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STRESS, DELAYED HARVEST AND FRUIT QUALITY IN FUERTE AVOCADO FRUIT

J G CUTTING & J P BOWER
CITRUS & SUBTROPICAL FRUIT RESEARCH INSTITUTE, NELSPRUIT
B N WOLSTENHOLME
DEPT. HORTICULTURAL SCIENCE, UNIVERSITY OF NATAL, PIETERMARITZBURG

SUMMARY

Changes in the levels of two plant growth substances, abscisic acid (ABA) and ethylene, as well as the browning enzyme polyphenol oxidase, were studied in ripening Fuerte avocado fruit. The fruit came from various localities, were subjected to various stress treatments or taken from different harvest dates. Results showed that stress or delayed harvest caused an increase in ABA with a resultant negative effect upon fruit quality, as measured by polyphenol oxidase. At high ABA levels the ethylene climacteric was reduced or collapsed. The study indicated a vital role for ABA in fruit ripening and quality.

OPSOMMING

Veranderinge in die vlakke van twee plantgroeistowwe, naamlikabsisiensuur (ABA) en etileen, sowelasdie verbruinigsensiem, polifenoioksidase, was bestudeer in rypwordende Fuerte avokadovrugte vanaf verskeie lokaliteite, onder verskillende spanningstoestande of oesdatums. Bevindinge toon dat spanning of 'n vertraging in oestyd 'n toename in ABA veroorsaak.

Vrugkwaliteit word hierdeur benadeel soos bepaal deur polifenoioksidase. By hoë ABA-vlakke het die etileen klimakteriese kurwe verlaag ofineengestort. Hierdie studie bevestig die deurslaggewende rol wat ABA speel by vrugrypwording en kwaliteit.

INTRODUCTION

Fruit quality is a prime concern for all involved in the avocado industry, particularly the exporter, who could face heavy losses should the fruit arrive at the overseas markets in an unsatisfactory condition. It was found (Bezuidenhout & Kuschke, 1982; Bezuidenhout, 1983) that 20% of all South African fruit arriving in France had internal physiological problems. The on-farm condition of the fruit is thought to be of vital importance in combating poor internal quality (Bower &Van Lelyveld, 1985).

Earlier work by Bower & Van Lelyveld (1985) indicated that long-term irrigation treatments could cause stress in fruit development and a decrease in fruit quality, as measured by polyphenol oxidase (PRO) (Kahn, 1975).

In view of this earlier work, it was decided to investigate any changes that might occur in the plant regulator levels, particularly abscisic acid (ABA) and ethylene, during ripening. Ample evidence already exists to suggest that ABA and stress are linked (Walton, 1980). This avenue was also to be investigated in the present study, as well as the effect of 'hanging' or delayed harvest on ABA and ethylene. It would then be possible to ascertain if there was any connection between ABA, ethylene, PPO and fruit quality.

MATERIALS AND METHODS

Biological material

Fruit used in the present study was of the Fuerte cultivar and from two sources, namely, the CSFRI research farm, Burgershall, near Nelspruit and Ukulinga, the University of Natal research farm outside Pietermaritzburg. Thirty to forty fruit were harvested in April and then again ¡n August. The trees on Burgershall were subjected to various stress treatments (Bower & Van Lelyveld, 1985).

The harvested fruit was stored at room temperature. Each day 2-3 fruit were destructively sampled. Firmometer readings were taken daily, and when remaining fruit reached a firmometer reading of 100, indicating eating ripeness (Swarts, 1981), the determinations were terminated. After each firmometer reading, avocado rind (exocarp) was removed and extracted separately. Meso- and endocarp (flesh) was pooled after removal of any attached testa.

Determination of ABA

Samples of 10 g were homogenized in 50 ml 90% methanol acidified with 1% acetic acid. After extraction (4°C for 48 hours) in the dark, material was centrifuged (1000 x g for 10 min). The supernatant was reduced to dryness under vacuum at 30°C and taken up in 5 ml methanol (1 ml per 2 g fresh material). Aliquots, suitably diluted, were used for the determination of total ABA.

One ml of extract was added to 10 ml potassium phosphate buffer (0,05 M, pH 8), mixed, reduced to the aqueous phase and extracted with 40 ml diethyl ether. The lower phase was retained, acidified to pH 3 and further extracted with 40 ml diethyl ether. The upper phase was retained and reduced to dryness. The residue was taken up in 2 ml diethyl ether and subjected to TLC (Merck 5554 silica gel plates, solvents, toluene: ethyl acetate: acetic acid (50:30:4). The Rf zone corresponding to authentic ABA was isolated and removed. Elution of the ABA was achieved using 20 ml methanol, which was reduced to 1 ml, split and assayed. The RIA for ABA has been described elsewhere (Cutting, Hofman, Lishman & Wolstenholme, 1985)

Determination of PPO activity

The method of Van Lelyveld & Bower (1984) was used without modification

Ethylene Determination

Ethylene evolution was determined by gas chromatograghy. During the ripening phase, percentage ripeness was estimated daily by firmometer. Each fruit was placed in a 1,5ℓ glass container, the open end being sealed by standing in water. Fruit was left for 30 or 60 min, depending upon the physiological state of the fruit, before withdrawal of the gas sample. A Carlo Erba 4 200 gas chromatograph with an injection temperature of 160°C, oven temperature of 125°C and detector temperature of 16°C, was used. Gas flow was: air 300 *µ*l min, the helium carrier gas 40 *µ*l min and hydrogen 50 *µ*l min. A 2 m long activated alumina column with flame ionisation detector was used. A 1 ml gas sample was injected. After calibration, results were computed on a Spectra Physics computing integrator.

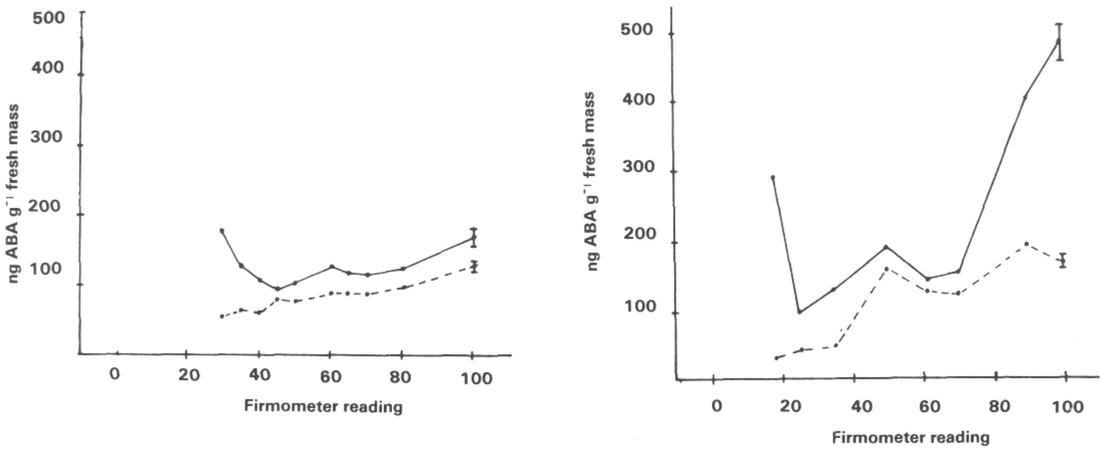

Fig. 1. Changes in the levels of free (brokenline) and total (solid line) ABA with ripening for Fuerte fruit harvested in (a) April and (b) August. Results are for distal flesh with the SE presented.

RESULTS

Changes in ABA equivalents that occurred with softening as monitored by firmometer for April and August fruit from Ukulinga are presented in Fig. 1. In April harvested fruits, the distal flesh contained about 170 ng/g total ABA at a firmometer reading of 25, about two days after harvesting. At this stage the free ABA concentration was, however, only about 55 ng/g. Free ABA levels increased as softening proceeded to about 120 ng/g at a firmometer reading of 100, rising up to about two thirds of the total ABA concentration in fully ripe fruits. The pattern for ABA in late harvested fruit was essentially similar, but with total ABA concentrations in the flesh always higher than in early harvested fruits. Free ABA concentrations were again comparatively low in firm unripe fruits, followed by a rising trend until a firmometer reading of 70, when the concentration of free ABA declined relative to that of total ABA. Generally, free ABA concentrations were higher in late harvested than in early harvested fruit approaching levels of 180 ng/g at full ripeness.

Concentrations of ABA in 50% soft fruit from Ukulinga (Natal) and Burgershall (Transvaal) are presented in Fig. 2. April harvested fruit from Ukulinga and the unstressed Burgershall fruit had the lowest ABA concentrations (between 90 and 105 ng/g) with the concentration in the Natal fruit being slightly lower. The stressed fruit from Burgershall and the August harvested fruit from Ukulinga were essentially similar but contained higher levels of free ABA (140 to 160 ng/g).

The ethylene levels for the water stressed and non-stressed fruit from Burgershall are given in Fig. 3. Ethylene levels peaked at about 50% softness for non-stressed fruit and showed a typical climacteric. In contrast the stressed fruit evolved less ethylene for a longer period as evidenced by the flatter curve in Fig. 3.

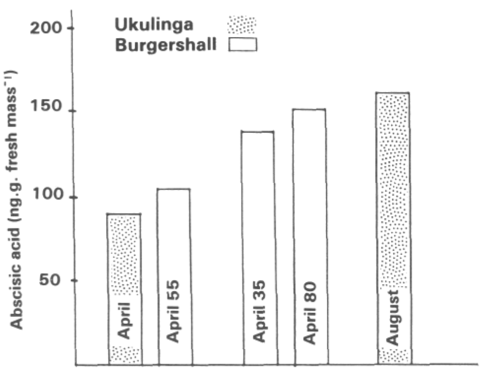

Fig. 2. Abscisic acid levels at 50% fruit softening for Ukulinga and Burgershall at different harvest dates and irrigation regimes respectively.

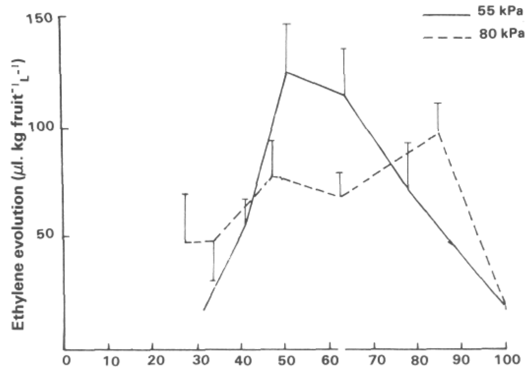

Fig. 3. Ethylene evolution during Fuerte fruit softening for two irrigation stress regimes. Bars indicate SE of means.

The times required by Fuerte fruit to reach various firmometer readings are given in Table 1.

TABLE 1

Mean time (days) required by Fuerte fruit from Ukulinga and Burgershall to reach various firmometer readings

	Ukulinga		Burgershall	
Firmo meter	April	August	Unstressed	Stressed
30	3	1	7 ± 2	6 ± 1
50	7 ± 1	3	8 ± 1	7 ± 3
100	9 ± 1	4	10 ± 3	8 ± 1

It is apparent from Table 1 that the late harvested fruit softened very rapidly as compared with the fruit harvested in April. While stress reduced the ripening time, it was not as pronounced as the four month 'tree storage'.

The ABA and PPO interaction is presented in Fig. 4. Of particular interest is that ABA and PPO mirrored each other. Soluble levels of PPO activity ranged from a change in OD_{420}min^{-1}. mg protein^{-1} of 0,55 for unstressed fruit to 0,95 for severely stressed fruit. The levels of total PPO activity ranged from just over 1 for unstressed fruit to just under 1,6 for the stressed treatment. From these results it would appear that high levels of ABA during the climacteric (50% softness) lead to increased levels of PPO activity at full ripeness.

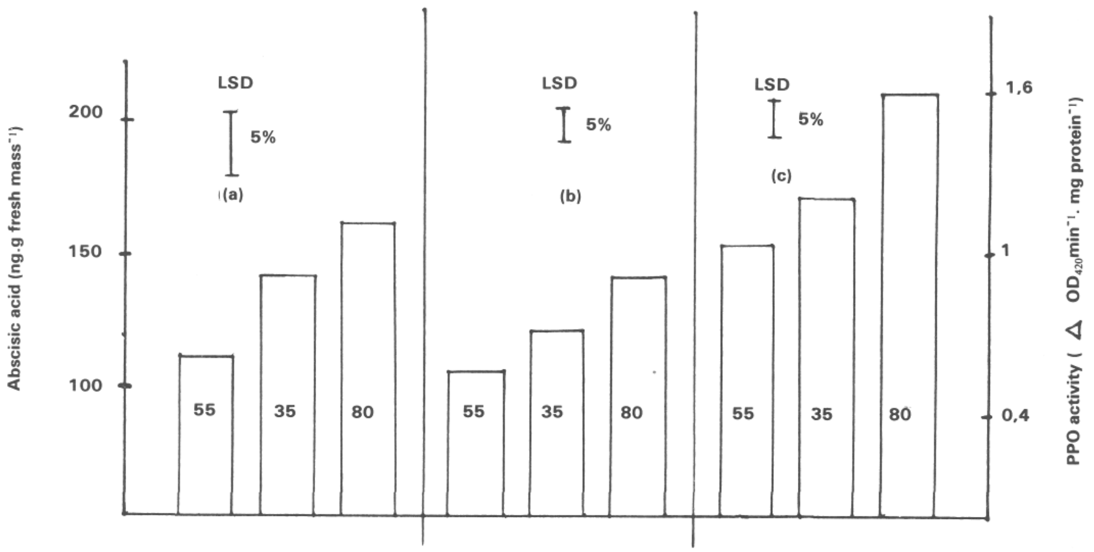

Fig. 4. Levels of (a) abscisic acid in 50% soft fruit, (b) soluble polyphenol oxidase and (c) total polyphenol oxidase in 100% soft fruit for the differing irrigation stress regimes.

DISCUSSION

There is still little consensus regarding the primary trigger for the commencement of avocado ripening. Adato & Gazit (1974) found a negative linear correlation between the rate of water loss from the avocado fruit and the rate of ripening. Infusion of water delayed ripening, and they concluded that moisture stress is a determining factor in the rate of ripening. Similarly, as avocado fruit mature, there is an increase in oil content and a significant decrease in water content (Pearson, 1975). The results obtained from this investigation support these findings, bearing in mind that there is ample evidence to link ABA and stress (Walton, 1980).

Assuming moisture stress is the major factor determining ripening rate, its physiological effect may be transmitted either via ABA or ethylene, both of which are known to increase during water stress. ABA has been shown, through various exogenous application experiments, to advance ripening in both climacteric and non-climacteric fruit (McGlasson, Wade & Adato, 1978). In the present study there are indications of an important role of ABA in avocado fruit ripening. Late harvested fruit showed the highest levels and fluctuations in both free and bound ABA.

Ethylene, unlike ABA, has been extensively studied and its importance in fruit ripening is well established. Unstressed avocado fruit evolved most ethylene between firmometer readings of 45 and 65. The behaviour of the fruit was essentially uniform, with all fruit evolving similar amounts of ethylene at the climacteric peak. The stressed fruit displayed considerable variation in ethylene evolution with several fruit failing to produce an ethylene climacteric. The majority of these fruit could be said not to have undergone a 'true' climacteric (lower levels of evolved ethylene over a longer period of time) as evidenced by the flat curve obtained. ABA is known to cause alterations to the levels of certain enzymes (Leopold & Kriedemann, 1975) and as a senescent agent the high levels of ABA could have caused premature membrane degradation, leading to fruit softening without 'true' ripening.

Lieberman, Baker & Sloger (1977) believe that ABA accelerates ageing during the ripening response. This implies rapid cell degeneration and associated fruit quality problems. Of particular interest is that 'tree stored' fruit (August fruit from Ukulinga) had similar ABA levels to stressed fruit from the April harvest from Burgershall. Should high levels of ABA influence PRO levels and hence quality (Bower, Cutting & Van Lelyveld, 1986) the 'merits' of hanging fruit late (delayed harvest) needs reappraisal in the light of a better understanding of the factors responsible for physiological problems associated with ripening. While more work needs to be done before recommendations can be made, it would appear from the present study that the Fuerte fruit with the best quality are those harvested as soon as possible after maturity.

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