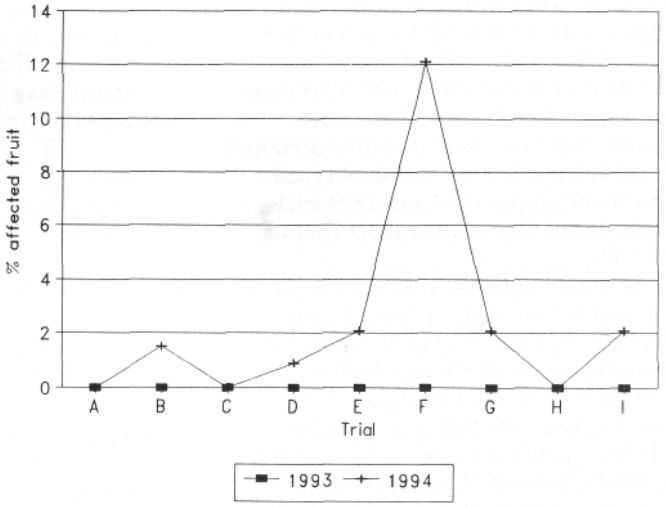

Figure 2
Incidence of distal end browning after 4 weeks at 5.5°C, during the 1993 and 1994 harvesting seasons.

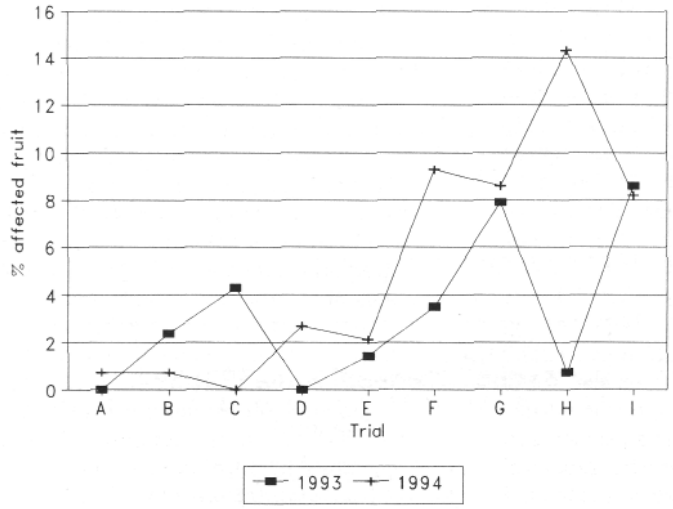

Figure 3
Incidence of pulp spot after 4 weeks at 5.5°C, during the 1993 and 1994 Fuerte harvesting seasons.

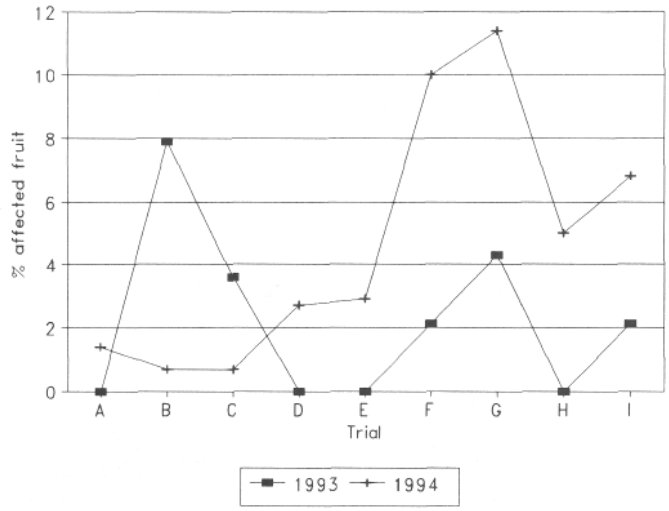

Figure 4
Incidence of grey pulp after 4 weeks at 5.5°C, during the 1993 and 1994 Fuerte harvesting seasons.

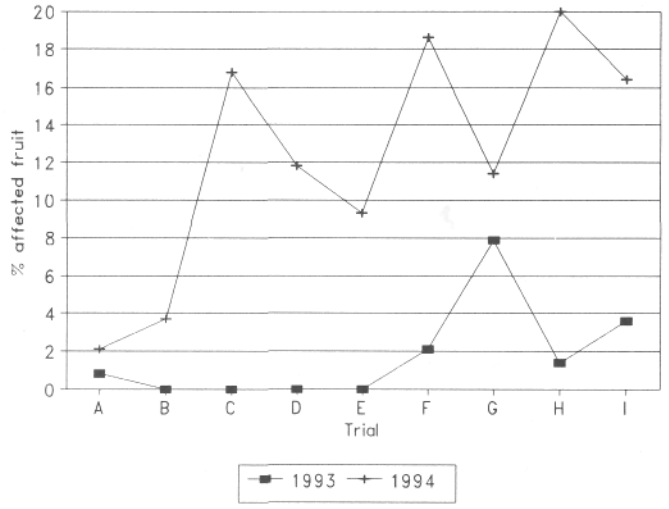

Figure 5
Incidence of vascular browning after 4 weeks at 5.5°C, during the 1993 and 1994 Fuerte harvesting seasons.

Distal end browning or "bolverkleuring" was only observed in a few fruits in the 1993 season and was not recorded as a disorder when evaluating the ripe fruit, but was more prevalent in the 1994 season where the disorder occurred in between 0 and 12 % of the fruit (Figure 2). Not all treatments had as high an incidence of the disorder in trial F of 1994. The incidence in treatments 4 and 5 was significantly lower than in treatment 6 (Table 4). The incidence of pulp spot (Figure 3), grey pulp (Figure 4) and vascular browning (Figure 5) was generally higher in 1994 than in 1993. This indicates that pre-

harvest factors play an important role in determining the quality of cold stored avocados. At Everdon Estate, the 1993 crop was heavier than the 1993 crop with less vegetative growth and the incidence of internal physiological disorders was higher in 1994, confirming industry experience that fruit physiological disorders are worse in seasons characterised by high tree vigour, usually associated with light cropping.

Susceptibility to mesocarp discolouration (van Rensburg and Engelbrecht, 1985; Bower and Cutting, 1988) and chilling injury (Chaplin and Scott, 1980) have been associated with low calcium levels in avocado fruit. Calcium is transported to plant organs by mass flow in the xylem. Polar transport of indoleacetic acid (IAA) from an organ is also thought to affect Ca transport (Bangerth, 1979). Physiologically active organs such as developing shoots and fruit are vigorous exporters of IAA. In avocado trees, the spring flush overlaps to varying extents with fruit set and development. Fruit and new shoots therefore compete for resources, one of which is Ca. Because of their high physiological activity and transpiration rate, leaves and shoots are stronger sinks for Ca than fruit (Witney *et al.,* 1990). It appears that in years of vigorous vegetative growth, less of the available calcium taken up by the tree is allocated to the fruit, resulting in a greater susceptibility to cold storage disorders.

It seems that the vegetative-reproductive balance in avocado trees is crucial in determining tolerance of the fruit to low storage temperatures. Cultural practices should be employed to reduce the vigour of the spring flush.

Phenolic quantification

There was no significant difference between total phenolic concentrations in the fruit mesocarp during the 1994 Fuerte harvesting season ($P < 0.05$) (Figure 6). Trials F, G and H of 1994 showed an increase in the incidence of pulp spot (Figure 3) and grey pulp (Figure 4) compared to the previous 5 weeks of the harvesting season. This increase in browning disorders was not accompanied by an increase in total phenolic levels. The results obtained in this experiment are in agreement with those of Cutting *et al.* (1992) where there was no marked increase in phenolic levels from May to July in Fuerte fruit harvested at Everdon Estate. Their work only showed an increase in total phenolics in September and October which falls outside of the Fuerte harvesting season at Everdon Estate. Although fruit phenolics are involved in mesocarp discolouration, their concentration does not seem to be a factor which influences susceptibility to mesocarp discolouration during the normal Fuerte harvesting season.

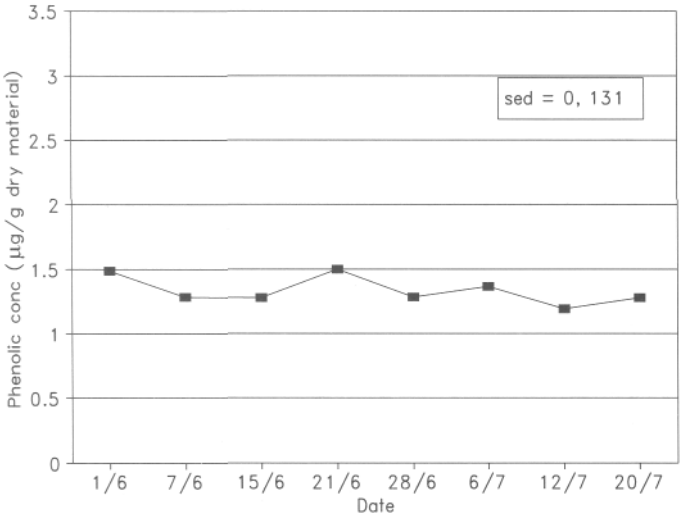

Figure 6
Total phenolic concentration in Fuerte avocado mesocarp harvested at weekly intervals during the 1994 Fuerte harvesting season.

Ethylene determination

During the 28 days of cold storage, there were no dramatic fluctuations in ethylene production. The levels of ethylene production during storage at 5.5°C and 7.5°C were very low viz. mostly between 0 and 5 $\mu\ell.kg^{-1}.h^{-1}$. On removal from cold storage, the fruit stored at 7.5°C had a higher rate of ethylene production, peaking at 109 $\mu\ell.kg^{-1}.h^{-1}$ as opposed to 75 $\mu\ell.kg^{-1}.h^{-1}$for the fruit stored at 5.5°C (Figure 7). Increased duration of storage was found to reduce the amount of ethylene produced at the climacteric peak after removal from cold storage in Hass fruit (Eaks, 1983). The degree of cold stress therefore seems to have an effect on the levels of ethylene produced after cold storage. Similar results were obtained by Zamorano *et al.* (1994) who found that avocado fruit softening was delayed at 7°C but was inhibited at 3°C during a storage period of 55 days and that ethylene production rates were drastically reduced by cold storage. Although ethylene production was inhibited, CO_2 production increased after 25 days of storage at 7°C which coincided with softening. In the light of the results obtained in this experiment and those obtained by Zamorano *et al.* (1994), it can be concluded that ethylene production rate in avocado fruit cannot be used to determine a change in chilling sensitivity during cold storage. It is also noteworthy that there was no evidence of a climacteric rise in respiration during the period of cold storage, contrary to a common industry perception that occurs during the sea voyage to Europe.

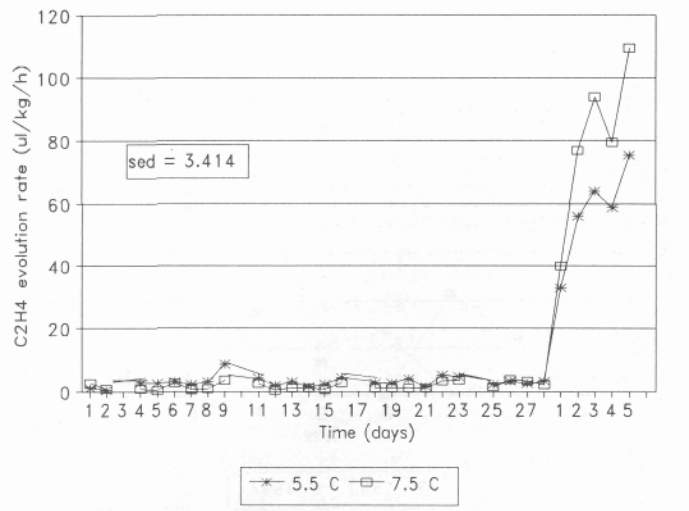

Figure 7
Ethylene evolution rate of Fuerte avocado fruit stored at 5.5°C and 7.5°C for 28 days and then allowed to ripen at room temperature.

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