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THE EFFECT OF LONGTERM IRRIGATION REGIME ON AVOCADO FRUIT POLYPHENOL OXIDASE BROWNING POTENTIAL

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SUMMARY

The effect of long-term irrigation on avocado fruit polyphenol oxidase (PPO) activity was investigated. Three irrigation regimes were imposed, namely rewetting to field capacity when soil moisture tension reached 80 kPa, 55 kPa and 35 kPa in the root zone. Fruits were analysed after harvest when hard, and after softening, with and without low temperature storage, for soluble and total PPO activity. Soluble and total PPO activity decreased during softening of unstored fruits, the activity in 55 kPa fruits decreasing the most. Final soft fruit soluble PPO activity was lowest in the 55 kPa fruit, followed by the 35 kPa with 80 kPa fruit the highest. Total PPO activity showed a similar trend. Low temperature storage increased PPO activity. The period during which supplementary irrigation was necessary occurred during the first three months after fruit set, implying that water stress at this time can affect postharvest polyphenol oxidase activity.

OPSOMMING

Die effek van langtermyn besproeiing op avokado polifenoloxidase (PFO) is ondersoek. Drie besproeiingspatrone is gebruik, nl. herbenatting tot veldkapasiteit wanneergrondvogspanning 35 kPa, SOkPa ofSOkPa binne die wortelsone bereik het. Vrugte is naoes ontleed, terwyl die vrugte nog hard was en na sagwording met en sonder koelopberging. Oplosbare sowel as totale PFO is gemeet. Oplosbare en totale PFO het tydens opberging verminder, met die grootste vermindering by die 55 kPa behandeling. Die finale sagvrug opiosbare PFO het die minste aktiwiteit gehad by die 55 kPa behandeling, gevolg deur die 35 kPa en 80 kPa, wat die hoogste was. Totale PFO het dieselfde tendens getoon. Lae temperatuur opberging het PFO aktiwiteit verhoog. Die tydperk wanneer besproeiing noodsaaklik was, was tydens die eerste drie maande na vrugset, wat ímpliseer dat waterstremming gedurende die tvd die naoes PFO aktiwiteit kan béinvloed.

INTRODUCTION

Disorders of the avocado fruit mesocarp cause considerable problems for exporters of fruit shipped and stored at low temperatures for long periods. The disorders range from a rapid blackening in the region of cut vascular bundles without affecting the fruit flesh (pulp spot), to a general grey to black discoloration. Symptoms are usually more severe in the distal half of the fruit. While such disorders are often associated with chilling injury

(Eaks, 1976; Chaplin, Wills and Graham, 1982 and Couey, 1982). Vakis (1982) indicated that chilling need not necessarily be required for symptom expression.

Van Lelyveld and Bower (1984) showed that fruit flesh discoloration could be induced by restricted ventilation of fruit, with considerable increases in polyphenol oxidase (PPO) activity. Kahn (1975) was able to correlate the intensity of browning with PPO activity.

The severity of symptoms can, however, vary within a consignment and even within a single carton. Thus, other preexisting factors must also play a role in the development of avocado fruit physiological disorders. In analysing factors affecting disorders in South African export avocados, Bezuidenhout (1983) found unknown orchard factors to be of considerable importance. The purpose of this investigation was to ascertain whether long-term irrigation (and thus plant water stress) could have an effect on PPO activity at the time of picking, as well as after softening with and without low temperature storage. Such factors may assist in defining orchard conditions contributing to avocado physiological disorders.

MATERIALS AND METHODS

The fruits used in the experiment were picked from 5yearold Fuerte trees on seedling stocks planted 7,5 m x 7,5 m apart. The experimental orchard was divided into blocks of 25 trees, each block having been randomly allocated one of three irrigation treatments. The soil had previously been shown to be uniform throughout the area, and the scion material originated from the same mother tree. Irrigation treatments had been imposed for a season previous to that analysed.

The irrigation treatments imposed, consisted of irrigation back to field capacity when soil moisture tension in the root zone reached:

1) 35 kPa (frequent irrigation)

2) 55 kPa (moderate irrigation)

3) 80 kPa (occasional irrigation)

During the period from fruit set (September) to the end of December, little rain fell. According to previous water stress experiments (Bower, Wolstenholme and De Jager, 1978), the 80 kPa treatment should have induced considerable moisture stress, and this should have decreased in the treatments with lower soil moisture tension thresholds. From January until the first week of April (picking) regular rains fell, so that supplementary irrigations were seldom necessary.

Legally mature fruits (less than 80% moisture) were picked during the first week of April. Maturity was determined using the dry matter method of Swarts (1978). Forty fruits were randomly picked from five data trees in each treatment.

Fruits were immediately packed in export cartons, but no wrappers or wax treatments were applied as this may have affected later results. The fruits were then divided into two samples, the one being stored at 5,5°C for 30 days before analysis and the other being analysed without storage. In each case, the samples were further randomly

subdivided so that 10 fruits were immediately analysed while the other 10 were first allowed to reach the soft-ripe stage, at 21°C, as determined by the firmometer of Swarts (1981).

Fruits were peeled, the seed removed, and the flesh divided into proximal and distal halves. The physiological disorders concerned are most intense in the distal half (Van Lelyveld and Bower, 1984), and this work reports on this region: In the case of hard fruit, the flesh was ground using a kitchen grater. Soft fruits were placed under nitrogen in polyethylene bags and mashed by hand. All fruit was then immediately frozen and placed at 20°C until further analysis.

Crude enzyme extraction for soluble PPO was made according to the method of Van Lelyveld and Bower (1984). A 2,5 g fruit sample was ground in 5 ml cold 10 mM acetate buffer pH 5,0, in a mortar and pestle at room temperature (Kahn, 1977; Golan, Kahn and Sadovski, 1977). Insoluble polyvinyl pyrrolidone (polyclar AT) was added during homogenisation (Loomis, 1973). In the case of hard fruit, chemically pure sand was added as an aid to homogenisation. The same method was used for the extraction of total (bound plus latent plus soluble) PPO, with the addition of 0,1% SDS (Roberts, 1974; Kahn, 1977). The homogenate was squeezed through gauze cloth and centrifuged in a Sorval Superspeed RC2 centrifuge at 18 000 X g for 45 min. at 0 to 4°C. The supernatant was used immediately for PPO assay.

The PPO assay was as described by Van Lelyveld, Gerrish and Dixon (1984), with the exception that 50 μl enzyme extract in 6 ml substratebuffer solution was used.

The protein content of the extracts was determined following precipitation of protein by trichloroacetic acid, by the method of Lowry, Rosebrough, Farr and Randall (1951) as modified by Leggett-Baily (1962).

The PPO activity is expressed as the OD change at 420 nm per min. per mg protein, at 25°C.

RESULTS

The mean PPO specific activity results are shown in Table 1.

The soluble PPO is probably the most important fraction from the marketing point of view, as it is this which will determine the immediate browning potential of the fruit. In the case of soft unstored fruit the moderate irrigation (55 kPa soil moisture tension) resulted in the lowest PPO activity, followed by the very frequent irrigation, and highest ($P<0.01$) in the infrequently irrigated fruit. Stored fruit showed a similar trend as unstored fruit, and in all cases a trend towards higher PPO values as a result of low temperature storage was evident. In the case of hard fruit, only unstored results are presented, as a change in PPO activity occurred during softening. As it was not possible to stop ripening entirely during the storage period, PPO activity in "hard" fruit after storage does not necessarily represent the influence of low temperature. In unstored hard fruit, a reverse trend to that in soft fruit existed.

Total PPO activity indicates the potential for browning should latent and/or bound PPO become active, as shown by Van Lelyveld, Gerrish and Dixon (1984). Soft, unstored

fruit again showed the same trend as the soluble PPO activity. Enzyme activation or solubilisation therefore occurs. Fruits from the 80 kPa treatment having the highest PPO activity, would be likely to show greater browning. In stored fruit, the trend shown in other treatments was not evident, implying that the low temperatures to which the fruit had been subjected, had in some way altered the total PPO activity.

Hard, unstored fruit showed the same trend for total PPO as for soluble PPO. The same trend of decreasing PPO activity occurred with fruit softening.

DISCUSSION

There is little doubt that the water relations history of avocado trees and fruits has an effect on PPO activity at harvest and after ripening.

An important result concerns that of the very frequent irrigation regime. The mean PPO activity was, on most occasions tested, intermediate between the moderate and occasional irrigation treatments. Some water relations evidence did exist to indicate that a degree of stress existed in these trees. Furthermore, the soil contained 40% clay, so that waterlogged conditions could have existed with very frequent irrigation. The soil moisture tension at 450 mm depth seldom rose to 35 kPa. It is also well-known that the avocado is very sensitive to a low soil oxygen content (Wager, 1942). This aspect will need further investigation.

Of particular consequence is the time during fruit development when tree and fruit water stress could have occurred. Neither tree nor fruit water status differed between the treatments at the time of picking. Considering the rainfall pattern for the period of fruit development, the most likely period of stress was during the first three months of fruit development. The avocado is unusual in the cell division takes place throughout development (Schroeder, 1960), although the most rapid division occurs in young fruits (Blumenfeld and Gazit, 1974). A large number of cells could therefore have developed under stress conditions, with later adverse consequences. The mode of action whereby stress affects PPO activity is unknown, but membrane structural differences may occur. This also needs further investigation.

In conclusion, water relations history, particularly early in fruit development, appears to have a profound effect on PRO activity changes during ripening. The mode of action, however, is not yet understood.

TABLE 1

Effect of irrigation on total and soluble polyphenol oxidase (PPO) in hard and soft Fuerte fruit before and after storage. PPO expressed as OD_{420} min^{-1}mg $protein^{-1}$. Significance at P = 0,05 is shown by *and at P = 0,01 by **. S.E. of means are shown in brackets.

	Total PPO			Soluble PPO		
Irrigation regime	unstored soft	hard	stored soft	unstored soft	hard	stored soft
35 kPa	1,167 (± 0,05)	3,867 (±0,31)	1,902 (± 0,13)	0,711 (± 0,03)	2,030 (± 0,19)	0,934 (± 0,08)
55 kPa	0,999 (± 0,03)	3,929 (± 0,11)	1,603 (± 0,11)	0,531 (± 0,03)	2,225 (± 0,13)	0,840 (± 0,04)
80 kPa	1,542 (± 0,05)	3,193 (± 0,22)	1,590 (± 0,06)	0,856 (±0,05)	1,330 (± 0,06)	1,115 (± 0,09)
Significance	**	NS	*	**	**	**
LSD P=0,05	0,153		0,268	0,126	0,497	0,165
P=0,01	0,201		0,368	0,172	0,682	0,226
CV%	13,3	25,9	16,9	19,2	28,4	18,0

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