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LONG-TERM IRRIGATION AS INFLUENCING AVOCADO ABSCISIC ACID CONTENT AND FRUIT QUALITY

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SUMMARY

Avocado fruit from three different irrigation stress treatments were harvested and subjected to abscisic acid (ABA) and polyphenol oxidase analysis at various stages of ripening/softening. The level of ABA at 50% soft (coinciding with the ethylene peak) had an effect upon the eventual fruit quality as determined by the polyphenol oxidase content (browning potential) at 100% soft. There are indications that water stress could be involved with ripening and fruit quality.

OPSOMMING

Avokadovrugte vanaf drie verskillende besproeiings-behandelings was ge-oes op verskeie stadia van rypwording/sagwording en vir absisiensuur (ABA) en polifenoloksidase ontleed. Dit is gevind dat die ABA-vlak by 50% sagwording (ooreenstemmend met die etileenpiek) 'n duidelike effek op die uiteindelike vrugkwaliteit by 100% sagwording het, soos bepaal deur die polifenoloksidase inhoud (verbruiningspotensiaal). Daar is ook aanduidings dat vogspanning 'n rol speel by rypwording en vrugkwaliteit.

INTRODUCTION

Avocado fruit quality is of prime importance, particularly for the exporter, who is faced with considerable losses should his fruit arrive on overseas markets in a poor condition.

It was estimated by Bezuidenhout & Kuschke (1982) that internal physiological problems occurred in more than 20% of all South African avocados that arrived in France in 1981. Bezuidenhout (1983) found a similar tendency for the 1982 season. In the former study, statistical analysis implicated on-farm fruit condition as being of great importance to final fruit quality, while the latter assigned more than 50% to unknown factors, once again implying original fruit condition before packing to be vitally important.

Previous work by the senior author (unpublished) indicated that long-term irrigation treatments could cause stress early in the development of the fruit, and resulted in increased levels of both bound and free polyphenol oxidase (PPO) after ripening, which was an indication of increased browning potential (Kahn, 1975). It was also found that the later in the season avocados were picked, the higher the PPO levels, perhaps implying a senescence factor.

Abscisic acid (ABA) is known as a promotor of ripening (Rhodes, 1981) and may accelerate fruit ageing (Lieberman, Baker & Sloger, 1977), while there is also ample evidence to suggest that ABA and stress are linked (Walton, 1980). Wills & Scott (1976) found that injection of ABA into apples increased storage breakdown, evidenced by a browning of cortical tissue. It is therefore tempting to link poor irrigation practices (thus causing stress) with increased ABA levels and browning potential. To investigate this possibility, the free ABA levels in avocado fruits were analysed, while the same fruit were checked for their PPO activities.

MATERIALS AND METHODS

Fruits were picked from five-year-old Fuerte trees on Duke seedling stocks which had been subjected to three different irrigation regimes, viz. replenishment of soil moisture to field capacity when soil moisture tension reached 35 kPa, 55 kPa and 80 kPa respectively. This is a long-term irrigation experiment, all irrigation regimes being imposed throughout the year. At the time of fruit picking, the experiment was in the second year, thus short term stress effects should not have been a problem. The experimental layout consisted of separate blocks randomly allocated for each regime, and consisting of 25 trees each. Double rows were left between each block, and no fruits were picked from outer rows. Further replication was impossible due to existing orchard layout, and management considerations. However, the area was small, microclimate and soils did not differ, all trees were planted at the same time and scion material came from the same source. The problems of replication should be considered in this light.

Thirty fruits were picked from each regime (fifteen from the north and fifteen from the south sides) in early April. Fruit were packed in export-type cartons. Ten fruit from each regime were peeled, the seed removed, and cut into proximal and distal halves before grating and storage at -18°C until analysis. The rest of the fruits were monitored daily for softness, using the firmometer of Swarts (1981). At 50% soft, another group of fruits were removed and prepared as described. This was repeated for 100% soft fruit.

Analysis for PPO was done on the distal halves of the hard and 100% soft fruit, while ABA was analysed in the 50% soft fruit as well.

ANALYSIS OF PPO

A crude enzyme extract of soluble enzymes was made by grinding 2,5 g of fruit tissue with 5 ml of cold 10 mM acetate buffer, pH 5,0 and insoluble polyvinylpyrrolidone (Polyclar AT) in a mortar and pestle at room temperature (Kahn, 1977; Golan, Kahn & Sadovski, 1977). The homogenate was squeezed through gauze cloth and centrifuged in a Sorval Superspeed RC-2B centrifuge at 18 000 X g for 45 min at 0 to 4°C. The supernatant was used immediately. Assay was based on the method of Kahn (1975). Substrate consisted of 3 ml 0,02 M4-methylcatechol in 3ml 10 mM pH 5,0 acetate buffer, to which 0,05 ml enzyme extract was added. The initial linear rate of change in absorbance at 420 nm and 25°C was measured, and specific activity expressed as OD_{420} min^{-1} mg $protein^{-1}$. Protein content was determined following precipitation by 5% trichloroacetic acid by the method of Lowry, Rosebrough, Farr & Randall (1951) as modified by Leggett-Baily (1962). Total PPO activity was analysed in a similar manner, except that 0,01% SDS was added during the initial grinding.

ANALYSIS OF ABSCISIC ACID

Abscisic acid was extracted and analysed according to the radioimmunoassay (RIA) technique of Cutting, Hofman, Lishman & Wolstenholme (1985).

Fruit samples of 5 g each were homogenised in 50 ml 80% methanol, and extraced at 5°C for 48 hrs in the dark. After centrifuging at 1 000 X g for 10 minutes, the supernatant was evaporated to dryness under vacuum at 30°C. The residue was dissolved in 2,5 ml methanol. The large quantities of oil and pigments present were removed by a pre-purification procedure.

The plant extract was placed in 10 ml 0,05 M potassium phosphate buffer pH 8,0, and reduced under vacuum to the aqueous phase. Diethyl ether (20 ml) was added, and after separation, the bottom phase was collected, and acidified to pH 3. A further 20 ml diethyl ether was added, and the mixture allowed to stand overnight. The top fraction was collected, reduced to dryness and the residue disolved in 2,5 ml diethyl ether. In order to separate the free ABA from the extract, thin layer chromatography using silica on aluminium plates was done. Extract volumes of 20 μl were chromatographed against authentic free ABA, using toluene: ethyl acetate: ascetic acid (50:30:4) as developer. The Rf zone corresponding to free (±) ABA was eluted in 2,5 ml methanol.

All samples were duplicated in the RIA step. A 250 μl alequot of sample was used, and the RIA was conducted in 10 X 75 mm rimless soda glass test tubes (Cutting, 1984). A known standard was subjected to the entire extraction and assay procedure, for the determination of recovery rate. Results were accordingly adjusted.

RESULTS

The changes in ABA during softening are shown in Fig. 1

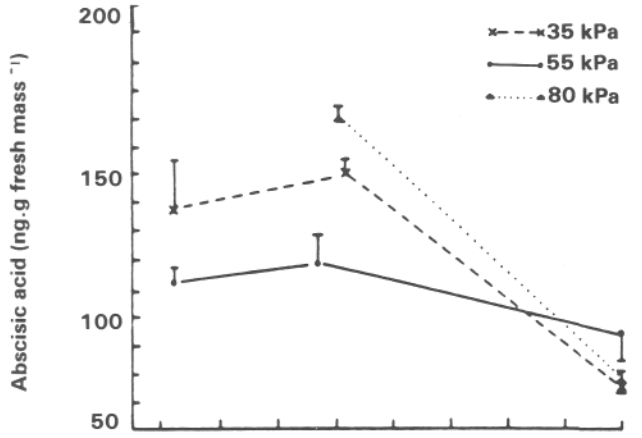

Fig. 1. Changes in ABA content of fruit mesocarp during softening.

Unfortunately, results of the hard fruit 80 kPa irrigation treatment were lost during the assay. Interesting trends can still, nevertheless, be determined.

Overall, there appears to be some increase in ABA as softening increases to the 50% level (which approximately corresponds to the ethylene peak) followed by a decline. This is in accordance with the findings of Adato, Gazit and Blumenfeld (1976), although the actual levels recorded are considerably lower.

It is particularly noticable that the least stressed treatment (irrigation at 55 kPa soil moisture tension) showed the smallest change in ABA during ripening, increasing only marginally, to the 50% level, thereafter showing a moderate decline. This treatment also showed a lower ABA level immediately after picking than did the 35 kPa soil moisture tension treatment, although it was not significant. The rate of increase in ABA level for the 55 kPa soil moisture tension treatment also appeared slower than for the 35 kPa treatment.

The PPO results are shown in the graphs of Fig. 2(b) and 2(c) for 100% soft fruit. It is evident that in both total and soluble PPO in soft fruit, an increasing trend in activity occurred with both over and under irrigation as compared with the 55 kPa treatment. In both cases the increases were significant. The under irrigation was particulary high, being significantly higher than the 55 kPa treatment at the 1% level.

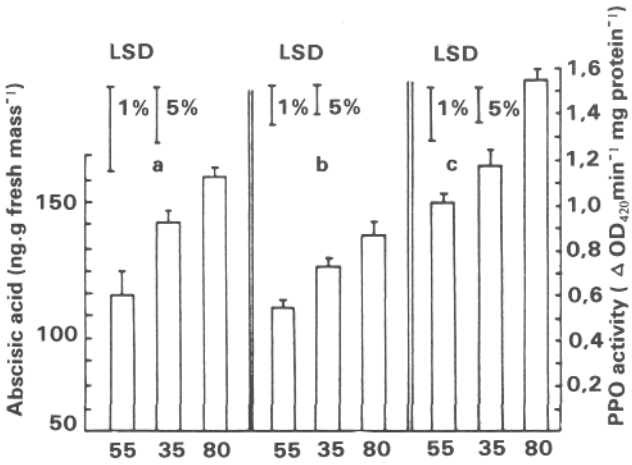

Fig. 2. **Relationship between (a) abscisic acid for 50% soft fruit, (b) soluble polyphenol oxidase and (c) total polyphenol oxidase in 100% soft fruit for three irrigation stress regimes.**

DISCUSSION AND CONCLUSIONS

The results seem to confirm the theory that water stress (including excess water) results in higher ABA levels. This is evidenced by the higher levels of ABA (significant at 1% level) obtained from the stressed treatments when fruits were 50% soft. However, rainfall and other tree water relations studies indicated little stress at the time of picking, implying that the ABA results were due to earlier events, possibly occurring shortly after fruit set.

The effects of the considerably higher levels of ABA in the cells of stressed fruits may be physiologically important. Lieberman, Baker & Sloger (1977) are of the opinion that ABA accelerates ageing during ripening. This would imply more rapid cell degeneration and therefore quality problems. While there seems to be no connection in the literature between ABA and PPO activity in avocados. Fig. 2 shows an interesting trend. Both the total and, especially, the soluble PPO activity show varying levels. Leopold & Kriedemann (1975) report that ABA is known to cause alterations in the activities of certain enzymes. While it is not known how PPO may be altered during ripening, a modification of a protease by high ABA activity could result in less removal of PPO, thus higher activity after ripening. Equally, an indirect route to the same result may be followed, such as ABA causing premature membrane degradation. It is interesting that recent work in apples has indicated a link between browning and ABA. Wills & Scott (1974) found that injection of ABA into apples causes increased storage breakdown (described as browning of cortical tissue). Scriven & Wills (1984) go further, showing that the amount of free ABA in cortical tissue was well correlated with the degree of browning occurring later. These authors do not, however, attempt to explain the link between ABA and storage disorders. It is nevertheless interesting that the level of free ABA was reduced by calcium (Wills, Franklin & Scott, 1978) and that calcium has, by so many workers, been linked to membrane stability.

It is thus concluded that although the actual mechanisims are unknown, the increase in browing potential of avocados due to poor long term irrigation (under or over) may be connected with abscisic acid metabolism, and that future study of this growth substance may result in a better understanding of the factors responsible for physiological problems related to ripening.

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