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MODE OF ACTION OF WATER LOSS ON FRUIT QUALITY OF 'HASS' AVOCADOS

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SUMMARY

In this study, increased water loss was induced in 'Hass' avocados at different stages of ripening (inhibition, preclimacteric, climacteric and postclimacteric phases) in fruit harvested early, mid and late season (designated H1, H2 and H3, respectively). The effect of water loss was investigated on ripening and the incidence of rots. In addition, to determine whether water loss was acting through, or independent of, ethylene, 1-aminocyclopropane carboxylic acid (ACC) content and ethylene forming enzyme (EFE) activity in fruit were determined after water loss treatments, and some fruit were treated with an ethylene binding inhibitor 1-methylcyclopropene (SmartFresh™) during the water loss treatments. To increase water loss during a ripening phase, fruit were transferred from high humidity conditions (>95% RH) to low humidity conditions (<20% RH) for the duration of that phase before returning to high humidity conditions to complete ripening. Fruit remained at 20°C throughout the water loss treatments and the duration of ripening.

Increased water loss from fruit in the early stages of ripening (inhibition and preclimacteric phases) resulted in accelerated ripening of fruit, although the degree of acceleration of ripening was reduced in fruit harvested later in the season. Ripening accelerated by water loss was associated with earlier ethylene production, higher EFE activity and ACC content, and SmartFresh™ negated the effect of water loss on ripening. The incidence of rots, and effect of water loss on rots, was inconsistent through the harvest season, but with a trend for water loss early in ripening to reduce the incidence of stem end rots.

It is concluded that increased water loss during the initial stages of ripening affects the rate of ripening and the incidence of rots and thereby strongly impacts on fruit quality. However, the capacity for increased water loss during the initial stages of ripening to accelerate fruit ripening is limited to fruit harvested early in the season. Similarly, increased water loss during the later stages of ripening, i.e. during rapid softening has little or no effect on ripening rate or rot incidence. The effects of water loss are most likely acting through ethylene biosynthesis.

Key words: avocado, water loss, mode of action, ripening, quality, ethylene

INTRODUCTION

Water loss after harvest affects the quality of avocados through an effect on the rate of ripening and/or an effect on the incidence of rots. High rates of water loss soon after harvest decrease the times to ripen during shelf life after storage but can increase the incidence of rots by 5-15% (Bower and Cutting 1988; Lallu *et al*., 2002, 2003). The mechanism by which water loss leads to the effects on quality is unknown, but such knowledge would be useful in developing harvesting and handling practices that minimise the negative effects of water loss on postharvest quality.

Typically, the transformation of an unripe avocado fruit at harvest into a ripe fruit is associated with an increase in ethylene production by the fruit. For a period lasting 24 to 72 hours immediately after harvest, avocado fruit are relatively insensitive to ethylene but thereafter become rapidly and increasingly sensitive to ethylene. The fruit begins to increase its rate of ethylene production that eventually peaks. Associated with the peak in ethylene production is an increase in respiration. Together the increase and peaks in respiration and ethylene production are referred to as the climacteric. The climacteric pattern of ripening behaviour can be divided into 4 phases: the inhibition phase that includes the time after harvest when fruit are relatively insensitive to ethylene, the preclimacteric phase that follows the inhibition phase and in which fruit become sensitive to ethylene but are not producing ethylene, the climacteric phase that includes the rise in ethylene production, and the postclimacteric phase that includes the period after the climacteric peak.

The ethylene and respiratory climacterics are considered to trigger the events that lead to ripening and the climacteric peak that may last for 2 to 4 days in avocados. The trigger for the climacteric is unknown but it is possible that water loss after harvest leads to the initiation of ethylene production since water stress is known to increase ethylene production in various plant tissues including avocados (Adato and Gazit, 1974).

The biosynthetic pathway for ethylene production involves the synthesis of ACC (1-aminocyclopropane carboxylic acid) and its conversion or oxidation to ethylene. The key enzymes involved are ACC synthase, which synthesizes ACC from SAM (S adenosyl methionine) and ACC oxidase, which converts ACC to ethylene. When ACC oxidase is not measured directly *in vitro* after purification, but instead measured *in vivo* by adding ACC to the tissue, it may be referred to as ethylene forming enzyme (EFE) activity. This pathway of ethylene synthesis is common to nearly all plant species.

After synthesis, ethylene binds to its receptor, which is a protein inserted in the membrane of cells, and a sequence of events are initiated that result in typical ethylene action or responses such as ripening. By preventing ethylene binding to its receptor, it is possible to prevent ethylene action. SmartFresh™ is a commercial formulation of 1-MCP (1 methylcyclopropene) that binds to the ethylene receptor and thereby prevents ethylene action.

Given both water loss and ethylene lead to an increase in the rate of ripening the question arises is water loss acting on ripening through ethylene, or independently of ethylene? Similarly, is the effect of water loss on rots through an effect on ripening or

directly on rots? In previous studies, the importance of the rates and timing of water loss were studied, and it was shown that water loss in the first 24 to 48 hours after harvest could affect the incidence of rots by approx. 5 to 15% (Lallu *et al.,* 2002, 2003).

In this study, water loss was induced at different stages of ripening (inhibition, preclimacteric, climacteric and postclimacteric phases) and its effect on ripening and the incidence of rots was quantified using fruit from an early (September), mid (November) and late (January) harvest. In addition, to determine whether water loss was acting through or independently of the ethylene pathway, the ACC content and EFE activity of fruit were determined after water loss treatments, and some fruit were treated with 1-MCP during the water loss treatments.

MATERIAL AND METHODS

FRUIT

Approx. count 23 'Hass' avocados that had been harvested from a commercial orchard around Katikati on 3rd September 2004, 19th November 2004 and 18th January 2005 were selected from bins within 1 hour of harvest and packed into single layer trays complete with a moulded cardboard pocket-pack. After packing, fruit were transported by van to HortResearch, Auckland and the trays randomly allocated to treatments.

TREATMENTS

Trays (8) of fruit were held at 20°C in chambers under high humidity conditions until subjected to high rates of water loss by transferring the trays to a low humidity chamber for the duration of the inhibition, preclimacteric, climacteric and postclimacteric phases of ripening. Overall, 5 chambers were set up, 1 each for the 4 phases of ripening and 1 (Control) in which fruit remained in a high relative humidity (RH) environment throughout ripening. Half of the fruit (4 trays) at each phase of ripening were treated with 300 ppb SmartFresh™ for 18 hours under low RH conditions in a separate chamber to the non SmartFresh™ treated fruit. The duration of treatments under low RH differed through the season with the natural change in ripening off the tree. In September the fruit were held for 3, 4, 4, and 4 days respectively for the inhibition, preclimacteric, climacteric and postclimacteric periods respectively. Fruit from the November harvest were held for 3 days for all phases, and fruit harvested in January for 2 days for each phase. After treatment with SmartFresh™ and/or for water loss, treated and untreated trays were returned to the high humidity chambers.

For high humidity conditions (>95% RH), 4 trays of fruit were held in 360 L PVC chambers through which water saturated air was passed at a rate of 1015 L/min. Within each chamber, approx. 5 kg of ethylene absorbent granules (Ethysorb®) was placed on top of the trays, which were stacked on top of approx. 20 mm of water. For low humidity conditions (<20% RH), compressed air rather than saturated air was used and water was omitted from within the chambers and replaced with pellets of sodium hydroxide. The flow rate was increased to approx. 220 L/min in order to maintain a low humidity in the chamber.

The ripening phases were determined by measuring the rates of ethylene production and respiration rates of 96 individual fruit that had been sampled from the trays of fruit.

The fruit were placed on an auto respiration system in 2 L jars with a humidified airflow of approx. 25 ml/min. per jar. For the duration of each ripening phase, sub samples of 12 fruit were removed from the auto respiration system and placed under the low humidity conditions before returning to the auto respiration system for the remaining phases of ripening. These sub samples of fruit were used for ACC and EFE determinations, which were carried out according to Lizada and Yang (1979) and Bufler (1984), respectively.

FRUIT ASSESSMENTS

At intervals after the water loss and/or SmartFresh™ treatments, all fruit in each chamber were examined and any fruit at the eating ripe stage were removed and assessed for the rate of ripening, the incidence and severity of rots and disorders, and weight loss. Subjective assessments of skin colour, stem end rots, body rots, external rots, and disorders were made according to the methods and 0-100% rating scales described in the New Zealand Avocado Industry Council (AIC) Assessment Manual 2001, Version 2.

Rate of ripening during shelf life

The time taken for fruit to reach eating ripeness was used to calculate days to ripe. The ripeness of avocados was determined by squeezing a fruit gently in the hand, and a fruit was considered to be at eating ripeness when the flesh could be depressed with light pressure, but left no indentation. Assessments for hand firmness were calibrated against ripe fruit that had a firmness of 8590 units when determined by Anderson firmometer.

Rots and disorders

Severity of rots and disorders was determined when fruit were eating ripe using 0 to 100% rating scales as described in the NZ AIC Assessment Manual. The incidence reported was based on fruit with a severity greater than 2%. The percentage surface area of fruit with external rots was assessed first, the stem button was then removed and the fruit cut into quarters to assess internal rots and disorders. The severity of stem end rots was assessed, the peel was then removed from each quarter and the severity of body rots (i.e. rots on the body of the fruit under the skin) was determined. Physiological disorders (vascular browning and grey pulp) were then assessed.

Weight loss

The weight of 10 fruit in each tray was measured prior to and after water loss treatments, and again when ripe, from which water loss was calculated.

DATA ANALYSIS

To identify significant effects of treatments at a $P<0.05$ level, untransformed data or arc sin square root transformed data were subjected to ANOVA using Genstat. All data in the tables are untransformed data.

RESULTS AND DISCUSSION

For this paper, mainly data from early season fruit (September) is presented, but a

comparison of the effects of water loss on the fruit from all 3 harvests is made. Patterns of change in ripening were similar between all harvests but there were quantitative differences and these are tabulated and discussed when present.

During ripening, water loss was approx. 6% by the end of the postclimacteric phase. Transferring fruit to low humidity conditions for the different phases of ripening resulted in increased water loss by approx. 3% at the end of the inhibition phase and by approx. 4% at the postclimacteric (Table 1). When fruit were treated with SmartFresh™ during the respective ripening phase, water loss was similar to that of untreated fruit (Table 1), and therefore the effect of SmartFresh™ was not through any difference in the amount of water lost.

Table 1. Water loss from early season 'Hass' avocados held at 20°C in a high humidity (>95% RH) during all phases of ripening (Control), or in a low humidity (<20% RH) for the duration of the Inhibition, Preclimacteric, Climacteric, or Postclimacteric phases of ripening, before returning to high humidity for the completion of ripening. Data in parenthesis is for fruit treated with SmartFresh™ (300 ppb) during the first 18 hours under low humidity. Control fruit were not treated with SmartFresh™. Values are the average of 40 fruit.

	Water loss (% of initial)			
Treatment	Inhibition	Preclimacteric	Climacteric	Postclimacteric
Control	1.3	3.1		
Inhibition	4.4 (4.6)			
Pre-climacteric		7.5 (7.2)		
Climacteric			8.8 (8.3)	
Post-climacteric				10.0 (9.9)

Table 2. Time taken to ripen by early, mid or late season 'Hass' avocados held at 20°C in a high humidity (>95% RH) during all phases of ripening (Control), or in a low humidity (<20% RH) for the duration of the Inhibition, Preclimacteric, Climacteric, or Postclimacteric phases of ripening before returning to high humidity for the completion of ripening. Values in parenthesis are for fruit treated with 300 ppb SmartFresh™ during the first 18 hours under low humidity. Values are the average of approx. 90 fruit.

	Time to ripen (days)		
Treatment	Early	Mid	Late
Control	16.4	13.2	10.3
Inhibition	14.1* (18.7)	12.4* (18.1)	10.3 (15.4)
Pre-climacteric	14.1* (22.1)	12.8 (20.4)	10.8* (18.8)
Climacteric	15.7* (23.3)	13.7* (17.4)	10.0* (10.0)
Post-climacteric	17.0* (16.2)	13.8* (13.9)	10.1 (10.1)

Values in a column marked * differ significantly from the control at P=0.05.

The natural time taken for early, mid and late season fruit to ripen was 16.4, 13.2 and 10.3 days, respectively (Table 2). Water loss during the early phases of ripening (inhibition and preclimacteric phases) accelerated ripening by approximately 2.3, 0.8 and 0 days for early, mid and late season fruit, respectively (Table 2). Hence as the

season progressed, and the untreated fruit ripened more rapidly after harvest, the potential for water loss to influence the rate of ripening was reduced. Water lost during the climacteric and postclimacteric phases of ripening had little or no effect on the rate of ripening, although the result for fruit from the early and mid season harvests suggests that water loss during the postclimacteric phase may slow ripening slightly by approx. 0.6 days.

For fruit from the early harvest, there was a clear increase in ethylene production during ripening from which the durations of the inhibition, preclimacteric, climacteric and postclimacteric phases of ripening were determined as being approximately 3, 4, 4 and 3 days, respectively (Figure 1). The duration of the low ethylene production periods (i.e. the inhibition and preclimacteric phases) declined from approximately 10 days in the early harvested fruit to approximately 6 days for fruit from the late harvest. SmartFresh™ treatment delayed the increase in ethylene production when inhibition, preclimacteric or climacteric fruit were treated, but had no or little effect on postclimacteric fruit.

Once ethylene production and fruit softening, i.e. ripening, had progressed from that in the inhibition and preclimacteric fruit, the effect of both water loss or SmartFresh™ treatment on fruit ripening were reduced. This reflects the natural ripening of the fruit having progressed beyond the point at which water loss, or SmartFresh™ treatment could influence ripening.

Table 3. The ACC content (nmol/gFW) of early season 'Hass' avocados held at 20°C in a high humidity (>95% RH) during all phases of ripening (Control), or in a low humidity (<20% RH) for the duration of the Inhibition, Preclimacteric, Climacteric, or Postclimacteric phases of ripening before returning to high humidity for the completion of ripening. ACC was determined at the end of the period in low humidity. Data for fruit treated with 300 ppb SmartFresh™ during the first 18 hours under low humidity in parenthesis. Values are the average of 6 fruit.

	ACC content (nmol/g) after			
Treatment	Inhibition	Preclimacteric	Climacteric	Postclimacteric
Control	1.1	0.3	1.6	42.6
+3Days				14.1
Inhibition	0.6 (0.6)			
+3Days	0.5 (0.5)			
Preclimacteric		0.3 (0.3)		
+4Days		21.4 (0.3)		
Climacteric			20.3 (1.0)	
+4Days			34.3 (99.3)	
Postclimacteric				65.4 (59.4)
+ 3Days				29.5 (36.4)

The levels of ACC in the untreated control fruit were low during the inhibition, preclimacteric and climacteric phases, but increased markedly during the postclimacteric period before declining as the fruit rapidly ripened (Table 3). For mid and

late season fruit, the peak in ACC levels occurred during the climacteric phase. The SmartFresh™ treatment delayed the increase in ACC. Water loss treatment resulted in a marked increase in ACC levels several days after the water loss treatment had ended, but not during the water loss treatment.

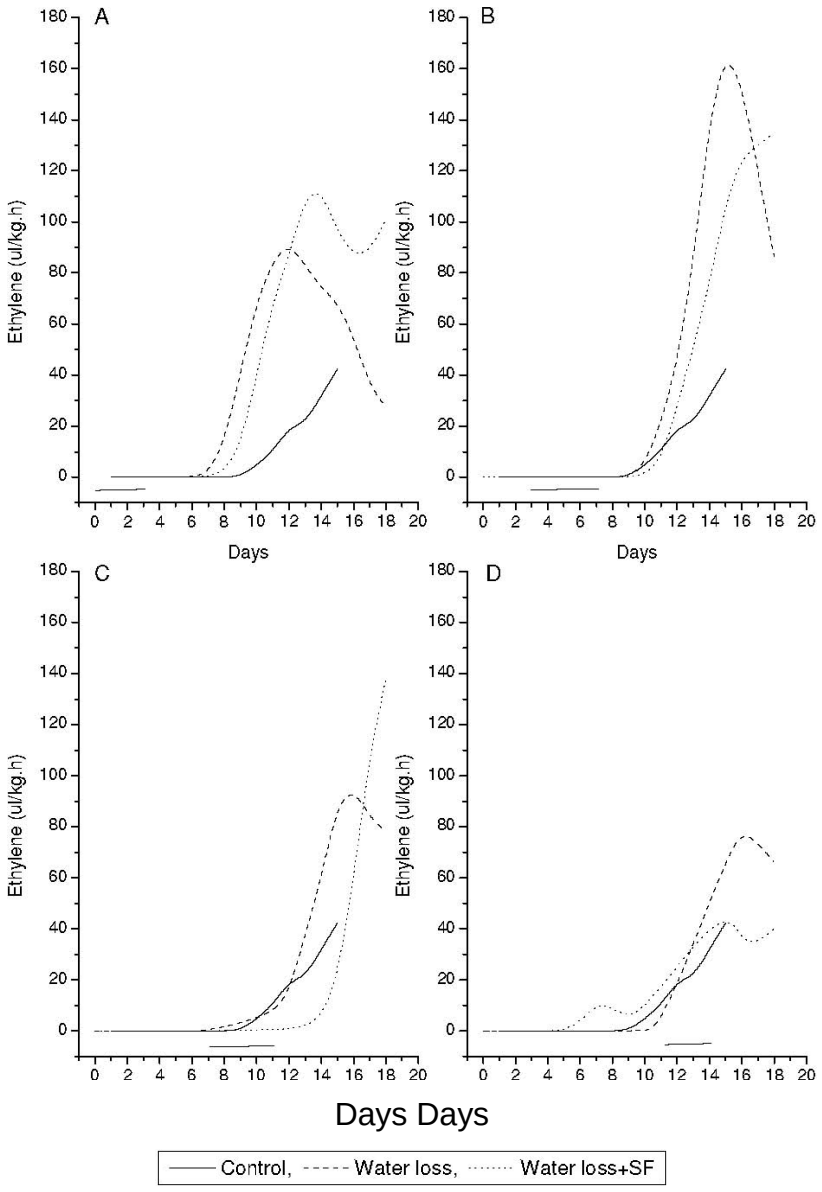

Figure 1. Rates of ethylene production of early season 'Hass' avocados held at 20°C in a high humidity (>95% RH) during all phases of ripening (Control), or in a low humidity (<20% RH) for the duration of the Inhibition (A), Preclimacteric (B), Climacteric (C), or Postclimacteric (D) phases of ripening before returning to high humidity for the completion of ripening. During holding under low humidity, half of the fruit were treated with SmartFresh™ (300 ppb, 18 hours). Horizontal bars indicate the duration of the respective ripening phases.

The activity of EFE in the control fruit increased steadily throughout the ripening phases and was coincident with the increased ethylene production (Table 4, Figure 1). Water loss treatments during the inhibition and preclimacteric phases resulted in slightly higher EFE activities both immediately at the end of treatment and also after a subsequent 3 or 4 days at 20°C. The SmartFresh™ treatment prevented the increase in EFE both at the end of treatment and also after a further 3 or 4 days. However, once fruit reached the climacteric phase, water loss and SmartFresh™ treatment had little effect on EFE activity.

Table 4. The EFE activity of early season 'Hass' avocados held at 20°C in a high humidity (>95% RH) during all phases of ripening (Control), or in a low humidity (<20% RH) for the duration of the Inhibition, Preclimacteric, Climacteric, or Postclimacteric phases of ripening before returning to high humidity for the completion of ripening. EFE activity was determined at the end of the period in low humidity. Data for fruit treated with 300 ppb SmartFresh™ during the first 18 hours under low humidity in parenthesis. Values (µL ethylene/kg.h) are the average of 6 fruit.

	EFE activity (µL ethylene/kg.h) after			
Treatment	Inhibition	Preclimacteric	Climacteric	Postclimacteric
Control	2.6	2.5	6.0	7.8
+3Days				8.9
Inhibition	0.7 (1.1)			
+3Days	5.3 (1.2)			
Preclimacteric		4.2 (4.8)		
+4Days		5.4 (1.4)		
Climacteric			6.6 (7.9)	
+4Days			14.6 (3.0)	
Postclimacteric				16.6 (38.4)
+ 3Days				14.0 (9.0)

In contrast to ethylene production, there was no clear peak in respiration, although there was an upward trend in respiration rate during ripening (Figure 2, which for fruit from the mid and late season harvests, was more consistent and pronounced and the overall pattern of respiration was more typical of a climacteric peak (data not shown).

Water loss during the preclimacteric, climacteric and postclimacteric phases resulted in lower incidences of stem end rot, vascular browning and body rot in the ripe fruit compared to the controls, although only the preclimacteric and climacteric treatments were statistically significant (Table 5). The effect of water loss treatment on the rot incidence is likely to reflect changes in the rate of ripening, with fruit taking less time to ripen providing less opportunity for rots to develop. In addition, the incidence of rots and the effects of water loss on rots were not consistent between harvests, and therefore, it is difficult to quantify the impact of water loss on rots in this set of experiments.

In the present study, fruit were ripened without a period of storage at low temperature, and therefore, the results cannot be directly applied to what may occur under commercial situations. Typically, fruit stored for 2 weeks prior to ripening have fewer rots than fruit ripened without storage, and as the storage time increases beyond 2

weeks, the incidence of stem and body rots increases (Dixon *et al.,* 2004). The inhibition and preclimacteric periods are likely to be extended in storage, as well as the climacteric phase in fruit prone to premature ripening. Water loss under low temperature storage conditions may attenuate or exacerbate the incidence of rots.

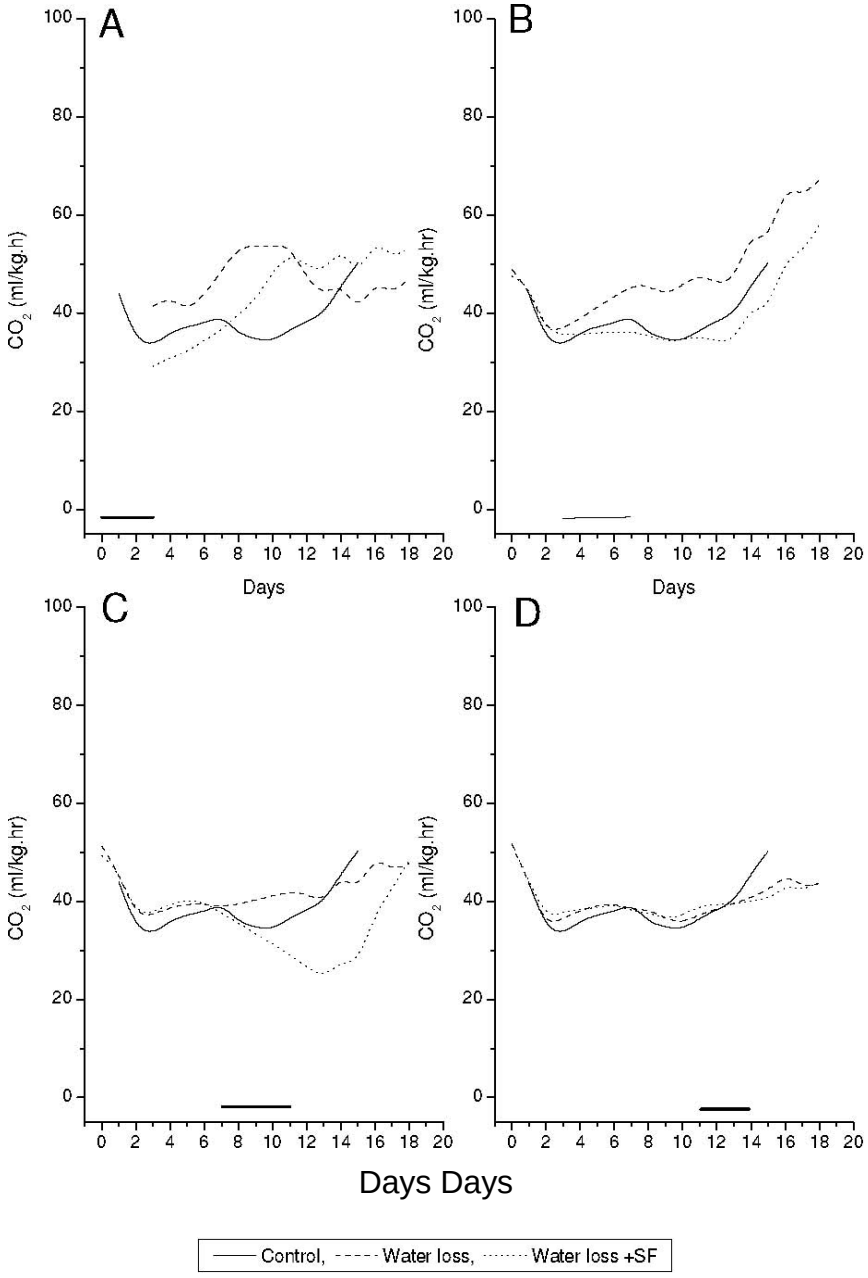

Figure 2. Rates of respiration of early season 'Hass' avocados held at 20°C in a high humidity (>95% RH) during all phases of ripening (Control), or in a low humidity (<20% RH) for the duration of the Inhibition (A), Preclimacteric (B), Climacteric (C), or Postclimacteric (D) phases of ripening before returning to high humidity for the completion of ripening. During holding under low humidity, half of the fruit were treated with SmartFresh™ (300 ppb, 18 hours). Horizontal bars indicate the duration of the respective ripening phases.

Table 5. Incidence of rots in early season 'Hass' avocados when ripe after holding at 20°C in a high humidity (>95% RH) during all phases of ripening (Control), or in a low humidity (<20% RH) for the duration of the Inhibition, Preclimacteric, Climacteric, or Postclimacteric phases of ripening before returning to high humidity for the completion of ripening. Values are the average of 4 replicates (approx. 90 fruit in total).

	Rot incidence (%)		
Treatment	Stem End Rot	Vascular Browning	Body Rot
Control	53.5	45.2	45.9
Inhibition	57.0	49.4	34.2
Preclimacteric	35.1*	27.3*	26.0*
Climacteric	35.5*	29.0*	22.4*
Postclimacteric	38.7	34.7	42.7

Values in a column marked * differ significantly from the control at P=0.05.

For fruit harvested early in the season (i.e. up to November) conditions under which fruit are held during the first 24-72 hours after harvest, appear to impact strongly on ripe fruit quality. Excessive water loss over this period can shorten ripening times, and depending on storage conditions, will have negative or positive effects on rot incidence (Lallu *et al.,* 2002, 2003). Therefore, maintaining minimal water loss conditions e.g. high RH, and preventing exposure to ethylene throughout holding, packing and shipping periods should be considered as a best practice goal.

In summary, the ripening stage at which water loss occurred differentially affected the rate of fruit ripening and the effect differed through the harvest season. In particular, water loss during the initial stages of ripening, i.e. the inhibition or preclimacteric phases, accelerated the rate of ripening, whereas water loss during later stages of ripening, i.e. postclimacteric phase when fruit were already softening rapidly, had little or no effect on the rate of ripening. This effect of water loss accelerating ripening was most noticeable in fruit harvested early in the season where the inhibition and preclimacteric phase of ripening were comparatively long compared to fruit harvested later. Hence there is greater scope for water loss to influence fruit ripening early in the season, whereas later, when fruit ripening occurs naturally more rapidly off the tree, there is less scope for water loss to affect the rate of ripening.

The effect of water loss on ripening appears to be through ethylene biosynthesis. Increased water loss during the inhibition and preclimacteric phases of fruit resulted in an earlier climacteric peak in ethylene production and subsequently earlier ripening. Furthermore, treatment with SmartFresh™ delayed the climacteric peak and ripening of fruit that had lost approx. the same amount of water as fruit not treated with SmartFresh™. The effect of water loss on rots appears to be related to the time taken for fruit to ripen, and is therefore, a result of the effect on ethylene production. The effect of water loss on ethylene metabolism was confirmed by the data on levels of ACC and EFE activity in the fruit. Water loss increased EFE activity and a greater accumulation of ACC in fruit prior to the climacteric.

CONCLUSIONS

It is concluded that:

- Water loss during the initial stages of ripening affects the rate of ripening and the incidence of rots and thereby strongly impacts on fruit quality.
- The capacity for water loss during the initial stages of ripening to accelerate fruit ripening is limited to fruit harvested early in the season.
- Water loss during the later stages of ripening, i.e. during rapid softening has little or no effect on ripening rate or rot incidence.
- The effects of water loss are most likely acting through ethylene biosynthesis.

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