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ROLE OF WATER LOSS IN RIPENING OF 'HASS' AVOCADOS

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ABSTRACT

Increased water loss was induced at different stages of ripening (inhibition, pre-climacteric, climacteric and post-climacteric phases) to determine its effect on ripening and the incidence of rots. In addition, to determine whether water loss was acting through or independently of the ethylene pathway, 1-aminocyclopropane carboxylic acid (ACC) content and ethylene forming enzyme (EFE) activity in fruit was determined after 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. Fruit remained at 20°C throughout the water loss treatments and the duration of ripening.

Transferring fruit to low humidity conditions during these ripening phases increased water loss by approx. 2.5-3.0%. Increased water loss during the inhibition phase increased the rate of respiration and ethylene production and advanced ripening by approx. 2 days, and decreased the incidence of stem-end rots from approx. 50% in control fruit to approx. 19%. Similarly, when water loss was increased during the pre-climacteric, climacteric and post-climacteric phases, stem-end rots were reduced to approx. 35%. Increasing water loss during the pre-climacteric, climacteric or post-climacteric phases also increased the rate of respiration but rates of ethylene production and ripening were not markedly affected. The ACC content increased from 0.12 nmol/g at the end of the inhibition phase to 5.86 nmol/g at the end of the climacteric phase. EFE activity increased with the progression of ripening from 1.261/kg.h at the end of the inhibition phase to 7.521/kg.h at the end of the post-climacteric phase. The greatest increase in ACC content and EFE activity occurred during the climacteric phase

It is concluded that water loss during initial stages of ripening affects the rate of ripening and the incidence of rots and thereby strongly impacts on fruit quality. Water loss during the later stages of ripening, i.e. during rapid softening have little or no effect. The effects of water loss on fruit physiology and quality are most likely acting through ethylene biosynthesis

Keywords: *ripening phases*

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 decreases the times to ripen during shelf life after storage but can increase the incidence of rots by 5-1 5% (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 minimize 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. The fruit begins to increase its rate of ethylene production that eventually peaks (Figure 1). 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 pre-climacteric 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 post-climacteric phase that includes the period after the climacteric peak.

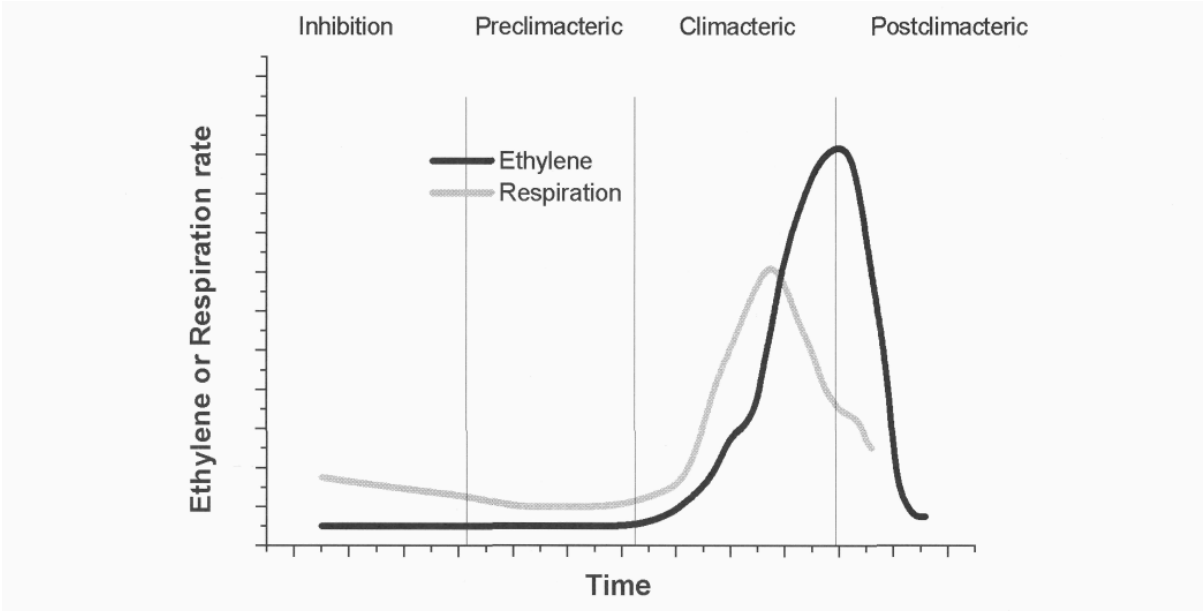

Figure 1. Diagram of typical patterns of ethylene production and respiration during 4 phases of ripening: Inhibition, Preclimacteric, Climacteric and Postclimacteric phases.

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.

Given that both water loss and ethylene lead to an increase in the rate of ripening the question arises is water loss acting through ethylene, or independently of ethylene on ripening? 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 the climacteric (inhibition, pre climacteric, climacteric and post climacteric phases) and its effect on ripening and the incidence of rots was quantified. 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.

MATERIAL AND METHODS

Fruit. Approx. count 23 'Hass' avocados that had been harvested from a commercial orchard in Katikati on January 30th 2004, 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 Hort Research, 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, pre-climacteric, climacteric and post-climacteric 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. After treatment 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 10-15 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 air flow 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, 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. Fruit firmness was determined using an Effigi penetrometer fitted with a 6 mm head for moderately firm fruit, or a 4 mm head for very firm fruit. Fruit were cut longitudinally, and readings (4; 2 per half fruit) within the flesh were taken on the cut surface in the region between the stone and the skin at the equator of fruit.

Data Analysis. Means for treatment effects were calculated based on the total fruit in each tray. To identify significant effects of treatments and/or orchards at a $p<0.05$ level, untransformed data or arc sin square root transformed data were subjected to ANOVA using Sigma Stat. All data in the tables are untransformed data.

RESULTS

The durations of the inhibition, pre-climacteric, climacteric and post-climacteric phases of ripening were 4, 3, 4 and 3 days, respectively (Figures 2 and 3). Transferring fruit to low humidity conditions during these ripening phases increased water loss by approx. 1.6-3.0% (Table 1).

Increasing water loss during the inhibition phase increased the rate of respiration and ethylene production and advanced ripening by approx. 2 days (Figures 2 and 3, Table 1). Although fruit treated during the inhibition phase ripened faster than control fruit, they had a darker skin colour when ripe than fruit treated during the pre-climacteric, climacteric and post-climacteric phases.

Increased water loss during the pre-climacteric, climacteric or post-climacteric phases also increased the rate of respiration, but rates of ethylene production were not markedly affected, although the maximum rates of ethylene production for fruit treated during the pre-climacteric and climacteric were higher than in control fruit and similar to

fruit treated during the inhibition phase. Water loss during the pre-climacteric phase advanced ripening by approx. half a day but had no effect in the climacteric and post-climacteric phases.

Table 1. Water loss from '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 are the average of 40 fruit.

	Cumulative water loss (%) after				
Treatment	Inhibition	Preclimacteric	Climacteric	Postclimacteric	Ripe
Control	1.28	2.15	3.56	4.80	4.80
Inhibition	4.20				7.87
Preclimacteric		3.80			6.43
Climacteric			6.17		7.33
Postclimacteric				6.67	6.67

Fruit firmness had decreased from approx. 7.0 kgf at the end of the inhibition phase to 6.0 kgf by the end of the pre-climacteric phase, and subsequently to approx. 4 kgf and 0.2 kgf by the end of the climacteric and post-climacteric phases, respectively (Table 2).

Table 2. Firmness of '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. Firmness was determined at the end of the period in low humidity. Values are the average of 12 fruit.

	Firmness (kgf) after			
Treatment	Inhibition	Preclimacteric	Climacteric	Postclimacteric
Control	6.99	5.99	4.10	0.21
Inhibition	6.91			
Preclimacteric		6.26		
Climacteric			4.21	
Postclimacteric				0.20

The timing of water loss had a significant effect on the incidence of stem-end rots but not body rots, external rots or the physiological disorder vascular browning (Table 3). Approx. 50% of the control fruit developed stem-end rots, whereas when water loss was increased during the inhibition phase, the incidence of stem-end rots was reduced to approx. 19%, or to approx. 35% if water loss was increased during the pre-climacteric, climacteric and post-climacteric phases.

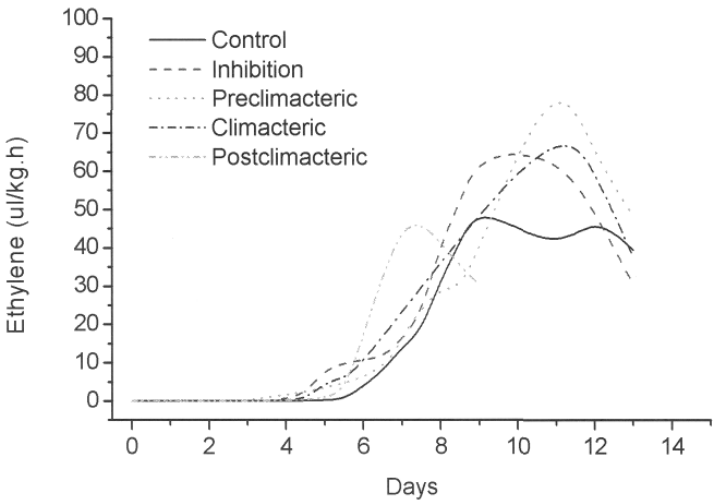

Figure 2. Rates of ethylene production of 'Hass' avocados held at 20°C in a high humidity (>95% RH) during all phases of ripening (Control), or in a low humidity (<20% RH) for the duration of the Inhibition, Preclimacteric, Climacteric, or Postclimacteric phases of ripening before returning to high humidity for the completion of ripening. Values are the average of 12 fruit.

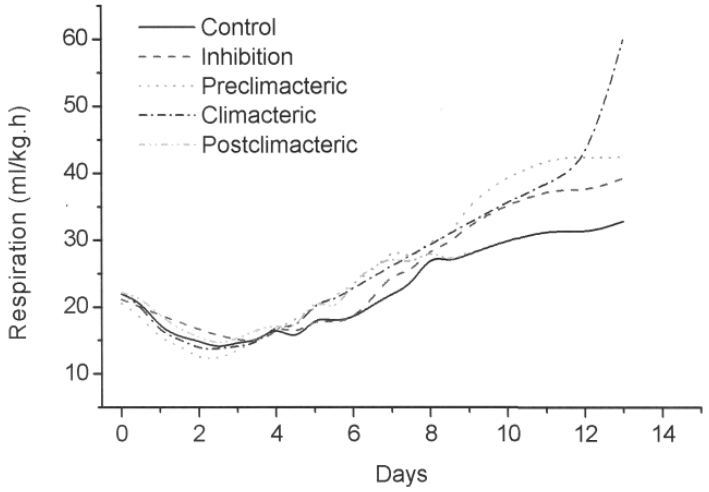

Figure 3. Rates of respiration production of 'Hass' avocados held at 20°C in a high humidity (>95% RH) during all phases of ripening (Control), or in a low humidity (<20% RH) for the duration of the Inhibition, Preclimacteric, Climacteric, or Postclimacteric phases of ripening before returning to high humidity for the completion of ripening. Values are the average of 12 fruit.

Table 3. Quality of 'Hass' avocados at the eating ripe stage after storage 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 approx. 90 fruit.

Treatment	Days to ripen	Skin Colour (0-100)	Stem-end Rots (%)	Body Rots (%)	Vascular Browning (%)	External Rots (%)
Control	12.3	79.9	51.1	17.0	1.1	5.7
Inhibition	10.2	83.1	19.3	25.0	4.5	0.0
Preclimacteric	11.8	79.7	34.1	17.0	5.7	2.3
Climacteric	12.4	78.6	35.2	18.2	6.8	3.4
Postclimacteric	12.1	78.8	38.6	13.6	5.7	3.4
Statistical analysis						
p values	<0.001	<0.001	0.027	0.326	0.270	0.544

The ACC content increased from 0.12 nmol/g at the end of the inhibition phase to 5.86 nmol/g at the end of the climacteric phase (Table 4). Increasing water loss during a specific ripening phase did not result in an immediate increase in ACC content, except in the climacteric phase where the ACC content increased from 5.86 nmol/g to 6.92 nmol/g.

Table 4. The ACC content of '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. Values are the average of 12 fruit.

	ACC content after (nmol/g) after			
Treatment	Inhibition	Preclimacteric	Climacteric	Postclimacteric
Control	0.12	0.14	5.86	not done
Inhibition	0.04			
Preclimacteric		0.03		
Climacteric			6.92	
Postclimacteric				not done

EFE activity increased with the progression of ripening from 1.26 l/kg.h at the end of the inhibition phase to 7.52 l/kg.h at the end of the postclimacteric phase (Table 5). As with ACC content, the greatest increase occurred during the climacteric phase with an increase in activity from 1.96 l/kg.h to 4.57 l/kg.h. Increased water loss had no marked or consistent effect on the EFE activity whilst fruit were under the low humidity conditions.

Table 5. The EFE activity of '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. Values are the average of 12 fruit.

	EFE activity after (l/kg.h) after			
Treatment	Inhibition	Preclimacteric	Climacteric	Postclimacteric
Control	1.26	1.96	4.57	7.52
Inhibition	1.04			
Preclimacteric		0.76		
Climacteric			4.86	
Postclimacteric				7.25

DISCUSSION

The ripening stage at which water loss occurred differentially affected fruit quality. In particular, water loss during initial stages of ripening, i.e. the inhibition phase

immediately after harvest, accelerated the rate of ripening and reduced the incidence of stem-end rots, whereas water loss during later stages of ripening, i.e. post-climacteric phase when fruit were softening rapidly had little or no effect on either the rate of ripening or the incidence of rots.

The effects of water loss on ripening and/or rots appear to be through ethylene biosynthesis. Increased water loss during the inhibition phase resulted in an earlier climacteric peak in ethylene production and subsequently earlier ripening. The effect of water loss on rots also appears to be through an effect on ethylene since the longer the fruit took to ripen, the higher the incidence of rots.

The ethylene biosynthesis data was limited to determinations at the end of the respective ripening phases only. Consequently the data was of limited use for confirmation that effects of water loss were acting through ethylene biosynthesis. Further determinations of ACC and EFE activity after the phase in which fruit were exposed to high water loss conditions were needed. Nevertheless, based on the respiratory and ethylene production patterns, and the slight increase in ACC and EFE activity in water stressed fruit in the climacteric phase, the biosynthetic evidence also suggests that water loss effects act through the ethylene pathway.

In this study fruit were ripened without a period of storage at low temperature, and therefore, the present results cannot be directly applied to what may occur under commercial situations. Typically, fruit stored for 2 weeks have less 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 pre-climacteric 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.

Despite storage information and the limitation of only one harvest of late season fruit, some practical implications can be suggested from the findings. The first 24-72 hours after harvest and the conditions under which fruit are held during this period 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.

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. However, 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. This trial should be repeated to include early, mid and late season fruit, and be extended to include fruit held in low temperature storage.

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