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Effect of harvest date and applied abscisic acid on browning potential of avocado fruit

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SYNOPSIS
The effect of harvest date and abscisic acid (ABA) on polyphenol oxidase (PPO) activity was investigated. Harvest date and applied ABA were both found to affect PPO activity. The later the fruit were harvested and the greater the quantity of infused ABA, the higher was the resultant PPO activity. Delayed harvest and stress strongly increased the potential for poor fruit quality.

INTRODUCTION

Avocado fruit quality is of prime concern to all involved in the avocado export industry. The avocado fruit is unusual in that it will only begin its ripening climacteric after harvest and not while attached to the tree (Leopold & Kriedemann, 1975). The pre-harvest condition of the fruit, particularly water stress history, is known to be important in determining poor internal quality (Bower & Van Lelyveld, 1986) as measured by the browning enzyme, polyphenol oxidase (PPO) (Kahn, 1975).

In view of this earlier work, it was decided to investigate the effect of the plant growth regulator, abscisic acid (ABA) on PPO during ripening. Earlier work by Cutting (1984) showed that the level of ABA varied with harvest date and increased as the season progressed. Ample evidence already exists to suggest that ABA and stress are linked (Walton, 1980). It was therefore decided to investigate the effect of applied ABA and harvest date upon fruit quality as measured by PPO activity.

MATERIALS AND METHODS

Fruit used in this two-year study, were of the Fuerte cultivar and came from the CSFRI research farm at Burgershall, near Nelspruit. Trees were not subjected to any cultural stress. The fruit used in Experiment 2 were randomly selected from an eight-year-old uniform orchard. Due to the high standard errors that resulted from this method, all the fruit used in Experiment 3 came from one representative tree in the same orchard.

Experiment 1: Uptake of ABA by the fruit

Three fruits with long pedicels were picked and transported to the laboratory where the pedicels were cut to leave approximately 10 mm attached. The unripened fruit were passively infused with 50 000 cpm ^{14}C-ABA (specific activity 25 mCu.mmol^{-1}) in water,

using silicone tubing attached to the pedicel (Figure 1). The fruit were left for 24 hours at room temperature.

Thereafter the fruit were longitudinally sectioned and autoradiography of the cut surface took place at -70°C for 30 days.

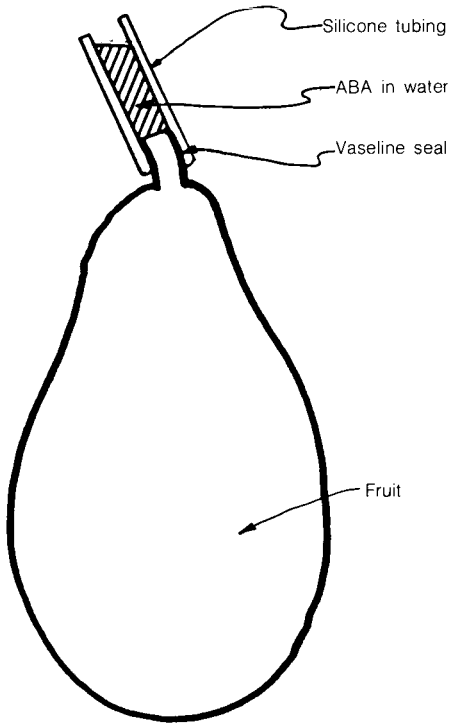

Fig 1 Diagrammatic representation of ABA infusion technique using silicone tubing.

Experiment 2: Effect of ABA upon polyphenol oxidase and determination of residual ABA in soft fruit

A stock solution of 1 $mg.ml^{-1}$ (±) ABA in methanol was diluted in water to give two levels of ABA, equivalent to 90 and 270 ng ABA per gram fruit. The ABA in 1,5 ml aliquots, was placed in a 50 mm length of silicone tubing attached to the pedicel of hard unripened fruit (Figure 1) harvested on 22/05/1985. There were five fruits per treatment. Passive infusion of the ABA took place over periods varying from one to six hours, while there was also a water and an untreated control.

The fruit were then allowed to ripen at room temperature (21 °C). When they reached a firmometer reading of 100, indicating eating ripeness (Swarts, 1981), the rinds (exocarp) were removed and the flesh (meso- and endocarp) from the distal half pulped, rapidly frozen and stored at -20°C.

PPO activity was determined by the method of Van Lelyveld and Bower (1984).

For the determination of ABA, samples of 10g were homogenised in 50 mf methanol acidified with one per cent acetic acid and with 20 mg BHT per litre added as an antioxidant. After extraction (4°C for 48 hours) in the dark, material was centrifuged (10000 g for 10 min). The supernatant was reduced to the aqueous phase under vacuum at 30°C and added to 10 ml potassium phosphate buffer (0,05M, pH 8,5). After extraction with diethyl ether (2 x 20 ml), the aqueous phase was retained, acidified to pH 2,5 and further extracted with 2 x 20 ml diethyl ether. The organic phase was retained and reduced to dryness.

The residue was taken up in 5 ml methylene chloride and subjected to seppak silica purification and isolation, according to the method of Hubick & Reid (1980). The radioimmuno-assay for ABA has been described and characterised (Cutting, Hofman, Lishman & Wolstenholme, 1986).

The experiment was repeated for fruit harvested on 12/06/1985. All results were subjected to simple random and factorial statistical analysis. Results from Experiment 3 were also analysed using the Student-Newman-Keuls comparison test.

Experiment 3: The effect of harvest date and ABA on polyphenol oxidase

The same methodology was used as for Experiment 2, but was carried out the following year with the following additions or omissions. Residual ABA was not analysed. The experiment was repeated for five harvest dates (April 2, April 22, May 12, June 13 and July 4, 1986). As a result of earlier work (Cutting, 1984) two levels of ABA, *viz* 90 and 270 $ng.g^{-1}$ fruit mass, were selected for Experiment 2, equivalent to the lowest and highest levels of free ABA detected at harvest. It was, however, difficult to draw conclusions from the results as the trends obtained were not significant. A third level of ABA, viz 550 $ng.g^{-1}$, which was equivalent to the highest level of total endogenous ABA detected at maturity, was therefore included in Experiment 3.

RESULTS

Experiment 1

Autoradiographs from the longitudinal sections showed that ABA was taken up by the fruit and deposited in the flesh. The pattern of accumulation of ^{14}C-ABA was similar in all three fruits. Figure 2 is a pooled diagrammatic representation of the results and shows the two major zones of accumulation within the distal half and the neck of the fruit.

Experiment 2

Results indicated that an undefined 'maturity factor' played a role in determining final PPO levels. Increasing the ABA concentration, increased the soluble PPO activity (from 0,92 to 1,34 $ng.g^{-1}$) in early-harvested fruit. The same treatment for later-harvested fruit significantly ($P < 0,01$) increased PPO activity (Table 1). The later harvest date also had higher overall levels of PPO at full softness.

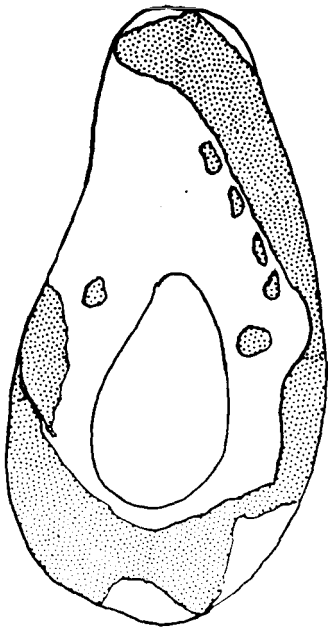

Fig 2 Diagrammatic representation of zones of ^{14}C-ABA accumulation from autoradiograph after passive infusion of ^{14}C-ABA into avocado fruit.

TABLE 1 Effect of applied ABA on PPO activity (OD_{420} min^{-1} mg $protein^{-1}$) and on ABA levels (ng.g-1) in soft Fuerte avocado fruit for two harvest dates. SE of means (five fruits) are shown in brackets.

	Harvested 22/05/85		Harvested 12/06/85	
Treatment	PPO	ABA	PPO	ABA
Water control	0,92 (±0,09)	62,4 (±10,7)	1,76 (±0,19)	33,3 (±11,0)
Control	1,54 (±0,31)	22,3 (±7,2)	2,04 (±0,16)	25 (±1,4)
90ng/g ABA	1,26 (±0,35)	46,2 (±9,2)	1,99 (±0,23)	26,8 (±5,1)
270 ng/g ABA	1,34 (±0,26)	56,8 (±3,6)	3,65 (±0,54)	17,3 (±0,7)
LSD P=0,05	NS	20,6	0,66	13,7
LSD P=0,01	NS	28,1	0,92	19,3

In all cases the level of ABA at full softness, was less than the ABA initially infused into the fruit. This indicated utilisation or catabolisation of ABA by the fruit, with greater utilisation or catabolisation later in the season. The less 'stressed' the fruit, in terms of applied ABA and infused water, the higher the ABA level at softness, ranging from 62,4

$ng.g^{-1}$ for the least stressed treatment (with the lowest PPO activity) to 13 $ng.g^{-1}$ for the most stressed treatment (with the highest PPO activity).

A linear correlation coefficient of -0,544 ($P < 0,001$) between PPO activity and ABA concentration in soft fruit was obtained, indicating that the greater the utilisation of ABA, the higher the PPO activity. Visual observations indicated three of the five high ABA treatment fruits to have internal browning.

Experiment 3

The levels and trends of soluble and total PPO are presented in Figures 3a and 3b. There was little or no response to the added ABA early in the season (the first two harvest dates). The high concentrations of ABA tended to depress PPO levels initially relative to the low ABA treatments. As the season progressed, however, PPO activity increased with the addition of ABA. This indicated some form of varying tissue sensitivity to ABA where the avocado fruit became more sensitive to ABA-induced PPO activity as the season progressed. The differences between the control and the water-only treatment, were consistently small for soluble and bound PPO and never differed significantly. The SEs for PPO, were much smaller than those obtained in Experiment 2 and show the advantage of selecting a single representative tree in an effort to reduce error in a crop renowned for its high variability (Jones, Embleton & Cree, 1957).

Of particular interest was the rise in total PPO in response to applied ABA for the last two harvest dates (Figure 3b) and the large rise in the level of soluble PPO for the last harvest date. The ratio of soluble to total PPO also changed after ABA infusion. While infused ABA increased, the potential for poor fruit quality for the last two harvest dates (as evidenced by the high total PPO levels) was only realised for the final harvest date (as evidenced by the large amounts of soluble PPO). Sixty per cent of fruit from the last harvest date and high ABA treatment showed visual browning.

DISCUSSION

Both the ABA treatments and harvest date affected fruit PPO levels, The ABA has been associated with avocado ripening (Adato, Gazit & Blumenfeld, 1976), but its function appears to be unknown. Water stress occurs in ripening fruit due to transpirational losses (Adato & Gazit, 1974). In this study, the later the fruit were harvested, the higher the final PPO level. This response does not appear to be reversible, as the trees were not subjected to any cultural stresses. Whilst the authors are uncertain as to applied ABA duplicating water stress, they believe that the elevated ABA levels may stimulate fruit into reacting as if they had recently experienced a water stress.

As avocado fruits mature, there is an increase in oil content and a significant decrease in water content (Pearson, 1975). The less the percentage water in fruit at harvest (due to maturity), the larger the resultant water stress. There is also ample evidence to link ABA and water stress (Walton, 1980). Two interacting mechanisms appear to affect the final PPO levels. These are a declining fruit water content, with associated internal water stress and an increasing tissue sensitivity to ABA as evidenced by the differing responses to the applied ABA as the season progressed.

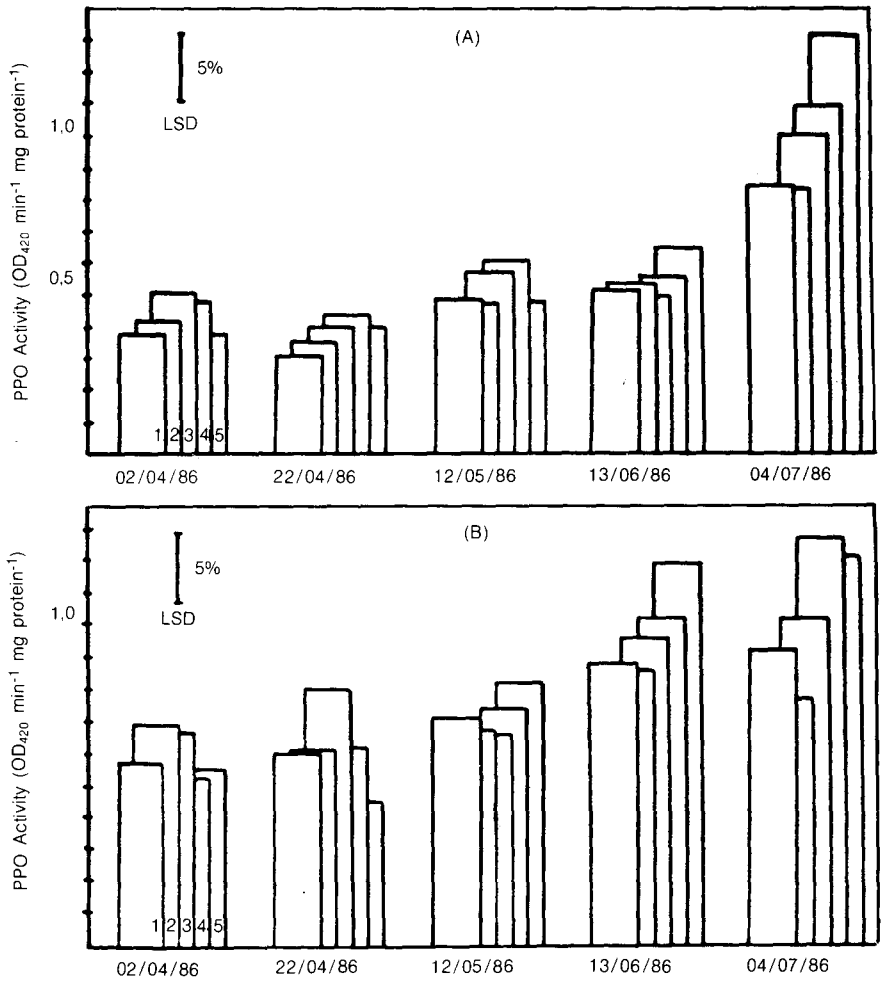

Fig 3 Influence of ABA on soluble (A) and total (B) PPO activity for five harvest dates. The treatments for each harvest date appear in the following order: 1 = water control, 2 = untreated control, 3 = 90 ng ABA, 4 = 270 ng ABA and 5 = 550 ng ABA per gram fruit. There are five samples per mean.

ABA is known to cause alterations to the levels of certain enzymes (Leopold & Kriedemann, 1975), which may explain the increase in total PPO activity. In addition, ABA is a known senescence agent and the infused ABA could have caused membrane degradation with subsequent release of bound PPO, thereby changing the ratio of soluble to total. Lieberman, Baker & Sloger (1977) believe that ABA accelerates ageing during the ripening response, and this could explain poor fruit quality from the final harvest dates in both Experiments 2 and 3.

It was of particular interest that early in the season, ABA had little effect on PPO levels, despite the fruit being mature. As the season progressed, the effect of the infused ABA became considerably greater, due to, the authors believe, a changing tissue sensitivity to ABA. As delayed harvest and water stress, particularly during the dry winter months, will greatly increase the percentage of fruit with physiological problems related to ripening, the best quality fruit are those harvested within two months of legal maturity.

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