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The involvement of polyphenol oxidase, abscisic acid and gibberellins in the expression of mesocarp discolouration in Fuerte avocado fruit

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SYNOPSIS

Mature Fuerte avocado fruits were harvested from three areas in the cool subtropics (Pietermaritzburg, SA) at fortnightly intervals from late April until mid-September. For each harvest, fruits were analysed immediately for polyphenol oxidase (PPO) activity and for abscisic acid (ABA) and gibberellin (GA) concentrations, while others were stored at 5,5°C for 28 days. After ripening at 22°C, the severity of pulp spot and grey pulp were monitored and PPO, ABA and GA quantified. Although there were locality differences, grey pulp intensity showed no consistent trend with harvest, but pulp spot tended to be less severe during midseason. PPO activity was high (6-28Δ_{420} min^{-1} mg protein^{-1}), but showed little obvious relation to the presence of pulp spot. Both free and bound ABA concentrations were low (6-25 ng g^{-1}) and showed little consistent variation with harvest, locality or storage. Gibberellin concentrations were higher in unripe fruit. In both unripe and stored ripe fruits, GA concentrations decreased with increasing maturity. These results provided neither evidence for a direct relation between PPO activity and pulp spot or grey pulp, nor any indication of an interaction with ABA and GA in the development of these disorders.

INTRODUCTION

The majority of the South African avocado crop is exported by refrigerated (theoretically at 5,5°C) transport to Europe and Britain. The time from harvest to sale is usually four to five weeks and during this time physiological disorders are often expressed, resulting in mesocarp discolouration. The severity of these disorders is known to be influenced by pre-harvest conditions, but it is not certain which factors are important, or what the influence of locality of production is.

It is generally agreed that the browning of plant tissue, including avocado fruit, is due to the activity of the enzyme PPO, polyphenol oxidase (Kahn, 1975; Mayer & Harel, 1979). Recent research on avocado fruit grown under warm subtropical conditions (Burgershall, Transvaal) has indicated a possible relationship between PPO activity, harvest date and potential flesh disorder expression, particularly pulp spot (Bower, pers com; CSFRI, Nelspruit). However, no comparable research has been conducted on fruit grown under cooler conditions.

The present investigation was aimed at determining the severity of flesh disorders after cold storage of fruits grown under relatively cool subtropical conditions. In an attempt to explain any trends observed, PPO activity and the concentrations of the plant hormones abscisic acid (ABA) and gibberellins (GAs), were also determined.

MATERIALS AND METHODS

Fuerte avocado fruits were obtained from three areas:

Wartburg (50 km north-east of Pietermaritzburg); vigorously growing, well-maintained four-year-old trees on Duke 7 rootstocks.

Thornville (13 km south of Pietermaritzburg); well-maintained six-year-old trees on Edranol rootstocks.

Baynesfield (15 km south of Pietermaritzburg); eight-year-old trees on seedling rootstocks.

From each farm 10 apparently healthy trees were selected and two average-sized fruits harvested from the north side of each tree at fortnightly intervals from the last week in April, until the second week in September, 1986. One fruit from each tree was stored at 5,5°C for 28 days, after which they were allowed to ripen at 22°C to a firmometer reading of 100 (Swarts, 1981). Each fruit was then cut longitudinally and one half rated for flesh disorders after 30 minutes as described below. Flesh from the distal portion of the remaining half was immediately macerated before being snap-frozen in liquid nitrogen and stored at -20°C. The second fruit was cut longitudinally as soon as possible after harvest, and the flesh from the distal section of one half grated, snap-frozen and stored as above. The presence and severity of grey pulp and pulp spot were monitored by estimating the total cross-sectional area affected, irrespective of colour intensity.

For PPO analysis, equal masses of flesh from two fruits were pooled, thus providing five replications per harvest. Soluble and total PPO activity was determined by the method of Bower (1985) and proteins by the method of Hartree (1972).

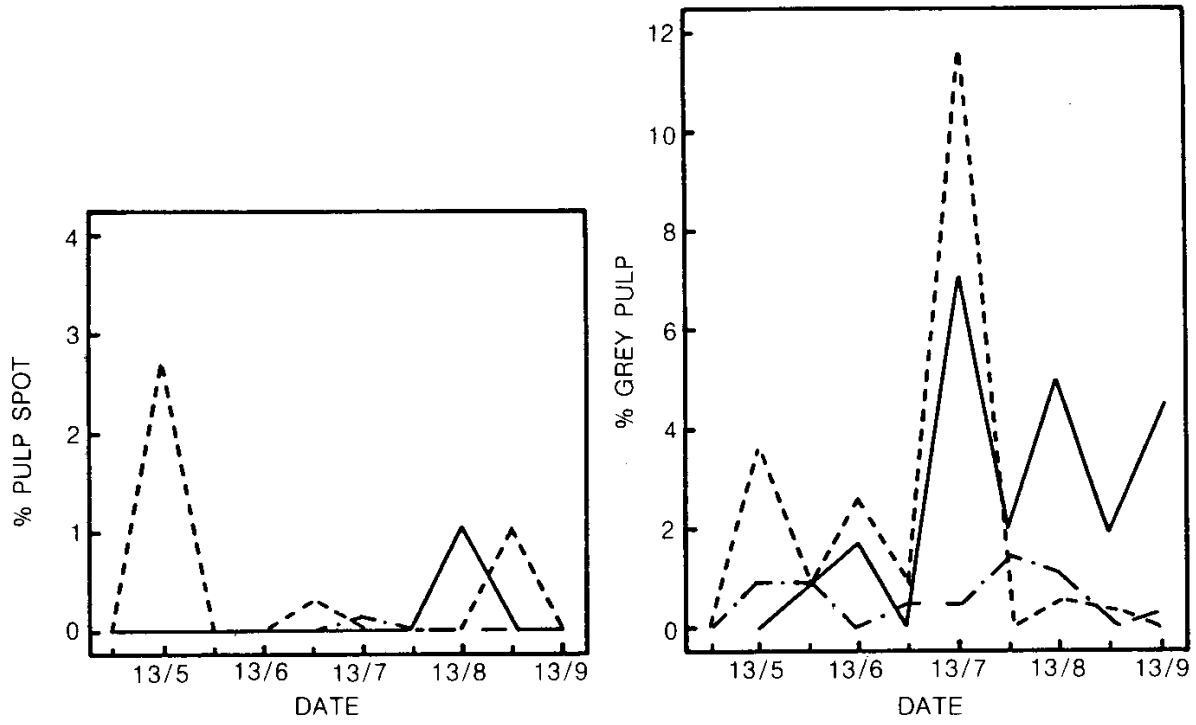
For hormone analysis, equal masses of avocado flesh from all samples within each harvest were combined to provide 5 g fresh mass, and the hormones extracted with 50 mf 80 per cent methanol (containing 20 mg l^{-1} BHT) overnight at 4°C. After filtration, the organic phase was evaporated and an equal volume of pH 8,5 phosphate buffer (1,0 M) added. Following partitioning against petroleum ether, the aqueous phase was adjusted to pH 2,5 and partitioned against ethyl acetate. For estimation of bound ABA, the remaining aqueous phase was adjusted to pH 11, incubated for one hour at 60°C, adjusted again to pH 2,5 and partitioned against ethyl acetate. The ethyl acetate fractions were evaporated to dryness and re-dissolved in 10 ml of pH 8,5 phosphate buffer (0,1 M). Two ml of this was subjected to PVP column chromatography and Sep-Pak C18 (Rosher *et al*, 1985). The final residue was dissolved in phosphate-buffered saline (PBS, pH 7,4) and radioimmuno-assayed.

The radioimmuno-assay procedure was similar to that of Weiler, (1979) except that 0,1 mf each of PBS, standard or unknown, radiolabelled hormone and suitably diluted antiserum, was used. Following incubation for two hours at room temperature or overnight at 4°C, bound and free fractions were separated using $(NH_4)_2SO_4$. Assay characteristics for ABA are described in Cutting *et al* (1986). The GA antiserum was raised against a GA_3 conjugate and the assay conducted using radiolabelled GA_1. The assay system had a measuring range for GA_3 conjugate and the assay conducted using radiolabelled GA_1. This assay system had a measuring range for GA_3 of 0,029-29 pmol, a 56 per cent cross-reaction to GA_1, but negligible response to GAs 4, 7, 9 and 13 (Hofman, unpublished data).

RESULTS

Flesh Disorders

Thornville fruit generally showed the lowest presence of the two disorders monitored, particularly for pulp spot (Figures 1 and 2). Of the disorders, pulp spot was the least severe and tended to show the lowest occurrence during mid-season. Grey pulp was generally of fairly high occurrence, but low colour intensity and tended to remain fairly constant throughout the season, apart from a major increase at 13/7.

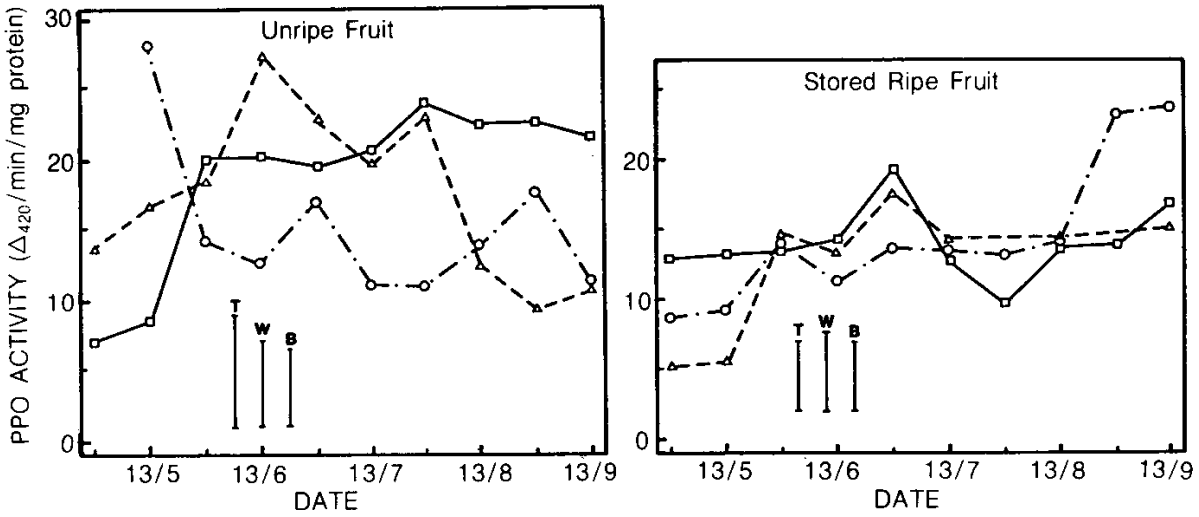


Figures 1 and 2 The effect of harvest date on the occurrence of pulp spot and grey pulp in stored ripe Fuerte avocado fruit obtained from Thornville (_._._), Wartburg (_____) and Baynesfield (— — —). Rating is given as the percentage of fruit cross-sectional area affected.

Polyphenol oxidase

Soluble and total PPO activity differed very little in all samples analysed. Therefore only total PPO data are presented (Figures 3 and 4).

In general, PPO activity in stored ripe fruit was lower than that in unripe fruit, indicating a decrease in PPO activity during storage and/or ripening. In all cases PPO activity remained fairly constant during mid-season, with a possible exception at 28/6, where Wartburg unripe and Baynesfield ripe fruits showed significant increases and similarly at 13/6 in unripe Thornville fruit. During the early and later stages PPO activities both increased and decreased, with no apparent pattern for locality or storage/ripening treatment.

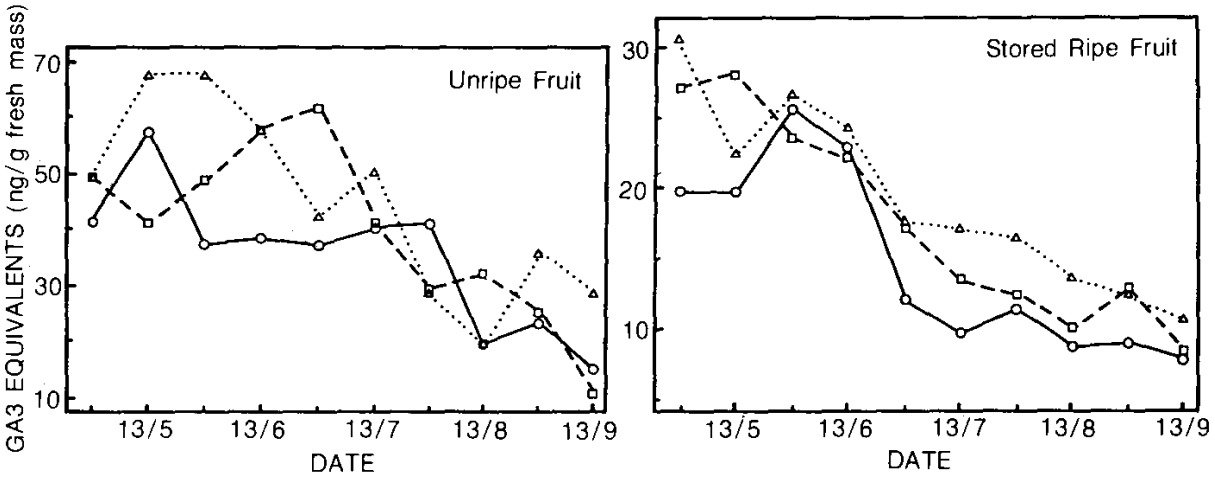


Figures 3 and 4 The effect of harvest date on the polyphenol oxidase activity in the mesocarp of unripe and stored ripe Fuerte avocado fruit obtained from Thornville (Δ——), Wartburg (o——.——) and Baynesfield (□——). Bars indicate LSD ($P<0,01$) for Thornville (T), Wartburg (W) and Baynesfield (B) fruit respectively.

Hormones

Neither harvest date, locality nor degree of ripeness had any consistent effect on free ABA concentrations. These varied from 12 to 25 ng g^{-1}. Bound ABA concentrations in unripe fruit were consistently low (6 to 9 ng g-1) in all fruit analysed, but concentrations in ripe fruit were higher and showed greater variation (8 to 21 ng g^{-1}). The only consistent trend observed in bound ABA was a fairly steady increase in ripe Wertburg fruit, from 11,8 to 21,3 ng g^{-1} between 28/6 and 13/9.

Gibberellin concentrations were generally higher in unripe than in ripe fruits (Figures 5 and 6). In addition, concentrations tended to be fairly constant between 28/4 and 28/5, but decreased thereafter, particularly between 13/6 and 13/7. This trend was more obvious and consistent in ripe fruit.



Figures 5 and 6 The effect of harvest date on the gibberellin concentration in the mesocarp of unripe and stored ripe Fuerte avocado fruit obtained from Thornville (Δ . . . .), Wartburg (o____) and Baynesfield (□ _ _)

DISCUSSION

Mesocarp discolouration severity is influenced by a number of both pre- and post-harvest factors (Ginsberg, 1985) and may therefore be expected to show seasonal and locality differences. Bower (personal communication) has found that generally, pulp spot severity tends to be most severe early and late in the season in fruit obtained from the northern Transvaal. This also appears to apply to fruit grown in cooler areas. There was considerable variation in disorder expression in fruit from the various areas around Pietermaritzburg, suggesting that soil and management conditions, rather than climate, may be more important in disorder expression.

Close relationships have been indicated between PPO activity and avocado mesocarp discolouration (Kahn, 1975; Van Lelyveld & Bower, 1984), but this may not be the only factor involved (Kahn, 1977). In this investigation, it is difficult to prove or disprove conclusively a relationship between PPO activity and browning potential, mainly because of a poor trend in mesocarp disorders with harvest. A strong relationship is not suggested however, since the observed reduction in pulp spot in the middle of the season was not accompanied by a reduction in PPO, and Thornville fruit did not have noticeably lower PPO activities, despite the lower occurrence of disorders in these fruits.

The concentrations of ABA detected here were low (Milborrow, 1984), and this, combined with a lack of an obvious pattern in both free and bound ABA with harvest, may suggest that ABA does not play an important role in fruit growth at this late stage of development. The decreasing GA concentrations may be associated with a reduction in growth rate that occurs in March/April under cool growing conditions (Van den Dool & Wolstenholme, 1983).

It is difficult from this investigation to determine the underlying causes behind mesocarp discolouration expression in avocado fruit, mainly because of the indistinct pattern of disorder occurrence with harvest. The possible link between PPO activity and disorders has already been discussed. These data also provide no indication of a relation between ABA and disorders or PPO concentrations, except perhaps, that the low ABA concentrations observed may be associated with the unusually high PPO activities. As mentioned, GA may be associated more with growth than fruit quality. In order to further clarify the relation between these factors, treatments are required which cause greater extremes of disorder expression and thus, if related, greater changes in PPO and hormones.

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