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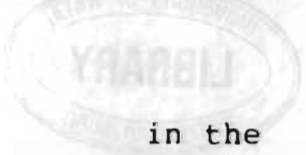
SOME ASPECTS OF WATER RELATIONS ON AVOCADO Persea americana
(Mill.) TREE AND FRUIT PHYSIOLOGY

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CONTENTS

	Page No.
DECLARATION	v
ACKNOWLEDGEMENTS	vi
SYMBOLS AND ABBREVIATIONS	vii
ABSTRACT	viii
INTRODUCTION	1
CHAPTER 1 REVIEW OF LITERATURE	6
1.1 PLANT WATER RELATIONS	6
1.2 FRUIT PHYSIOLOGY	13
1.2.1 POLYPHENOL OXIDASE AND PHENOLICS	13
1.2.2 FRUIT RIPENING	20
1.2.2.1 Ethylene	20
1.2.2.2 Calcium	23
1.2.2.3 Ultrastructural changes	28
1.2.2.4 Enzymes	29
1.2.2.5 Plant growth regulators	30
CHAPTER 2 TREE AND FRUIT WATER RELATIONS	34
2.1 TREE WATER RELATIONS	34
2.1.1 SEASONAL TRENDS	34
2.1.1.1 MATERIALS AND METHODS	34
2.1.1.1.1 Osmotic adjustment	36
2.1.1.1.1.1 Calculation of results	39
2.1.1.1.2 Leaf water potential	41

2.1.1.2 RESULTS AND DISCUSSION	42
2.1.1.2.1 Osmotic pressure	42
2.1.1.2.2 Osmotic pressure at zero turgor	46
2.1.1.2.3 Leaf water potential	48
2.1.2 DIURNAL WATER RELATIONS	51
2.1.2.1 MATERIALS AND METHODS	51
2.1.2.1.1 Stomatal resistance and transpiration	52
2.1.2.1.2 Leaf water potential	53
2.1.2.1.3 Environmental stress parameters	54
2.1.2.2 RESULTS AND DISCUSSION	55
2.1.2.2.1 Summer trends	55
2.1.2.2.1.1 Leaf water potential	55
2.1.2.2.1.2 Stomatal resistance and transpiration	58
2.1.2.2.2 Winter trends	63
2.1.2.2.2.1 Stomatal resistance and transpiration	63
2.1.2.2.3 Spring trends	68
2.2 FRUIT WATER RELATIONS	71
2.2.1 MATERIALS AND METHODS	71
2.2.2 RESULTS AND DISCUSSION	78
2.3 GENERAL DISCUSSION	82
CHAPTER 3 INFLUENCE OF WATER RELATIONS ON FRUIT PHYSIOLOGY	87
3.1 POLYPHENOL OXIDASE AND PHENOLICS	87
3.1.1 MATERIALS AND METHODS	87
3.1.1.1 Extraction of PPO	89
3.1.1.2 Assay of PPO	90
3.1.1.3 Protein determinations	91

3.1.1.4	Extraction of total phenols	91
3.1.1.5	Assay of total phenols	93
3.1.2	RESULTS AND DISCUSSION	94
3.1.2.1	Polyphenol oxidase	94
3.1.2.1.1	First picking date	94
3.1.2.1.1.1	Soluble PPO activity	94
3.1.2.1.1.1.1	Unstored fruit	94
3.1.2.1.1.1.2	Stored fruit	96
3.1.2.1.1.2	Total PPO activity	98
3.1.2.1.1.2.1	Unstored fruit	98
3.1.2.1.1.2.2	Stored fruit	101
3.1.2.1.2	Second picking date	101
3.1.2.1.2.1	Soluble PPO activity	103
3.1.2.1.2.1.1	Unstored fruit	103
3.1.2.1.2.1.2	Stored fruit	103
3.1.2.1.2.2	Total PPO activity	106
3.1.2.1.3	Effect of irrigation on PPO activity in the 1984/1985 season	107
3.1.2.1.4	Effect of irrigation regime and restricted ventilation on PPO activity	109
3.1.2.1.4.1	Soluble PPO activity	109
3.1.2.1.4.2	Total PPO activity	109
3.1.2.2	Total phenols	112
3.2	FRUIT RIPENING	113
3.2.1	MATERIALS AND METHODS	113
3.2.1.1	Fruit softening	113
3.2.1.2	Ethylene evolution	114
3.2.2	RESULTS AND DISCUSSION	117
3.2.2.1	Fruit softening	117
3.2.2.1.1	Harvest 1982	117
3.2.2.1.2	Harvest 1983	118
3.2.2.1.3	Harvest 1984	121

3.2.2.2 Effect of irrigation regime on ethylene evolution	122
3.3 FRUIT CALCIUM RELATIONS	129
3.3.1 MATERIALS AND METHODS	129
3.3.2 RESULTS AND DISCUSSION	131
3.3.2.1 The 1982/1983 season	131
3.3.2.2 The 1983/1984 season	135
3.4 ABSCISIC ACID	138
3.4.1 MATERIALS AND METHODS	138
3.4.1.1 Extraction and assay of ABA	138
3.4.1.1.1 Extraction of plant material	139
3.4.1.1.2 Radioimmunoassay	140
3.4.1.2 Calculation of results	142
3.4.2 RESULTS AND DISCUSSION	143
3.5 GENERAL DISCUSSION	146
CONCLUSIONS	151
SUMMARY	158
LITERATURE CITED	163

DECLARATION

I declare that the results contained in this thesis are from my own original work except as acknowledged herein

J.P. Bower

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SYMBOLS AND ABBREVIATIONS

The following are important symbols and abbreviations used in the text:

ABA	Abscisic acid
ACC	1-Aminocyclopropane-1-carboxylic acid
BSA	Bovine serum albumin
e	Ambient vapour pressure
e_s	Saturated vapour pressure at ambient temperature
IAA	Indole acetic acid
MM	Molecular mass
NSB	Non specific binding
PAR	Photosynthetically active radiation
PBS	Phosphate buffered saline
PPO	Polyphenol oxidase
PVP	Polyvinyl pyrrolidone
RH	Relative humidity
RIA	Radioimmunoassay
SD	Saturation deficit
SDS	Sodium dodecylsulphate
Ψ	Water potential

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ABSTRACT

The effect of long-term irrigation on water relations of 'Fuerte' avocado trees, and the consequences for fruit ripening and physiology, particularly physiological disorders, were studied.

Four irrigation regimes were used, namely dryland relying on rainfall, occasional (irrigation when soil moisture tension reached 80 kPa), regular (soil moisture tension 55 kPa) and frequent (soil moisture 35 kPa).

Seasonal tree leaf water potential was studied. This became more negative during the dry season (winter and spring) and less negative during the period of summer rainfall. During the dry periods, the dryland and 80 kPa treatments had considerably more negative leaf water potential than the 55 kPa and 35 kPa regimes. These differences decreased during the summer rainfall period.

Acclimation was studied by measuring leaf osmotic pressure and osmotic pressure at zero turgor. A pattern similar to seasonal leaf water potential emerged. Further, the dryland treatment showed higher osmotic pressure, particularly at zero turgor, during winter and spring. It was concluded that these trees may have acclimated. Diurnal cycles of stomatal resistance, transpiration and leaf water potential on a summer, two winter and a spring day were monitored. Dryland

trees showed acclimation, with delayed reaction to environmental water demand and decreasing soil moisture. Trees of the 80 kPa treatment showed greatest stress.

Fruit water potential became more negative between April and July, with fruit softening becoming more rapid. Treatment differences were inconclusive.

Polyphenol oxidase activity (PPO), soluble and total, was measured. For fruit picked in April and July 1983, the 55 kPa treatment showed the lowest activity and the 80 kPa the highest. Storage at 5,5°C for 30 days increased the activity, while fruit softening decreased it. July activity was higher than the April-harvested fruit. The same pattern emerged for fruit harvested in April 1985, although treatment differences were not significant. Rainfall was considerably higher during the fruit development period of 1985 fruit as compared with that of 1983. A significant interaction between restricted container ventilation during ripening and irrigation history was obtained, the 80 kPa fruit showing higher PPO activity than 55 kPa fruit.

Ethylene evolution during ripening showed a normal climacteric pattern for 55 kPa and dryland fruit, but a delayed peak for 80 kPa fruit.

Fruit calcium concentrations showed rapid changes between 7 and 16 weeks after fruit set thereafter remaining constant to harvest. There were no clear treatment differences.

Fruit abscisic acid levels at 50% soft (100% is eating soft) were lowest in 55 kPa fruit, and highest in 80 kPa. A significant correlation between these values and soft fruit PPO activity was found.

A preliminary fruit quality prediction model is suggested.

INTRODUCTION

The South African avocado industry has, in recent years, shown a rapid expansion. There are now over two million trees, double the 1981 population. The 1985 production was 25 000 tonnes, of which 68% was exported. This compares with a 1978 production of 15 000 tonnes of which approximately 47% was exported (Lourens, 1979). The present value of the industry to South Africa is thus considerable, being approximately 21 million Rand. The majority of exports are of one cultivar, 'Fuerte', with 'Hass' showing a rising trend.

The 'Fuerte' is thought to be a natural hybrid of the Guatemalan and Mexican races (Bergh, 1975). As such, the natural habitat of the tree is considered to be the cool, subtropical to tropical highlands of Mexico and Guatemala. Work on the ecophysiology of the tree (Bower, 1978; Scholefield, Walcott, Kriedemann & Ramadasan, 1980) indicates mesic adaptations including shade adapted leaves, and best performance in reasonably cool, moist and humid climates. In fact, the trees appear susceptible to stress, from the point of view that stomatal closure for survival appears to occur at relatively moderate moisture stress levels (Bower, 1978).

Tree stress is essentially an interactive function of the environment with the tree. Atmospheric variables (radiant flux density, wind, temperature and relative humidity) create a demand for water from the tree. If this cannot be met by an equivalent water uptake from the soil and its movement through the tree, water stress will occur (Hsiao, 1973). The majority of avocado areas in South Africa do not satisfy optimal environmental requirements, particularly during the fruit set and early growth period (August to November). Air temperatures are often high, humidity low, and soil moisture

depleted as the normal summer rainfall does not contribute substantially to the soil moisture reservoir during this period. Supplementary irrigation should therefore be regarded as obligatory if tree stress is to be avoided, even in high rainfall areas normally receiving 850 to 1200 mm annually.

Thus far there has been very little work of direct applicability to the South African avocado grower investigating the effect of irrigation of field-grown trees over an extended period. The previous work of Bower (1978) was based entirely on container-grown glasshouse trees, which is not ideal due to root volume restriction which could affect response to water stress (Pereira & Kozlowski, 1976). Scholefield et al. (1980) did examine water relations of field trees, but only on recently-irrigated (thus implying sufficient water) trees, and only for a single, hot day. A number of experiments have been conducted in winter rainfall Israel, including extensive, long-term trials. In a 6-year trial, Lahav & Kalmar (1977) studied four different irrigation intervals (from 7 to 28 days). In the case of 'Fuerte' these authors were unable to find any differences as far as fruit growth, size or numbers were concerned, although oil accumulation was more rapid with shorter irrigation intervals.

Later work in Israel (Lahav & Kalmar, 1983) examined the effects of irrigation during spring and autumn. Supplementary irrigation was applied at soil moisture tensions of either 25 kPa or 40 kPa at 300 mm depth. Neither of these, according to the previous work of Bower (1978), would be likely to constitute a stress treatment. Only small differences (the wet regime being marginally better) were found for fruit size or total yield. There was also little effect on fruit quality, although no extensive physiological work was conducted, nor was fruit stored for long periods (up to 30 days) before quality analysis. If anything, it appeared that

the wetter regime was detrimental to fruit quality.

These results should not be assumed to be directly applicable under South African conditions, where many environmental conditions differ from those in Israel. The soils in the Western Galilee contain 60 to 63% clay, whereas some of the heaviest Transvaal avocado soils have approximately 40% clay. The water holding capacity in such Israeli soils is therefore higher than that of South African soils. Perhaps the most important aspect is that Israel has a Mediterranean climate. If, as Lahav & Kalmar (1983) mention, the flowering and fruit set period is the most important during which to minimize all forms of stress, this is a vital difference. In Israel, fruit set and early development occur after winter, when the soil is still moist from the winter rainfall. In South Africa, by contrast, the early fruit set period occurs in late winter and spring, during the driest time of the year. In addition, evaporative demand in spring is very variable and often high. Israeli work should therefore be interpreted with caution in the South African context.

Irrigation is considered essential for avocados in California (Gustafson, 1976), as rainfall is generally low and erratic. California also has a Mediterranean climate, which raises the same questions as Israeli work as far as South African applicability is concerned. The soils (and therefore drainage patterns and water holding capacity) are also generally very different from those in South Africa. The extremely high cost of water in California also influences irrigation decisions. While this is not as yet of prime importance in South Africa, increasing industrialization and thus competition for rather limited water resources is likely to increase. The grower will therefore have to minimize water applications as far as possible, without creating plant stress. Some work on the water stress characteristics of avocados has also been done in California by Sterne, Kaufmann & Zentmyer (1977). However,

the study was not conducted throughout the season, nor did it investigate fruit quality. Further, the work was on the cv. Bacon, not grown commercially in South Africa. It is thus not possible to apply Californian work directly in the South African context.

Knowledge of tree reactions to stress is not necessarily the most important aspect to the grower. Production of fruit, the end product, is the most important aim. The best possible yield in relation to input, and the best possible quality have to be aimed for. The aspect of quality is of particular importance to the South African grower, due to the high proportion of exported fruit. Approximately half the gross return (Toerien, Meyer & Milne, 1984) is spent on transport and marketing. Apart from the losses due to pathological problems which are not within the scope of this work, two major aspects are of importance. Firstly, fruit may be soft on arrival overseas or ripen very rapidly thereafter. This leads to trade reluctance to purchase, which in turn depresses prices. The second, more damaging aspect is that of internal physiological disorders. During the 1981 and 1982 seasons, the South African Avocado Grower's Association conducted extensive surveys of fruit quality in Europe. Bezuidenhout & Kuschke (1982; 1983) found more than 20% of all fruit had internal physiological disorders, the most common being vascular bundle discoloration and a grey discoloration of the fruit flesh. This is particularly damaging to the industry, as the disorders are usually not detected until after sale, causing consumer resistance to avocados. Large retail outlets often purchase on the understanding that quality will be of a certain standard, and below-standard fruit may be returned to distributors with heavy losses to the grower.

While van Lelyveld & Bower (1984) concluded that the atmosphere in containers, particularly after offloading, was

likely to affect internal fruit quality, other factors are clearly involved. Bezuidennout (1983) found that many of the reasons for internal disorders were unexplained, and were probably attributable to on-farm factors.

The quality of irrigation in South Africa is tremendously variable. Further, little guide could be found in the literature as to the important effect of water relations on fruit physiology, which could affect ripening and physiological disorders. Knowledge of the link between long-term water relations and fruit quality in avocados is particularly lacking.

The major objective of the research reported here was to study the effects of long-term irrigation on some aspects of fruit physiology, particularly as it related to fruit disorders. It was also decided to examine tree water relations under the irrigation regimes imposed, to gain a background to the conditions under which fruits were developing. Fruit physiology, particularly post-harvest, was investigated with special attention to ripening (including the presumably ethylene-induced climacteric), and the potential for browning resulting from the activity of the enzyme polyphenol oxidase. As lack of calcium has also been associated with internal disorders of many fruits (Bangerth, 1979), this aspect was also examined, as well as abscisic acid, the plant growth substance most associated with moisture stress (Leopold & Kriedemann, 1975). With the knowledge gained, it was hoped that the role of stress, particularly water stress, in the development of physiological disorders would be better understood, thereby allowing the avocado industry to improve crop quality, and predict problem areas. Hopefully, a more scientific basis for the irrigation requirements of avocados, for producing high-quality export fruit under local conditions, would also be gained.

CHAPTER 1

REVIEW OF LITERATURE

1.1 PLANT WATER RELATIONS

Before discussing the effects of changing water relations on avocado trees, it is necessary to briefly summarize water relations in general. The specific effects of water relations on fruit physiology will be covered later. Certain aspects more relevant to experimental technique are included under Materials and Methods.

Under normal conditions, a soil-plant-atmospheric water continuum exists. Atmospheric conditions, via their effect on transpiration, act as the driving force behind the movement of water through the plant (Leopold & Kriedemann, 1975). Under normal conditions atmospheric air is not saturated, while sub-stomatal spaces are. A diffusion pressure gradient therefore occurs, with water moving out of the leaves. Air temperature coupled with vapour pressure deficit is of particular importance. The higher the temperature of the air the more water vapour it can hold, and the steeper the gradient from the sub-stomatal spaces to the atmosphere, with consequently increased transpiration rates (Bidwell, 1974).

Transpiration causes a decreased water potential (ψ) in the leaves, which is transmitted to the roots via tensions in the xylem. Water must then be taken up from the soil to replace that lost, or a deficit (and therefore stress) will occur. A water gradient within the plant will be set up (Miller & Denmead, 1976), although the importance and reasons for this are disputed. Olsson (1977) indicated that water potentials for fully sunlit leaves are similar throughout the canopy, and as a result Chalmers, Olsson & Jones (1983) concluded that resistance to water flow within the tree is of lesser

importance, and that leaf environmental conditions are of prime importance. Nevertheless, from the viewpoint of practical water potential measurement, the possibility of such a gradient should not be ignored. As water potential is an important feature of the process of water movement in the plant, it is probably a good indicator of the plant water status at any one time. Hsiao (1973) concluded that xylem water potential is widely accepted as the fundamental indicator of plant water status.

Other parameters of plant water status have been used, but all have serious disadvantages. Hsiao (1973) mentioned that as far back as 1950, Weatherley indicated relative water content (the water content as a percentage of the water content at full turgor) as a measure of water relations. Among the disadvantages of this parameter is that it shows poor response at moderate water deficit levels. The same arguments hold for total water content. More indirect methods such as shrinkage of fruit (Tromp, 1984), or tree branches (Kozlowski, 1972) may be useful, but generally do not provide very much information about tree water status. Even worse are visual symptoms such as wilting. Recently, canopy temperature as measured by infrared radiant flux estimation has become popular as a means of water stress indication (Sumayao, Kanemasu & Brakke, 1980). While this is a stress indicator, it does not provide an accurate interpretation of plant water status. Water potential therefore remains an acceptable measure of the plant water status.

There are at least two important definitions of water potential, and according to Savage (1978) considerable confusion exists. Slatyer (1967), O'Leary (1970), Brown & van Haveren (1972) and Hsiao (1973) all defined water potential in terms of volume, as the potential energy needed to move a unit volume of water from the system in question to a reference point, usually pure, free water. This is volumetric

water potential, with units of Jm^{-3} , Nm^{-2} or Pa. On the other hand, Taylor (1968), Salisbury & Ross (1969) and Taylor & Ashcroft (1972) preferred specific water potential, defined in terms of mass as the potential energy needed to move a unit mass of water from the system to the reference position, again pure free water. The units are J mole^{-1} or J kg^{-1} . As the units used throughout this thesis are in kPa, volumetric water potential is being referred to.

The total water potential consists of a number of components, which affect the final value by decreasing the potential energy. The most notable are: hydrostatic pressure or tension, due to the osmotic uptake of water by roots; the effect of solutes dissolved in the plant water (solute potential); and adsorption to cell walls and the interaction with macromolecules (matric potential). The situation is summed up mathematically by Leopold & Kriedemann (1975) as follows:

$$\Psi_{\text{plant}} = \Psi_{\text{s}} + \Psi_{\text{m}} + \Psi_{\text{t}}$$

where: Ψ_{t} = turgor potential (pressure)

Ψ_{m} = matric potential

Ψ_{s} = solute potential

Initially, as tissue water decreases due to restricted inflow or excessive atmospheric demand, a rapid decrease in turgor pressure potential occurs. Once zero turgor is reached, solute potential becomes the most important component, and follows the simple osmotic relationship with solution volume. The change in water potential per unit change in tissue water becomes small compared to the situation where turgor potential is predominant (Hsiao, 1973). Matric potential only becomes important once considerable dehydration has occurred (around 50% according to Wiebe (1966)).

While overall water potential of the plant is important, an

assessment of the water relations of the plant can be difficult if total (or predominantly turgor) potential only is measured. This is due to the large changes in potential during the day (Hanson & Hitz, 1982). This also does not mean that plants which experience a large decrease in water potential during the day are, in the long term, markedly affected. Both the duration of stress and plant species would probably be important. Further, the potential of individual leaves at any one time does not necessarily reflect atmospheric or soil conditions, and the plant as a whole should be considered. A better understanding of plant water relations can, however, be obtained if the components of water potential are estimated. Kyriakopoulos & Richter (1981) stated that the pressure-volume technique is particularly useful in examining plants subjected to long-term stress, in that tolerance or adaptation can be detected.

It is known that osmotic adjustment can take place in water-stressed plants (Hsiao, Acevedo, Fereres & Henderson, 1976). Turner & Jones (1980) indicated that solute potential increases as a result of solute accumulation. This can have the effect of maintaining normal or near normal turgor. Osmotic adjustment normally only occurs where stress develops slowly, and over a long period (Turner & Jones, 1980). This is an important consideration where the long-term effects of irrigation are being studied. Apart from pure solute changes, other cell adaptations such as changes in wall elasticity may also occur (Jones & Turner, 1978). The exact mechanism of solute adjustment is not known, but Jones, Osmond & Turner (1980) consider it to be a general accumulation of solutes, and possibly an accumulation of unused assimilates. While inorganic as well as organic ions can accumulate, with the range being similar to normal cells, Munns, Brady & Barlow (1979) found considerable increases of proline in stressed wheat. No specific mechanism was advanced for osmotic adjustment, but changes in biochemical reactions during the

onset of stress may eventually result in such adjustment, thus allowing plants to operate normally under conditions which would otherwise result in considerable stress.

Should water stress occur, the effects on the plant are numerous. Perhaps the best known is stomatal closure. It is accepted from the work of many authors that as water potential decreases, (becomes more negative) so a threshold value is reached after which rapid stomatal closure occurs, which is apparently species specific (Boyer, 1970; Turner, 1974). Bower, Wolstenholme & de Jager (1979) found avocados to be sensitive to water stress, in that closure occurred relatively early (approximately -900 kPa) in cv. Edranol. Sterne, Kaufmann & Zentmyer (1977) found closure at -1 200 kPa in 'Bacon'. In the cotton plant stomatal closure occurred at -2 200 kPa (Thomas, Brown & Jordan, 1976). Past stress history can, however, influence this point, the latter authors finding a threshold of about -3 000 kPa after previous stress.

Perhaps one of the most important effects of stomatal closure is on carbon dioxide assimilation. Hsiao (1973) noted that a close correlation has been shown between closure and carbon dioxide uptake. Bower et al. (1979) and Kumar & Tieszen (1980) indicated this to be essentially correct with avocados and coffee respectively. However, non-stomatal effects may also be important, and acclimation of the process to low water potentials may also take place (Matthews & Boyer, 1984). Hanson & Hitz (1982) concluded that carbon dioxide starvation causes considerable disruption of leaf metabolism. Within a short while this will be transmitted via the phloem, due to changes in various substrates carried by the phloem. As a result many other processes in the plant could be affected.

A decrease in photosynthesis is likely to affect carbohydrate

accumulation, and Scholefield (1979) showed a clear influence on the well-known alternate bearing habit of avocados. Considerable variations in fruit-shoot ratios may occur, which may or may not be important for fruit physiology due to altering source : sink relationships. There is, nevertheless, a known decrease in fruit growth due to water stress, although the direct influence of carbon assimilation is not known. A more subtle effect on fruit physiology may be, that during periods of low tree water content, water may move out of fruits to supply leaves (Kozlowski, 1972). The result would be moisture stress in the fruit, and possibly some loss of minerals such as calcium, thought to be important in fruit physiology (Bangerth, 1979).

Cell enlargement is extremely sensitive to water stress, particularly loss of turgor (Hsiao, 1973). Leaf expansion may therefore take place predominantly at night. The response is also very rapid (Acevedo, Hsiao & Henderson, 1971). This may be important in avocados in South Africa, in that the spring flush often develops during a period of environmental stress. Soil water is at a minimum after the dry winter, while air temperature is often high, coupled with low relative humidity. The overall result could be a decreased individual leaf photosynthetic capability due to both a reduced leaf area as well as the effect of water stress on photosynthesis. Osmotic adjustment could alleviate some of the stress effects, but as Hsiao (1973) noted, this only takes place slowly, so that much loss of growth could in any event occur. Cell wall synthesis is also sensitive to decreased plant water content (Cleland, 1967).

Decreased plant water status will also affect plant growth regulator concentrations. Since abscisic acid (ABA) is well known as a stress-related hormone, a significant change in its content could be expected. It has also been suggested that ABA may be instrumental in stimulating the accumulation

of proline (Hanson & Hitz, 1982). This would imply a role in moisture stress acclimation. Abscisic acid is discussed in greater detail later with regard to fruit physiology, and only a very brief discussion is given here. It is also evident that interactions between plant growth regulators should be considered.

It is generally agreed that ABA plays a role in stomatal control (Kriedemann, Loveys, Fuller & Leopold, 1972), but Horton (1971) proposed that a break or decrease in cytokinin supply to the leaves may be involved in the build-up of ABA. Itai & Vaadia (1965) found water stress to decrease cytokinin activity in root xylem extracts. Similarly, auxins are known to be affected by water stress, perhaps due to increased activity of indole acetic acid oxidase (Darbyshire, 1971). The role of ethylene will be discussed later in relation to fruit ripening. However, it could also be important earlier in fruit growth, as stress-induced ethylene could enhance fruit drop as appears to be the case in cotton (Hsiao, 1973), with a resultant change in leaf-fruit ratio. Little work seems to have been done concerning the long-term changes in plant growth regulator substances due to water deficits, and it is therefore difficult to evaluate the effects that they may have on overall tree growth and response to stress, especially if osmotic adjustment occurs.

An aspect presumably affected by water stress is protein metabolism, and by implication also enzymes. Hanson & Hitz (1982) indicated that gene expression can change, thus altering the pattern of enzymes produced. The extent, however, to which long-term stress, perhaps accompanied by osmotic adjustment, alters enzymes and the overall effect on plant growth, fruit development and quality, seems to be largely unknown.

An effect of tree water stress, especially long-term stress

would be a change in mineral uptake (Hsiao, 1973). This may be due to a decrease in transpiration and therefore overall flow in the soil-plant-atmosphere system, as well as a decrease in active uptake mechanisms. Overall plant metabolism would be affected, dependent on which ions were reduced and by how much. Fruit physiology could be particularly affected by calcium availability as discussed below.

1.2 FRUIT PHYSIOLOGY

As previously mentioned, the fruit physiological problems encountered by consumers relate primarily to internal browning where polyphenol oxidase (PPO) and phenolics evidently play a role. Premature fruit ripening is also of some importance. This portion of the review is thus divided into two sections, that dealing with PPO and phenolics, followed by a discussion of fruit ripening physiology.

1.2.1 POLYPHENOL OXIDASE AND PHENOLICS

The polyphenol oxidases in higher plants have been shown to be of two main forms. In their review of the subject, Mayer & Harel (1979) describe them as laccase (p-diphenol oxygen oxidoreductase) and catechol oxidase (o-diphenol oxygen oxidoreductase). The latter is often referred to as phenolase, polyphenol oxidase, tyrosinase, catecholase or cresolase. The distinction between the two groups lies in the substrates oxidised. While the laccases can catalyse the oxidation of a wide range of substrates, catechol oxidase cannot catalyse oxidation of p-diphenols. Some monophenols can, however, be oxidised. Kahn (1977a) indicated that no laccase or cresolase activity could be found in avocado fruit. Mason (1955) proposed that cresolase has a different function to polyphenol oxidase, and this review will therefore be limited to the catechol oxidases, which will be

referred to under the broad heading of polyphenol oxidase EC 1.14.18.1 (PPO). The lack of cresolase activity should perhaps be viewed with some caution, as Harel, Mayer & Shain (1964; 1965) pointed out that solubilising of membrane-bound PPO can lead to a reduction or absence of cresolase activity.

There is still considerable argument over the mode of action of PPO. In general, however, it is thought that *o*-diphenols are oxidised to the corresponding quinone, with the loss of hydrogen. Brooks & Dawson (1966) concluded that copper is lost from the enzyme at the same time. Mayer & Harel (1979) in discussing this, point out a number of difficulties, including transition of forms, conformational changes (Lerner, Mayer & Harel, 1972; Lerner & Mayer, 1975) and activation by the substrate. Some of these factors may be of importance in the reaction occurring in avocados, and will be further discussed later in this review.

PPO is known to consist of a number of different components. Dizik & Knapp (1970) using gel filtration with "Sephadex G150" resolved avocado PPO into five fractions with molecular masses of 14 000, 28 000, 56 000, 112 000 and 400 000 Daltons. The 28 000 fraction was shown to be the most active. It was in turn differentiated by gel electrophoresis into six components, one of these being the most active. Overall, the 28 000 fraction was the major component, with the 400 000 fraction being minor but nevertheless important. Kahn (1976a) using a "Sephadex G100" column found two important components, the major one having a molecular mass of 35 000 Daltons. Similar work has shown multiplicity of forms in potatoes (Balasingam & Ferdinand, 1970), pears (Rivas & Whitaker, 1973), apples (Satjawatcharaphong, Rymal, Dozier & Smith, 1983) and an apparently single form in olives (Ben-Shalom, Kahn, Harel & Mayer, 1977a). There is not always agreement on the characterization of forms in a particular plant, probably, according to Kahn (1976a), due to

differences in techniques. It is nevertheless evident that multiples of sub-units can exist and that certain of these show greater substrate affinity than others, as shown by Sakamura, Shibusa & Obata (1966) in eggplant. Interconversion of sub-units can also take place under a variety of conditions, for example storage (Cheung & Henderson, 1972).

The extremely important aspect of activation and latency seems to have first been studied by Kenten (1957). PPO can exist in two forms, soluble and bound (Kahn, 1977b). Apparent activation of latent soluble PPO was achieved by the alteration of pH. Addition of anionic detergents had a similar effect, although in the case of avocados (Kahn, 1977b) non-ionic detergents had the opposite effect. Cationic detergents increased the specific activity.

Storage and temperature may also cause a change in the latency of the enzyme (Kenten, 1958; Tolbert, 1973). The work of Tolbert (1973) may be of particular importance for avocado fruit stored for long periods during shipment, as discussed later. Certain treatments such as proteolytic enzymes, detergents and salts can also release (solubilise) additional PPO from a normally insoluble fraction (Roberts, 1974). Sodium dodecylsulphate (SDS) was particularly effective, increasing the PPO activity as well as protein solubilisation, but doubling specific activity.

Various theories have been proposed to explain the activation of PPO. Dissociation of an enzyme inhibitor complex was proposed by Kenten (1958) and supported by the work of Tolbert (1973). The latter author considers that regulation or suppression of PPO normally occurs, but that abnormal conditions (such as damage to cells or ageing) cause a removal of this suppression, resulting in uncontrolled PPO activity and tissue browning.

Conformational changes due to pH changes or detergents, have been advanced as a likely mode of action by Swain, Mapson & Robb (1966), who were able to reverse the activity by removal of the agents. Lerner et al. (1972) showed that this type of activation in grapes was due to a change in the enzyme kinetics and could be reversed, although conformational or aggregational changes due to prolonged exposure to low pH were not. Lerner & Mayer (1975) also favoured conformational changes, which they describe as an unfolding of the enzyme.

A third activation mechanism advanced by some authors involves de novo synthesis. Bastin (1968) and a number of authors (e.g. Mayer & Harel, 1979), base this supposition on the fact that various metabolic inhibitors can block the activation of the enzyme. Mayer & Harel (1979) did, however, appear unconvinced and considered that better evidence of de novo synthesis was required.

A mechanism which does not appear to have received much attention, is the possibility of aggregation or dissolution of sub-units. The findings of Dizik & Knapp (1970) are of possible importance. As the 28 000 MM unit was found to be largely responsible for the activity of the enzyme, splitting of the 56 000 MM tetramer could enhance activity. Van Lelyveld, Gerrish & Dixon (1984) also found a change in sub-unit constituents in avocado fruit showing flesh discoloration as opposed to normal fruit.

Conflicting evidence exists as to the role of substrates in the overall activity of PPO. Mondy, Gedde-Dahl & Mobley (1966) correlated phenolic content with browning in potatoes, while Luh & Phithakpol (1972) correlated browning in peaches with PPO activity. Kahn (1975) considered that differences in the rate of browning of three avocado cultivars were directly related to PPO levels in the fruit. Kahn (1977a) however, expressed the opinion that the concentration of natural

substrates may be important. Ramirez-Martinez & Luh (1973) isolated 15 phenolic compounds in the avocado but as pointed out by Kahn (1976b), only the o-di-hydroxyphenols act as substrates for PPO. The latter author found that some of the phenols present could act as inhibitors, and others as activators of PPO in the avocado. Golan, Kahn & Sadovski (1977) concluded that both the PPO activity per se as well as the total phenol content present, influence the final rate of PPO reaction in the fruit. There are also many other inhibitors of PPO (Baldry, Bucke & Coombs, 1970; Dizik & Knapp, 1970).

The overall browning potential of fruit, although highly correlated with PPO activity per se, can therefore be modified by inhibitors, activators and phenolic substrates, should they come into contact with each other. They may also affect the latency properties of the enzyme. It is therefore essential that during extraction of PPO for activity assay, these substances be prevented as far as possible from reacting with PPO. Polyvinyl pyrrolidone (Loomis, 1973) is often used for this purpose.

The location of PPO in the cells is of particular interest in the study of fruit browning. An understanding of this aspect may help to illustrate the effects of various environmental and physiological factors on the behaviour (and thus browning potential) of the enzyme. Mayer & Harel (1979) indicated that in higher plants PPO is invariably intracellular. It has been found in a free soluble form in the cell cytoplasm of senescing tissues such as ripening peach fruit (Flurkey & Jen, 1978).

Vaughn & Duke (1984) believed that in normal healthy tissue PPO is associated solely with plastids. Tolbert (1973) was essentially of the same opinion, indicating that spinach PPO is situated in chloroplasts, and that membranes are involved.

Henry (1976) has cited considerable evidence to support the presence of PPO in chloroplasts. Vaughn & Duke (1984) cite work of Lieberei & Biehl (1976) as showing that PPO is located in the thylakoids of chloroplasts. Other plastids such as leucoplasts may also be involved, and these can occur in divergent tissue types, for example roots (Mueller & Beckman, 1978) and carrot tissue cultures (Olah & Mueller, 1981). Vaughn & Duke (1984) concluded that PPO is involved with no organelle other than the plastids, and to claim any other result is due to errors in identification.

An interesting and perhaps important difference of opinion occurs in the case of avocado fruit. Engelbrecht (1982) indicated PPO to be associated with thylakoids in chloroplasts, but Sharon & Kahn (1979a) claimed that the enzyme was contained in small particulate bodies, which they suggested were microbodies. Ruis (1972) suggested the same location for a large proportion of PPO in potatoes. Sharon & Kahn (1979a) ruled out the possibility of mitochondria (as indicated by Mayer & Friend, 1960), or chloroplasts or chromoplasts as being the predominant site of PPO in avocados. Sharon-Raber & Kahn (1983) also found no correlation between carotenoids and PPO activity in avocados. The significance of these findings in relation to avocado flesh browning is difficult to evaluate.

Microbodies (Esau, 1960) are often associated with chloroplasts, and are known to contain various enzyme systems. A single membrane surrounds a granular or crystalline matrix. The plastids are far more complex organelles, consisting of a double membrane surrounding a plate-like structure of membranes known as the thylakoids. It is evident that the majority of PPO is normally enclosed within a cell organelle, and strong evidence exists to indicate that a large proportion of the enzyme is bound to membranes of these structures. Any factors affecting membrane

stability could thus make more PPO available for reaction, should suitable substrates be present.

While various methods of creating increased PPO activity have been mentioned, it is important to investigate which factors may be responsible, thus leading to natural browning of fruits. As mentioned by Tolbert (1973), Flurkey & Jen (1978) and Volk, Harel, Mayer & Gan-Zvi (1978), tissue age (thus implying senescence) causes considerable increases in active soluble PPO. An increase in soluble PPO may also occur during fruit ripening (Harel, Mayer & Shain, 1966; Ben-Shalom, Kahn, Harel & Mayer, 1977b). The avocado may, however, be an exception, in that Sharon & Kahn (1979b) found a decrease in browning potential during normal ripening. Low temperature storage is also reported to result in an increase in PPO activity as judged by the browning which occurs in avocados (Sharon & Kahn, 1979b), and other tropical and subtropical fruits (Couey, 1982). The latter author concludes that damage is caused by deep-seated membrane dysfunction, which may be minimised by acclimation to lower temperatures before picking.

Increasing length of storage period (perhaps again related to senescence) is also correlated to increasing browning potential in avocado (Golan & Sadovski, 1977). The atmosphere ($O_2:CO_2$ ratio) in which fruit is stored also affects PPO activity and browning. Low O_2 and high CO_2 levels (Spalding & Marousky, 1981) caused damage in avocados, and van Lelyveld & Bower (1984) showed that "suffocation" resulted in increased levels of soluble active PPO. Again, this is probably the result of membrane damage. Similar enhancement of browning was achieved by moderate radiation of potatoes (Cheung & Henderson, 1972). As far as water stress is concerned, not many direct correlations seem to have been made, but Volk, Harel, Mayer & Gan-Zvi (1977) found increased PPO activity in apple tissue cultures stressed by low humidity.

1.2.2 FRUIT RIPENING

Biale (1975) described fruit ripening as the processes resulting in changes in colour, taste and texture, which make the fruit acceptable for consumption. The processes involve many complex catabolic and anabolic changes resulting ultimately in senescence. These processes, once started, cannot be reversed. The avocado differs from many fruits in that ripening does not normally take place on the tree, but only after picking (Schroeder, 1953). This fundamental difference should always be borne in mind when discussing avocado fruit ripening. The reasons for this phenomenon are not well understood, but Tingwa & Young (1975) postulated that some substance, possibly an anion, acts as a ripening regulator and moves either to or from the fruit pedicel once detached from the tree.

Normal avocado fruit softening with acceptable taste occurs only once a certain level of maturity has been reached. Before this, only slight softening occurs due predominantly to shrivelling resulting from water loss (Barmore, 1977), and flavour is poor (Nagy & Shaw, 1980). Once horticultural (legal) maturity has been reached, Barmore (1977) notes that the rate of ripening (picking to softening) becomes progressively shorter with increasing maturity. Maturity in the avocado has traditionally been based on the oil content, as it has been noted (Kikuta & Erickson, 1968) that rapid oil accumulation occurs only at the time of growth decrease and maturity. There is an increasing tendency, however, to base legal maturity on percentage dry mass of the flesh, or its reciprocal, percentage moisture content (Swarts, 1978).

1.2.2.1 Ethylene

The avocado is a climacteric fruit. This implies a marked

rise in respiration rate at the onset of ripening, followed by a decline. Ethylene is known to play a role in ripening, leading Rhodes (1981) to define the climacteric as "a period in the ontogeny of certain fruits during which a series of biochemical changes is initiated by the autocatalytic production of ethylene, marking the change from growth to senescence and involving an increase in respiration and leading to ripening". In fact Yang (1985) considered ethylene to be essential to the ripening of climacteric fruits. It is appropriate to briefly examine the possible role of ethylene, particularly in avocado ripening, as well as the factors resulting in the autocatalytic rise in ethylene evolution.

As previously mentioned, the avocado fruit is different to most others, as ripening does not occur until after picking. If ethylene is the stimulus for ripening in the avocado, exogenous applications should cause ripening even on the tree. Gazit & Blumenfeld (1970a) were unable to demonstrate this, although it is known that exogenous applications of ethylene after harvest do cause an earlier climacteric with consequential ripening (Eaks, 1966). The response was also not induced until 25 hours after harvest (Gazit & Blumenfeld, 1970a), although this was not confirmed by Zauberman & Fuchs (1973). In addition Biale & Young (1971) indicated that fairly high levels of ethylene are required to elicit a response. Lieberman (1979) concluded that some anti-ethylene factor must be removed, or tissue must acquire increased sensitivity to ethylene before a reaction will occur. McGlasson (1985) postulated such sensitivity changes to be crucial to ethylene response.

The maturity of treated avocado fruit also affected the response obtained (Eaks, 1980). Biale & Young (1981) found that the climacteric rise could be delayed by ethylene if applied to early season fruit just after picking. There is further conflicting evidence as to the role of ethylene in

initiating ripening in that Burg & Burg (1962a), using the little known cultivar Choquette, found the ethylene peak to precede the respiratory rise, while perhaps more importantly Kosiychinda & Young (1975) found the opposite in 'Fuerte'. Zauberman & Fuchs (1981) also noted that ethylene formation was not the earliest event in ripening. Kosiychinda & Young (1975) concluded that some other factor is important in the initiation of ripening in the avocado, and Rhodes & Reid (1975) reached a similar conclusion for apples. Hoffman & Yang (1980) showed that a rapid rise in ACC (1-aminocyclopropane-1-carboxylic acid) accompanied the ethylene rise in avocados. From these results, one could believe that ethylene plays no role in avocado fruit ripening, and that the rise in ethylene is merely a product of ripening. Quazi & Freebairn (1970) disagreed, indicating that certain aspects of ripening may well require ethylene.

Some of the most important work in this regard is that of Tucker & Laties (1984), who found a clear relationship between ethylene and polysome prevalence early in the climacteric of avocado fruit, which Richmond & Biale (1966) considered as demonstrating an increase in protein synthesis. Christoffersen, Warm & Laties (1982) showed an increase in at least three mRNA's with the translation products having molecular masses of 16 500, 36 000 and 80 000 Daltons. Tucker & Laties (1984) indicated that at about this time, a new gene product (identified as cellulase) appeared, with a molecular mass of 53 000 Daltons, and speculated that ethylene may be directly involved. A number of enzymes may therefore be initiated by ethylene. Thus, although other factors initiate the respiratory rise in ripening fruit, and perhaps alter sensitivity to ethylene, the latter and its autocatalytic production are of vital importance to normal avocado ripening. Factors which may affect ethylene, the climacteric and therefore normal ripening in avocados must be examined.

Temperature before and during the ripening phase is a major environmental factor. Erickson & Takaake (1964) and Zauberman, Schiffman-Nadel & Yanko (1977) found that temperatures above 30°C had adverse effects on avocado ripening. Eaks (1978) indicated that the ethylene rise appeared earlier as ripening temperature increased from 20°C to 25°C, while the peak value decreased at higher temperatures. Respiration increased with increasing temperatures, but above 35°C ripening was abnormal. Low temperature storage also affects ripening. Eaks (1976) found that unripe avocado fruit held at 5°C or 0°C for longer than a week failed to show a climacteric when ripened at 20°C, while storage of up to a week at these temperatures induced an earlier climacteric. Low temperatures before harvest may affect ripening. Fuchs, Zauberman & Yanko (1975) found orchard freeze damage enhanced ethylene production, respiration, enzyme activities and softening after harvest.

Water stress also has profound effects on avocado ripening physiology. Adato & Gazit (1974) found that the faster avocado fruits lost water after picking, the faster they ripened. The ethylene peak occurred earlier if fruits were allowed to become dehydrated, whereas the reverse was true if harvested fruits were infiltrated with water. Similar findings were reported by Fukushima, Yamazaki & Odazima (1977) and Fukushima, Yarimizu, Kitamura & Iwata (1980), for a number of other fruits. There appears, however, to be very little information concerning the long-term effects of fluctuating water conditions per se on fruit ripening physiology.

1.2.2.2 Calcium

An important link between water relations and mineral nutrition is possible, due to the effect of transpiration on mineral movement in the plant. Calcium in particular, has

been associated with many fruit disorders (Millaway & Wiersholm 1979), bitter pit in apples being one of the best known. Bangerth (1979) noted four important functions for calcium in plants, these being their effect on enzymes, membranes, cell walls, and interactions with plant growth regulators. While Kretsinger (1976) indicated that calcium can bind to approximately 70 different proteins, the concentration of calcium in the cytoplasm is thought to be generally too low for the binding affinity of these enzymes. Bangerth (1979) therefore considered membrane-bound proteins of more importance, particularly as some membranes are calcium-rich. Membrane-bound ATPases, probably involved in membrane transport, are thought to be influenced by calcium (Kylin & Kahr, 1973). Bangerth (1979) indicated that a group of membrane-bound protein kinases are similarly affected. This could have considerable importance for membrane transport, and as a result, on the cells as a whole.

Recently, a low molecular mass cytoplasmic group of proteins (calmodulin) with the ability to bind calcium, has been discovered. Their importance in animal cells, in their role of enzyme modulators, has been known for some time (Cheung, 1980), but in plants was first identified in peas by Anderson & Cormier (1978). Calmodulin has only been isolated and characterised in a small number of plants thus far (avocado is not among them), but Muto & Miyachi (1984) indicate that it is probably ubiquitous. The radioimmunoassay developed by the latter workers will probably help considerably in elucidating the cellular occurrence and functions of this protein-calcium complex. Roux & Slocum (1982) sum up by saying that calcium plays an important regulatory role in cells by modulating, via calcium-binding activator enzymes, the activity of several other enzymes. It is thought that once calcium in the cells reaches a certain level (it is considerably higher outside, and entry through the cell membrane may in itself be an important aspect) it combines

with calmodulin. This activates the complex via conformational changes, which in turn enhances the catalytic activity and thus the effect on other enzymes (Cheung, 1982). This reaction is reversible. Calmodulin may also be involved in actively removing excess calcium from the cells (Cheung, 1982).

Bangerth (1974) indicated that many calcium-related fruit disorders are probably membrane-related, and this could be explained by the known effect of calcium on permeability control and stability under stress (Roux & Slocum, 1982). Control of membrane constituent turnover, cell wall precursors and microtubule assembly all appear affected by calcium. Roux & Slocum (1982) also indicated a differential permeability function as well as membrane-bound enzyme activity changes through membrane fluidity alterations which are calcium-related.

Calcium seems to play an important role in cell wall construction during cell division, as well as later maintenance. Bangerth (1979) considered that cell wall structure depends on calcium cross-linkage with pectins in the middle lamella, which is a result of gel formation due to the effect of calcium on pectic acid (Demarty, Morvan & Thellier, 1984). Cell wall rigidity and cell expansion are also affected.

Interactions between plant growth regulators and calcium seem to exist. Whether this is direct or not, is open to question. Poovaiah (1985) indicates that calcium concentration changes are influenced by internal and external stimuli, which in turn affect cell structure and function. Hepler & Wayne (1985) come to similar conclusions. Lieberman & Wang (1982) found that the ethylene-forming system from ACC (1-aminocyclopropane-1-carboxylic acid) relies on membrane integrity, and that senescence in tomato fruits could be

slowed down (Poovaiah, 1979) by calcium, through maintenance of cell wall structure and membranes. Tirmazi & Wills (1981) were able to slow down ripening of mangoes by calcium infiltration, and in the case of avocados, Tingwa & Young (1974) found slower ripening with higher endogenous fruit calcium levels. In further work, Wills & Tirmazi (1982) showed that calcium infiltration of avocados greatly reduced the ethylene peak and respiratory rise, with a longer ripening period. In an extensive review, Ferguson (1984) concluded that the role of calcium in ripening and senescence lies in its effect on membrane and cell wall structure and function. It is perhaps important that a high calcium concentration outside the cell, acting on the cell walls and plasma membrane, and low cytosolic calcium are necessary for normal cell function. During senescence the regulatory mechanism maintaining this gradient malfunctions, resulting in increased cytosolic calcium levels, which in turn may affect membrane-bound enzymes. Ferguson (1984) does, however, say that considerable work is still necessary for a good understanding of calcium metabolism.

Auxin binding to membranes has been shown to be calcium-dependent (Pooviah & Leopold, 1976). Bangerth (1979) also suggested that very high levels of auxin may cause rapid cell expansion, and if the demand for calcium in the cell walls and membranes cannot be met, membrane collapse could result. Collier & Huntington (1983) found that the most rapidly growing inner leaves of lettuce were the most susceptible to tipburn, a calcium-related disorder.

Uptake and movement of calcium in the plant is an important aspect. Uptake occurs predominantly in the young, non-suberized portion of the root (Lauchli, 1976) after which xylem loading may be either active or passive. Thereafter, according to Bangerth (1979), calcium moves unidirectionally towards young, actively growing tissues. Once in the xylem,

calcium seems to move by a combination of mass flow and ion exchange. Calcium is thought to bind weakly to substances such as lignins (Shear & Faust, 1970). This could explain the apparent accumulation of calcium in trees, with later remobilisation, as noted by Bangerth (1979). Removal from such a binding site could allow replacement with further calcium. Removal could be facilitated by metabolising activity, hence movement towards more active regions. The link with auxin transport (Lee, Mulkey & Evans, 1984) may be important. The effect of transpiration and mass flow should, however, not be ignored, as the faster the transpirational flow, the faster the movement between binding sites (Hanger, 1979).

While transpiration and calcium movement may not be proportional, Bangerth (1979) considers that water movement and the potentials in various plant parts are decisive factors in calcium distribution. Rapidly transpiring regions (leaves) may out-compete regions of low transpiration (fruits) (Shear & Faust, 1970), particularly if leaves are also rapidly growing as normally occurs during and shortly after fruit set in the avocado, when the spring vegetative flush develops. Under conditions of high water deficits, some calcium may even flow out of the fruits (Bangerth, 1979).

Some phloem transport of calcium has also been postulated, with Shear & Faust (1970) favouring this method for apples. On the other hand Raven (1977) considered calcium movement in phloem as unlikely to be rapid enough, and Hanger (1979) even found exclusion of calcium from phloem. Clarkson (1984) indicated the majority of calcium in the phloem to be bound, and movement, if any, would be very limited. As water movement into the fruit is thought to become more phloem-orientated as assimilate demand increases, so calcium movement into the fruit could be expected to decrease, thus explaining the decrease in uptake rate later in the season.

1.2.2.3 Ultrastructural changes

Certain cellular structural changes take place during ripening, but overall cell and particularly membrane integrity must be maintained for normal ripening to occur. A number of studies in various fruit types show large increases in enzymes (Dilley, 1970) and their activities. Thomson & Platt-Aloia (1976) showed that membrane degeneration in citrus fruits occurred only after ripening was complete.

Detailed ultrastructural studies have been made of the cell wall (Platt-Aloia, Thomson & Young, 1980) and of the cell contents (Platt-Aloia & Thomson, 1981) in ripening avocados. With ripening it was found that the middle lamella begins to disappear, with pectin removal from the matrix of the cell walls. Later, a loss of organisation and density in the walls occurs. Finally, Platt-Aloia & Thomson (1981) found that the walls almost completely disappeared during the post-climacteric phase.

Internally, the fruit cell constituents can be divided into a group of organelles which do not change during ripening and those which do. Platt-Aloia & Thomson (1981) found that chloroplasts, dictyosomes, microbodies, the nucleus, vacuoles and ribosomes do not alter. The structural integrity of these bodies and of the mitochondria was unchanged although the latter bodies did seem to lengthen, presumably to cater for increased energy demand during ripening. The most profound changes occurred in the rough endoplasmic reticulum, which showed considerable swelling and vesiculation, and seemed, with ribosomes, to be associated with, or fused with the plasmamembrane. This work indicates that membrane systems remain intact. Further, the changes in endoplasmic reticulum suggested to these authors considerable enzyme synthesis, presumably those involved in ripening and cell wall degradation. Similar changes were found by Sexton & Hall

(1974) in leaf abscission zones, where cell wall degradative enzymes become active.

1.2.2.4 Enzymes

The ripening process is the result of a number of enzyme changes. Some of the more important of these have been studied in the avocado. Scott, Bystrom & Bowler (1963) reported that cellulose is the major constituent of avocado cell walls. If these walls degrade during ripening, it is reasonable to expect cellulase activation during ripening. Pesis, Fuchs & Zauberman (1978) found an increase in this enzyme accompanying softening, which was closely correlated with the respiratory climacteric and ethylene. Addition of ethylene also caused an increase in cellulase activity, which is in agreement with the work of Tucker & Laties (1984). Awad & Young (1979) found that cellulase activity in avocado fruits rose rapidly during the respiratory climacteric, reaching extremely high levels (116 000 units cellulase g⁻¹ fresh mass). They compared these results with those of Hobson (1968) for tomatoes, peaches and pears, and concluded that cellulase is particularly important in avocado softening, unlike many other fruits. The early stages of softening in the avocado are due to cellulase, with polygalacturonase responsible for the final softening. Polygalacturonase began increasing in activity after the rise in ethylene was well established and softening had begun.

In the avocado, pectinmethylesterase shows a decrease with ripening (Rouse & Barmore, 1974). Awad & Young (1979) believed that partial de-methylation of pectin is required, before it becomes a suitable substrate for polygalacturonase. The likely sequence of events in avocado softening seems to be an unknown factor which stimulates an ethylene increase, which in turn via gene transcription alteration, results in a number of enzymes being produced, including cellulase which

allows cell wall degradation. During the pre-climacteric phase but after the initiation of ripening, an unknown factor causes pectinmethylesterase to bind to the cell wall, resulting in inactivation (Jansen & Jang, 1960), but at the same time preparing the cell wall substrate for hydrolysis in the presence of polygalacturonase. Intact membrane systems would be vital for the functioning of this chain of events. There is also a possibility that certain phenols may have an effect, acting as promoters or inhibitors, as found in bananas by de Swart & Maxie (1967).

1.2.2.5 Plant growth regulators

Plant growth regulators play a major role in fruit ripening. The role of ethylene has already been discussed, but the other plant growth regulators are almost certainly involved, if one considers their overall role in plant growth and development (Dilley, 1969; Leopold & Kriedemann, 1975). The control of maturation and especially the initiation of ripening is thought to be a balance between promoting and inhibiting factors. Apart from ethylene, abscisic acid (ABA) appears to be a ripening promotor. Auxins, as indole acetic acid (IAA), cytokinins and gibberellins, are inhibitors of fruit ripening (Rhodes, 1981).

There is some disagreement about the role of auxins. While auxin applications can stimulate ethylene synthesis and maturity, as shown by Maxie & Crane (1967) in figs, Frenkel & Dyck (1973) found a delay in ripening of pears after auxin treatment, in spite of increased ethylene synthesis. Rhodes (1981) indicated that products of IAA catabolism due to increased IAA oxidase activity may act as ripening stimulators during the early pre-climacteric phase. Adato & Gazit (1976) were unable to elicit initiation of avocado fruit ripening except with very high doses of IAA.

As far as cytokinins are concerned, Lieberman, Baker & Sloger (1977) found a decrease in ethylene production in avocados with increasing isopentenyl adenosine. However, the work of Gazit & Blumenfeld (1970b) seems to imply that avocados have a low cytokinin content by the time fruit is mature. The role of cytokinins in avocado ripening is probably limited. This trend was confirmed by Wolstenholme, Hofman, Cutting & Lishman (1985). Lieberman, Baker & Sloger (1977) found that the same probably applies to gibberellins, as exogenous addition had very little effect on fruit ripening. Dilley (1969) suggested a link with ABA in general fruit ripening, whereby the gibberellins decrease and ABA increases, to the point where ethylene production is no longer inhibited.

ABA appears to play a key role in fruit ripening in general (Goodwin, 1978). Rhodes (1981) referred to ABA as a ripening promotor. In their work on tomatoes, apples and avocados, Lieberman, Baker & Sloger (1977) showed an increase in ethylene and ripening following application of ABA before the climacteric peak, but depressed ethylene evolution if applied after the peak. They concluded that ABA accelerates ageing. Gazit & Blumenfeld (1972) reported little change in ABA level takes place during avocado fruit development, but during ripening a considerable increase occurs (Adato, Gazit & Blumenfeld, 1976). These workers also found that the increase in ABA closely followed the ethylene curve, with peaks at about the same time. Further, the ratio of free (active) to bound ABA remained about the same, indicating that the free active ABA was not merely the result of activation, but due to synthesis. This is in agreement with Milborrow & Robinson (1973), who found that ripening avocado fruit could convert labelled mevalonate, which is believed to be an ABA precursor (Walton, 1980), to ABA.

In his review, Rhodes (1981) found that all fruits studied contained ABA, and that ABA is somehow involved in the

ripening process. The role does not seem to be direct, because Bangerth (1980) noted that ripening can be inhibited by ethylene inhibition. Bruinsma (1981) considered the likely role to be the stimulation of ethylene biosynthesis, or the creation of tissue sensitivity to ethylene (Rhodes, 1981) once ripening inhibitors from the tree are no longer available after picking. An increase in ABA levels to a certain threshold could thus be required for ethylene stimulation. This is in accordance with the postulations of Hobson (1979). Factors which affect ABA synthesis would therefore be important.

Stress is a major factor affecting ABA levels. It has long been known that ABA is linked with leaf stomatal closure during water stress conditions. Kriedemann, Loveys, Fuller & Leopold (1972) found a 40 times normal concentration of ABA in leaves within 4 hours of wilting, while Wright (1977) found a rapid increase in ABA at approximately -900 kPa internal water potential in wheat. Considerable work with mutant tomato plants which are unable to close their stomates under stress conditions, has also involved ABA, as applications of ABA were able to reverse this trend (Imber & Tal, 1970; Tal & Imber, 1970). Hiron & Wright (1973) in a wide-ranging series of experiments were able to show linkage between leaf water deficits due to extreme environmental conditions and increased ABA levels. The same applied to flooded conditions. It is well known that the avocado is highly sensitive to waterlogging (Zentmyer, Paulus, Gustafson, Wallace & Burns, 1965).

Similar responses to stress may occur in fruit. Once picked, considerable loss of water from fruit (including avocados) by transpiration normally takes place. This could create "stress" conditions in the fruit, leading to ABA accumulation once the fruit water potential approaches zero turgor, as reported by Ackerson (1982) for cotton leaves. Milborrow &

Robinson (1973) found that rapid dehydration of avocado slices did not cause an increase in ABA levels. However, no explanation of the known increase in ABA levels during ripening was given.

The regulation of ABA response to water stress is not understood (Walton, 1980), but there are suggestions. It is assumed (Walton, 1980) that the site of ABA synthesis in leaves is the chloroplasts, although this is not certain. He also suggested that stress causes a change in chloroplast membrane permeability, which allows ABA to move into the rest of the cell. At the same time, product inhibition decreases, allowing further ABA synthesis. Any factors which affect membrane permeability could thus result in accelerated ABA synthesis.

Factors other than water stress can also affect ABA synthesis, the most notable in avocado fruits being temperature. Wang, Wang & Mellethin (1972) found that pears subjected to low orchard temperatures tended to ripen faster, and also contained higher ABA levels. This would be important where fruit are allowed to hang into the winter, as well as fruit stored for long periods at low temperatures.

CHAPTER 2

TREE AND FRUIT WATER RELATIONS

2.1 TREE WATER RELATIONS

2.1.1 SEASONAL TRENDS

2.1.1.1 MATERIALS AND METHODS

The trees used in this and all subsequent experiments detailed in this thesis were five year old (1983) 'Fuerte' on 'Duke' seedling rootstocks. The block of 100 trees was divided randomly into four blocks of 25 trees each, for the imposition of varying irrigation regimes. Due to the existing layout of the orchard, the limitations on irrigation equipment and management considerations, it was not possible to randomise further. The resulting restrictions on the interpretation of statistical analysis must be borne in mind, but it should also be remembered that a fairly small block of uniform soil, trees of the same age and of the same scion source, and a random allocation of irrigation regime were used. Spacing of the trees was 7,5 m x 7,5 m, with a vacant row between each block. In order to minimise the influence of other irrigation treatments or tree competition on tree and fruit physiology (Chapter 3), no data from outside rows were utilized.

The experimental site was on the Burgershall Research Station, 65 km north of the authors headquarters at the Citrus and Subtropical Fruit Research Institute, Nelspruit. The station is at 25° 7' south latitude, and an altitude of 720 m. The mean monthly maximum temperature is 25°C with the highest in January (27,8°C). The mean monthly minimum is 14,7°C with the lowest in July, at 10,5°C). The highest mean monthly evaporation is 6,2 mm per day in December, with an

annual mean of 4,9. The mean relative humidity at 14h00 is 49%. Soils are described as a Hutton form of the Doveton series (approximately 40% clay), and have an orthic A and a red apedal B horizon. Figure 2 shows the mean monthly rainfall.

Supplementary irrigation regimes were imposed during the 1981 season, to allow for possible tree adaptation before any data was collected. Apart from a dryland treatment where no supplementary irrigation was given, irrigation regimes were based on soil moisture tension. Soil moisture was determined by two groups of two tensiometers per block, with the ceramic cups at 300 and 450 mm depth just inside the drip line on the north-west side of the trees. A mean of the daily 300 mm depth tensiometer readings was used for irrigation treatments, which were implemented as follows:-

- 1) Irrigation at 35 kPa soil moisture tension.
- 2) Irrigation at 55 kPa soil moisture tension.
- 3) Irrigation at 80 kPa soil moisture tension.
- 4) Dryland.

The 450 mm tensiometers were used to indicate the moisture regime in the lower half of the expected wetted profile. In each case, sufficient water was applied by a microjet system delivering $120 \text{ l h}^{-1} \text{ tree}^{-1}$ to bring the soil to field capacity at 600 mm depth. This was calculated in accordance with known soil moisture retention curves, and amounted to 360 l, 600 l and 660 l per tree for the 35 kPa, 55 kPa and 80 kPa regimes respectively. According to the avocado pot experiments of Bower et al. (1979), irrigation at 80 kPa soil moisture tension should have induced moisture stress, with 55 kPa being adequate. The 35 kPa tension treatment should have allowed very little soil drying, but at the same time would hopefully avoid an increased Phytophthora cinnamomi risk,

said by Sterne, Zentmyer & Kaufmann (1977) to occur at soil moisture tensions lower than 25 kPa.

The irrigation regimes were aimed at applying the same total quantity of water to each supplementary irrigation treatment but at varying frequencies. In practice, however, some variation occurred due to the occurrence of rain. During the approximately 2 years of study, the 35 kPa treatment received 38 supplementary irrigations, the 55 kPa 24, and the 80 kPa regime 20. The total quantity of water applied was highest in the 55 kPa treatment and lowest in the 80 kPa, but only by the equivalent of two 80 kPa irrigations.

Where irrigation treatments are shown in figures and tables, they are as described in this section.

2.1.1.1.1 Osmotic adjustment

The effects of long-term stress which may result in osmotic adjustment can best be studied by examining the components of water potential, and checking in particular for changes in osmotic potential, especially at turgor loss point. The pressure-volume technique is, according to Kyriakopoulos & Richter (1981), a suitable method. Richter, Duhme, Glatzel, Hinckley & Karlic (1980) defined pressure-volume as "the graphical display and analysis of data relating changes in water potential to changes in cell, tissue or organ content."

There are a number of variations in pressure-volume technique in the literature. The most common is that based on the pressure chamber method of Scholander, Hammel, Bradstreet & Hemmingsen (1965). Other methods include thermocouple psychrometry (Talbot, Tyree & Dainty, 1975) and other less used techniques such as equilibration with solutions of known osmotic pressures. It is also possible to draw pressure-volume curves in two ways, either as water potential⁻¹

against water loss or relative water content, or as relative water content⁻¹ against water potential. Theoretically, these should give the same result, based on Boyle's law where osmotic potential x volume = constant. This describes a rectangular hyperbole (Noy-Meir & Ginzburg, 1967) and is the pressure-volume curve. However, in practice these are not equal, due primarily to the existence of bound (apoplastic) water (Richter et al., 1980). This is water separate from that in the vacuole and is considered to be part of cell walls, or bound to macromolecules (Tyree & Hammel, 1972). These authors discuss the theory of the pressure-volume technique. Tyree & Richter (1981), discussing the method of drawing pressure-volume curves, concluded that the water potential⁻¹ vs water loss is the most suitable. The latter option was therefore chosen for this work.

The method chosen for this investigation was the pressure chamber technique of Scholander et al. (1965), as described by Tyree & Hammel (1972) and modified for single leaves instead of twigs by Cheung, Tyree & Dainty (1975). Wilson, Fisher, Schulze & Dolby (1979), comparing the pressure-volume technique using a pressure chamber, with dewpoint hygrometry, found the two methods agreed well for osmotic potential at full and zero turgor. The pressure chamber was, however, more reliable for determining bound water and modulus of elasticity. For these reasons and ease of operation and availability, the pressure chamber was chosen.

Twigs containing a number of leaves were cut from a date tree randomly selected in the treatment being tested. A plastic bag was placed over the leaves for a few minutes. The bag, containing leaves still attached to the twig, was sealed and taken to the laboratory, situated approximately 300 m from the orchard. This was to ensure a minimum amount of water loss before measurement, approximately 5 minutes after picking. Leaves were not rehydrated to maximum turgor before

measurement. This decision was in accordance with Wenkert, Lemon & Sinclair (1978), who concluded that results would be more realistic without rehydration. Leaves were picked from the northern sides of the trees, and were representative of overall leaf condition. In the case of a new flush, leaves were fully expanded. Leaves were, where possible, only picked shortly before irrigation, although this was not always possible during periods of high rainfall. Leaves were picked at approximately 09h00, so as to avoid the possibility of mid-day stress.

A single leaf was removed from the twig and sealed in the pressure chamber after placing the petiole through a rubber bung in the chamber top. The hole in the bung was just large enough for the petiole not to be loose, but at the same time not excessively tight. To ensure sealing, "Prestik" was placed around the petiole and the hole in the bung. The technique of Tyree & Hammel (1972) was then followed. The pressure then slowly increased, until the first sign of free water at the petiole surface. This was regarded as the first balance pressure recorded. An over-pressure of about 300 kPa was then applied, and the sap which was forced out of the petiole at this pressure collected, using a roll of filter paper in a small bottle placed over the petiole. The filter paper absorbed the water from the petiole surface, and by weighing the bottle and filter paper before and afterwards (sealed to prevent water loss) on a "Sartorius" electronic balance reading to four decimal places, the mass of water forced out of the leaf could be determined. The mass was then converted to volume.

Once no further water was released from the leaf, pressure was decreased to below the balance pressure before being increased to a new balance pressure, (second balance pressure) which in practice was nearly equal to the previous over-pressure. Once more, an over-pressure of about 300 kPa was

maintained, until no further water was forced out of the petiole at that pressure, and sap mass recorded. The process was repeated until a pressure of approximately 5 000 kPa was reached. A curve of the inverse of pressure vs accumulated volume was plotted in accordance with Tyree & Hammel (1972), and used for the determination of various parameters to be discussed below. After the collection of all results the leaf was removed, weighed, dried to constant mass and then reweighed. It was also noted that at pressures greater than approximately 5 000 kPa, deviations in the linear portion of the pressure-volume curve occurred, possibly due to cell damage.

Pressure-volume curves were first plotted in October, 1982, and continued at intervals until July, 1984. An attempt was made to evaluate the degree of acclimation before the normal summer rains and during fruit set (October); after the spring flush had hardened and normal rains had begun (January); during winter (June) and the early flowering period (August). The most comprehensive results pertain to the 1982/83 season, as most of the fruit physiology investigations were conducted on this fruit. Leaves from all four irrigation treatments were tested, except early in the sequence of experiments, where the 35 kPa soil moisture regime was omitted, as at this stage it was not thought that this treatment could show more acclimation than the 55 kPa regime.

2.1.1.1.1 Calculation of results

Once the characteristics of the pressure-volume curve were known (see Fig. 1 for a typical curve), a linear regression was performed on the linear portion of the curve. If this curve is described by the linear curve $1/P = A + BV$, then the point of interception on the Y-axis describes the inverse of the osmotic pressure at the start, or $1/A$. The point of inflection (as determined by deviation from the linear curve)

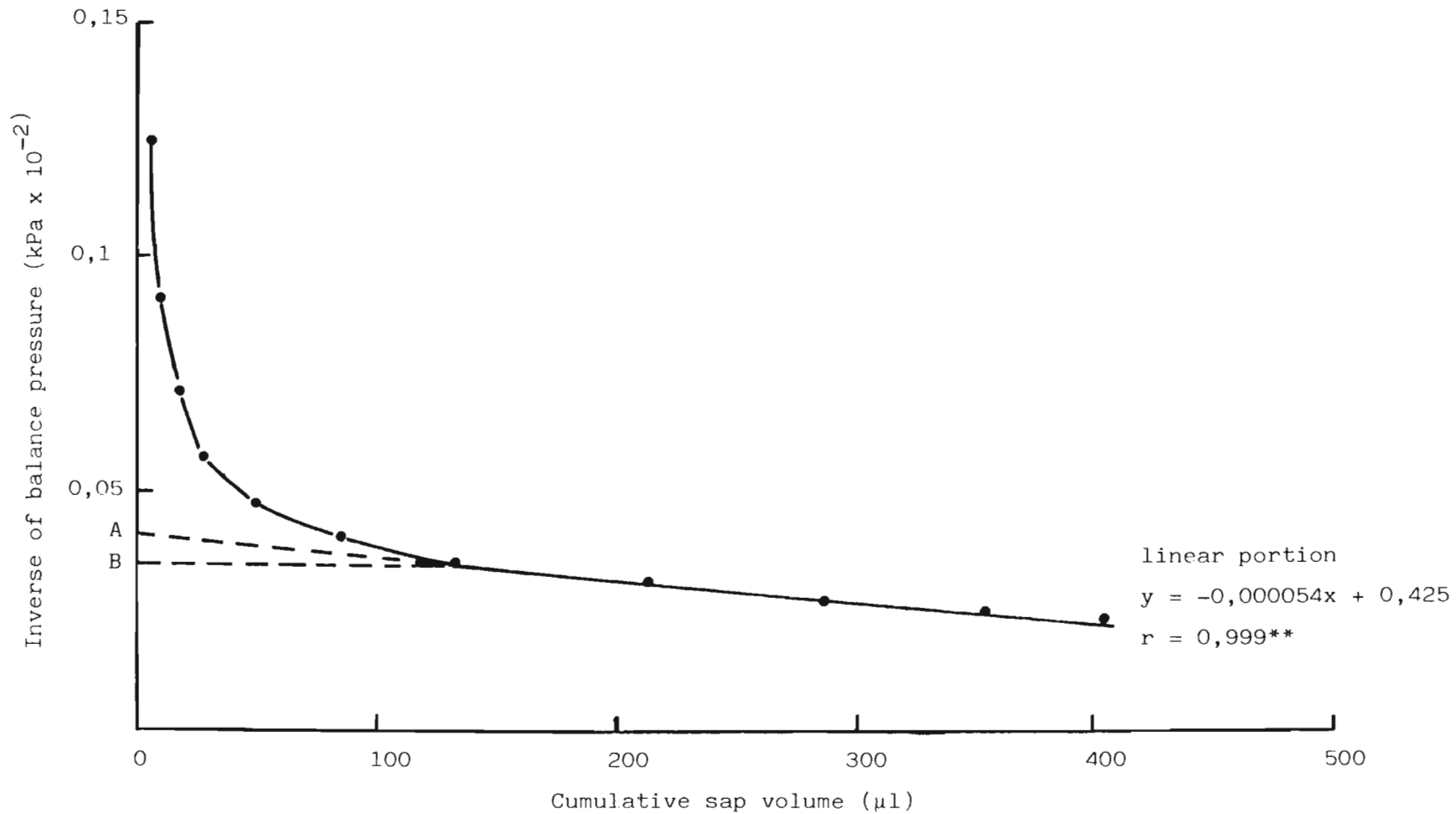


Fig. 1 Example of a pressure-volume curve. The curve depicts a dryland leaf during January, 1984. Point A=inverse of osmotic pressure and E=inverse of osmotic pressure at zero turgor

read on the Y-axis (B in Fig. 1) is the inverse of osmotic pressure at zero turgor (Tyree & Hammel, 1972).

2.1.1.1.2 Leaf water potential

Leaf water potential measurements were made at regular intervals (the majority were monthly) from September, 1982 through January, 1984. These were taken to augment the osmotic adjustment work in describing the stress background of the trees, particularly during fruit development.

Five data trees were randomly selected from each irrigation treatment, and a healthy leaf selected from the northern side of each. Measurements were done between 09h00 and 10h00, to avoid the effects of temporary afternoon stress. Where possible, measurements were made just prior to irrigation as explained in the osmotic adjustment section. Leaves were chosen from a height of approximately 1,6 m to minimise the effects of water gradients within the trees (Miller & Denmead, 1976). Thereafter the pressure chamber technique of Scholander et al. (1965) was used, in a manner similar to that of Rutherford & de Jager (1975). The leaf was placed in the pressure chamber as described in the osmotic adjustment work (section 2.1.1.1.1) and the pressure increased slowly, as too rapid an increase can result in cell bursting (Baughn & Tanner, 1976). The xylem pressure potential was taken as representative of leaf water potential when sap first appeared at the cut surface of the petiole.

2.1.1.2 RESULTS AND DISCUSSION

In order to gain a better perspective of the tree water relations results, in particular the seasonal trends in rainfall data in Fig. 2 should be consulted.

2.1.1.2.1 Osmotic pressure

Fig. 1 represents an example of a pressure-volume curve constructed to obtain each result in the osmotic adjustment study. Tyree (1976) concluded that high osmotic pressure indicates a low solute potential (accumulation of solutes), and this should impart drought tolerance. The osmotic pressure at zero turgor would indicate the lowest water potential which a cell can tolerate before serious damage occurs due to plasmolysis. While this occurs at a water potential more negative than would normally cause stomatal closure, there may well be a correlation with drought tolerance. If this is so, then this parameter would be of particular interest. Studying the trends of these parameters throughout the year should indicate the extent to which acclimation may have taken place.

The trend in leaf osmotic pressure for the four irrigation regimes is indicated in Fig. 3. Results for the 35 kPa regime were only taken once it was realised that this treatment could have been inducing some differences as compared to the 55 kPa treatment. The discussion will therefore concentrate on the other three regimes. The most notable overall feature is that the dryland treatment had, during most of the period studied, a higher osmotic pressure than other treatments, particularly the 55 kPa soil moisture irrigation regime. Taken through the season, Fig. 3 shows that in October, 1982, the dryland treatment had a considerably higher osmotic pressure than either the 55 kPa or 80 kPa treatments. This trend continued until the following June during a season of

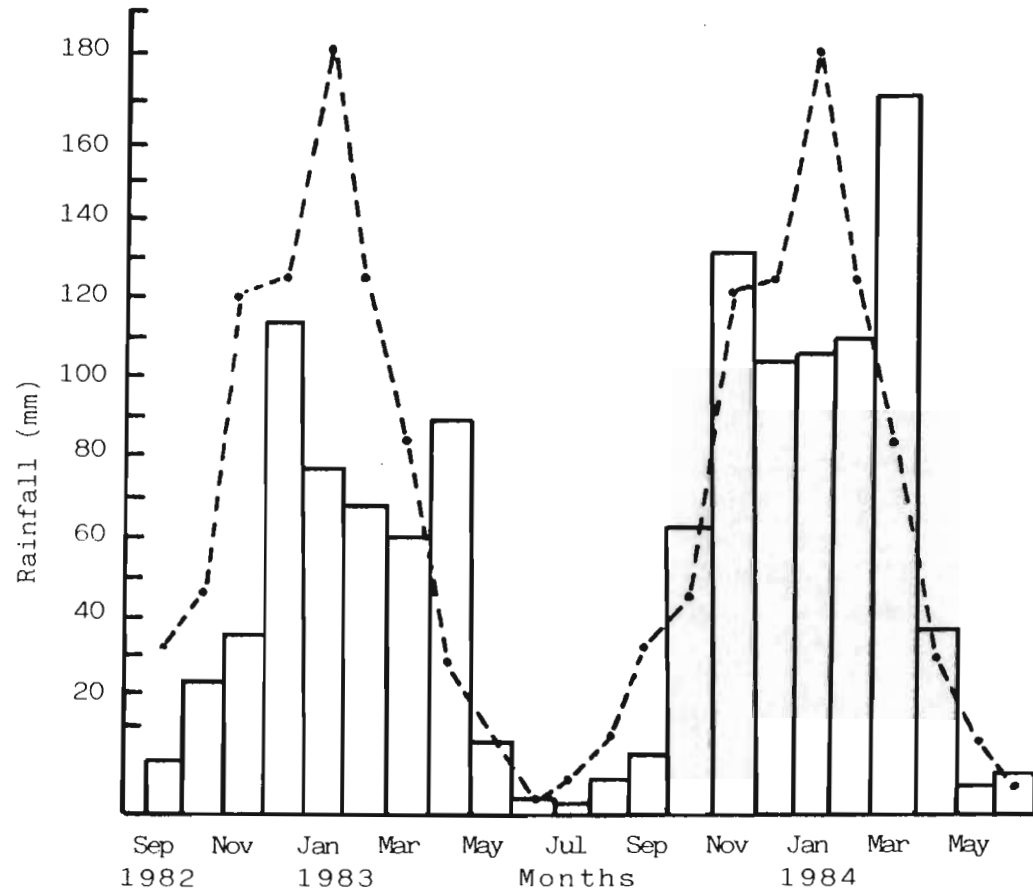


Fig. 2 Monthly rainfall at Burgershall Research Station for the period September 1982 to June 1984. Dotted line indicates long-term mean

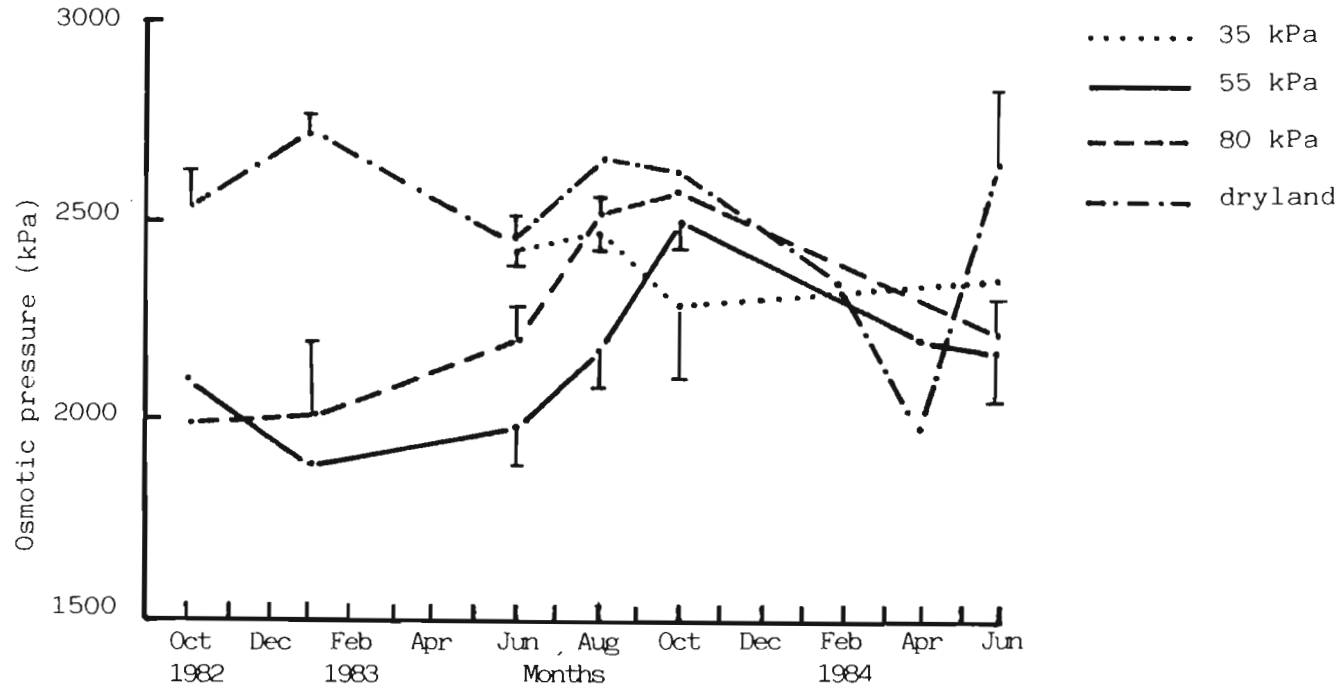


Fig. 3 Changes in avocado leaf osmotic pressure during three seasons for four irrigation regimes. Bars indicate S.E. of means. Where not shown insufficient data available

below average rainfall. The 55 kPa regime showed almost no increase during this period, while only a small increase occurred in the 80 kPa regime. The osmotic pressure of dryland leaves, although decreasing probably in response to summer rainfall, was still almost 450 kPa higher than the 55 kPa treatment in June, 1983. The avocado literature does not give an indication of whether these osmotic pressure differences actually constitute osmotic adjustment or not. However, Oosterhuis & Walker (1982) obtained similar actual values and differences between irrigated and stressed wheat, and concluded that adjustment had taken place. Fanjul & Rosher (1984) considered 300 kPa to be significant adjustment in apples.

After June, 1983 an overall increase in leaf osmotic pressure occurred in almost all treatments, until October. Osmotic pressure of dryland leaves remained highest, although differences between this and other treatments decreased considerably. The 35 kPa treatment showed an opposite trend, the reason being unclear. The reason for higher osmotic pressures than October the previous year may lie in leaf age. The October, 1983 results were conducted on old leaves, the normal spring flush being later than the previous year, resulting in the new flush still being too soft for consistent results. Wenkert *et al.* (1978) found soya bean leaves to show a similar trend with leaf age. An overall decrease in osmotic pressure occurred through the summer of 1983 to 1984. An examination of the rainfall for this period could explain this trend, particularly for dryland trees.

It was noticeable that osmotic potential seemed to alter rapidly in response to rainfall. A decrease occurred in the dryland treatment between February and April, 1984, and it can be seen from Fig. 2 that March rainfall was particularly high. Rainfall decreased considerably from April, and by June osmotic pressure had increased in the dryland treatment while

showing virtually no change in the 55 kPa regime leaves, resulting in a difference of approximately 500 kPa.

From these results there is an indication that little or no acclimation to stress occurred in a well irrigated (55 kPa) treatment under the conditions of this field experiment. A limited change occurred in trees irrigated to a lesser or greater extent, while considerably more acclimation, according to the criteria of Fanjul & Rosher (1984), resulted in dryland trees, although this was liable to alter in response to rain. Morgan (1984) does, however, mention that turgor pressure results should be cautiously interpreted, due to the effect of cell water changes on turgor pressure.

2.1.1.2.2 Osmotic pressure at zero turgor

The osmotic pressure at zero turgor is perhaps an even more important parameter of acclimation, in that cellular changes protecting the cells from plasmolysis could be indicated. Fig. 4 indicates the changes which took place from October, 1982 through June, 1984.

A very similar but more pronounced trend was apparent than shown by the osmotic pressure. The dryland treatment, except during the autumn of 1984, again showed the highest osmotic pressure at zero turgor. On the other hand, the 55 kPa treatment showed a lower osmotic pressure at zero turgor, even for the old leaves analysed in October 1983. Osmotic pressure at zero turgor decreased in response to summer rainfall in the case of dryland trees, but not completely during the 1982/83 summer. The higher rainfall early in 1984 did, however, appear to reduce acclimation in this treatment. Once rainfall decreased in autumn, stress presumably re-appeared and rapid acclimation once again occurred in the dryland treatment, which was not the case for the other treatments which received additional irrigation. Due to the

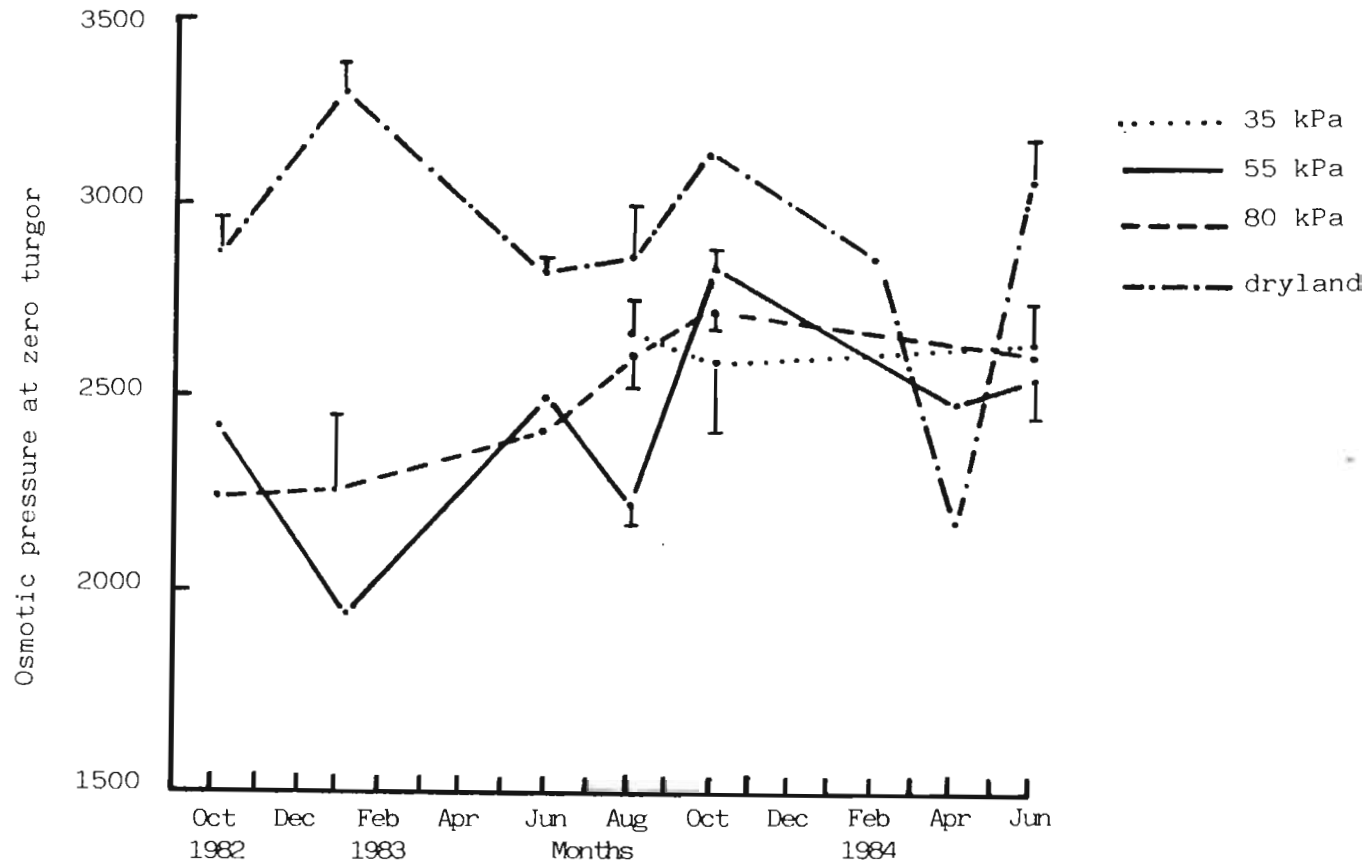


Fig. 4 Changes in avocado leaf osmotic pressure at zero turgor during three seasons for four irrigation regimes. Bars indicate S.E. of means. Where not shown insufficient data available

effects of leaf age and other unknown factors, caution is necessary in interpreting results of a particular treatment over time. Nevertheless, when comparing treatments, there is evidence that dryland conditions do result in some stress acclimation during winter at Burgershall, a relatively high rainfall area. Under-irrigation (80 kPa) is likely to cause adverse effects in that due to a lack of overall acclimation, such trees will be susceptible to short-term transient stress between irrigations. However, substantial rainfall appears to rapidly decrease acclimation where it occurs, and if followed by a dry period, substantial water stress with its physiological consequences would be likely. This situation would be damaging if it happened during a critical phase of fruit development.

Alterations in acclimation appeared to follow water availability by one to two months. Trees will only be protected from physiological damage after stress has occurred, and it should be noted that flowering and fruit set normally take place between mid-August and mid-October, which is normally a stressful period. Trees which have had some irrigation (such as the 80 kPa regime) through winter would not acquire any marked osmotic adjustment under Burgershall conditions, and would be sensitive to harsh environmental conditions at that time.

2.1.1.2.3 Leaf water potential

The seasonal leaf water potential results from September, 1982 through January, 1984 are shown in Fig. 5. If small fluctuations which could be due to rain or irrigations are ignored, then two characteristics of the data are apparent.

Firstly, a clear seasonal trend is visible. During the dry spring of 1982, leaf water potentials generally increased considerably. Once significant summer rainfall (Fig. 2)

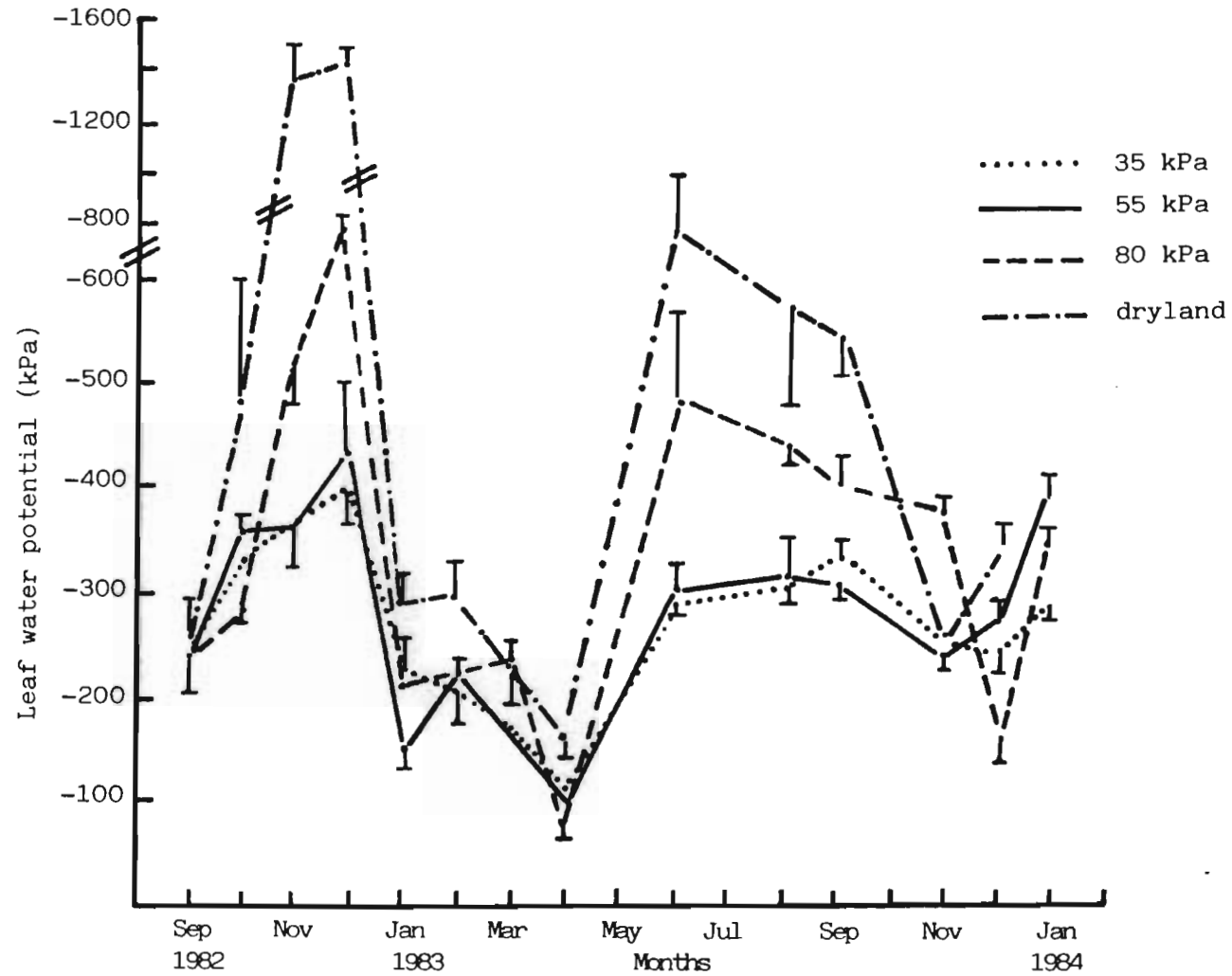


Fig. 5 Changes in leaf water potential during two seasons for four irrigation regimes. Bars indicate S.E. of means, five leaves per treatment mean

occurred, leaf water potentials rapidly became less negative (more favourable) and remained so until autumn, whereafter they once again became more negative, the cyclical response to overall rainfall again being exhibited. The pattern is helpful in explaining the fruit physiology results (Chapter 3). There is a clear indication, particularly for the 1982/1983 season, that the period of maximum tree water stress occurred during the early fruit development phase in spring, with considerably lower stress levels during the latter part of the fruit development period.

Secondly, the data can be divided into two groups, with the dryland and 80 kPa treatments in the one, and the more frequently irrigated 55 kPa and 35 kPa treatments in the other. During the spring and early summer of 1982 (also important in the context of fruit physiology results in Chapter 3) the dryland and 80 kPa treatments showed considerably more negative leaf water potentials than the 55 kPa and 35 kPa treatments. With summer rainfall, this difference diminished, but once again became apparent during the 1983 spring. Thus, trees of the dryland and 80 kPa treatments indicated higher stress levels during the early fruit development period than did trees with more frequent irrigation. These differences were not evident during the late summer and autumn, presumably due to rainfall effects.

2.1.2 DIURNAL WATER RELATIONS

2.1.2.1 MATERIALS AND METHODS

An examination of diurnal trends in leaf water relations should enable some evaluation of the degree to which trees react to environmental conditions, the tree water stress condition and the potential demand for water. While it is probably ideal to evaluate these parameters under varying soil moisture and environmental conditions, suitable equipment was not, until recently, available. It was therefore decided to select a typical summer day, which would, hopefully, provide sufficient but not excessive environmental stress.

Leaf water potential, stomatal resistance, transpiration and environmental conditions (in the form of saturation deficit) were evaluated at 2-hourly intervals between 07h00 and 17h00 on 10 January, 1984. Tensiometer readings at 300 mm depth were 36 kPa, 32 kPa, 47 kPa and 66 kPa for the 35 kPa, 55 kPa, 80 kPa and dryland treatments respectively. While soil moisture tensions were not at their respective treatments limits due to some rain the previous week, differences nevertheless existed. Two winter days, one in 1984 and another in 1985 were chosen to evaluate lower environmental stress combined with decreased soil moisture. It was necessary that each day, as far as possible, be cloudless, as clouds interfere considerably with transpiration characteristics. For the winter days, readings were increased to hourly intervals, but leaf water potential was not determined as this parameter was found in the summer readings to be less useful. In order to clarify the effects of winter water stress conditions, particularly in terms of acclimation, on the water relations of trees during spring when the early period of fruit growth occurs, and before

normal summer rains begin, a typical warm spring day was chosen for a diurnal study of leaf water relations. Leaf water potential, stomatal resistance and transpiration were evaluated during a day in October, 1985 at 2-hourly intervals between 07h00 and 17h00, for the 55 Kpa, 80 kPa and dryland treatments. Single days of diurnal readings were considered sufficient to illustrate the reactions of the trees to the environment, as evaluating several days would not necessarily improve accuracy. Each day is different, and one cannot take a mean of several days as this would give a meaningless set of data and obscure specific responses. It is thus more appropriate to take a particular day and relate the plant reactions to the particular environmental conditions of that day. This is in accordance with the work of Scholefield et al. (1980).

2.1.2.1.1 Stomatal resistance and transpiration

Stomatal resistance and transpiration were measured using a Licor "LI 1600" steady state porometer. This instrument has a number of advantages over the transient porometers of the type designed by Kanemasu, Thyrtell & Tanner (1969). These are ease of operation; accuracy due to the steady state conditions surrounding the leaf, which can be held at near atmospheric conditions; a factory calibration which need only be checked periodically; and a microprocessor evaluation of input variables to give a direct readout of stomatal resistance, transpiration rate, leaf temperature and relative humidity in the cuvette.

Five data trees from each treatment were randomly chosen, and a leaf from both the northern and southern sides of each tree was measured on each occasion. Mature leaves in the sun were randomly selected from a height of approximately 1,6 m (shoulder height) in order to minimise the possibility of errors caused by water gradients within the trees (Miller &

Denmead, 1976). In the case of the spring, 1985 day, five leaves chosen from the northern side of the data trees were used. After attaching the porometer cup to the leaves for measuring stomatal resistance of the underside of the leaves, steady state conditions were obtained, and stability in readings allowed to occur, according to the instructions of the manufacturer. Readings were then recorded.

2.1.2.1.2 Leaf water potential

Leaf water potential was measured using the pressure chamber technique of Scholander et al. (1965), in a manner similar to that of Rutherford & de Jager (1975). After measurement of stomatal resistance and transpiration parameters, the leaf was picked and immediately placed in the pressure chamber as described in section 2.1.1.1.2.

The use of pre-dawn water potential is said to be a useful means of estimating the ability of the plant to regain normal turgor at night. Covered leaf water potential was shown by Meyer & Green (1980) to be a suitable estimate of pre-dawn water potential, at the same time eliminating the problems of pre-dawn field measurements. Leaves were accordingly enclosed in aluminium envelopes during the day preceding measurement, so as to cause stomatal closure and to allow regaining of leaf water content. Aluminium foil was used for its reflective properties, thus protecting the leaves from solar radiation and temperature increase which could have resulted in leaf damage. Shortly after dawn on the day of measurement, leaves were cut from the trees, removed from the foil envelopes and placed immediately in the pressure chamber. Measurements were then taken as previously outlined in section 2.1.1.1.2.

2.1.2.1.3 Environmental stress parameters

Environmental conditions, being the driving force for transpiration, must be taken into account. At the start of each measuring period, relative humidity and air temperature (shade) were measured. The results were then combined to form the saturation vapour pressure deficit, otherwise known as the saturation deficit (SD) defined by de Bruin & Holtslag (1982) as:

$$SD = e_s - e \quad \text{where:}$$

e_s = saturated vapour pressure at ambient temperature

e = ambient vapour pressure.

In practice, SD is calculated (Schulze, 1965) as:

$$SD = SP - RH(SP)$$

where: SP = saturated vapour pressure at ambient temperature

RH = relative humidity.

Saturated vapour pressure over pure water at ambient temperature was obtained from the Smithsonian meteorological tables (List, 1958).

2.1.2.2 RESULTS AND DISCUSSION

2.1.2.2.1 Summer trends

The results of the diurnal trends in leaf water potential, stomatal resistance and transpiration, should be seen in terms of the atmospheric conditions as indicated by the saturation deficit (SD), air temperature and relative humidity at the time of measurement (Fig. 6). As no differences were found between the northern and southern side leaves by T-test, data was combined.

2.1.2.2.1.1 Leaf water potential

Pre-dawn water potential was essentially similar for all treatments except the dryland, which was approximately 100 kPa more negative. This indicates dryland trees were less able to regain leaf water potential during the night than the other treatments. Whether this difference can be considered to be physiologically significant or not, is debatable, and it appears that soil water at the time of measurement was not sufficiently low to cause a marked tree water stress in any of the treatments.

The changes in leaf water potential during the day are shown in Fig. 7. Overall, there was a slight decrease (less negative) in water potential between 07h00 and 09h00, followed by an increase to approximately mid-day, followed by a decreasing trend towards evening. This trend can be seen as an indication of net water loss from the leaves during the day. This forms a background to understanding the physiological effects on the leaves, as indicated by stomatal resistance and transpiration. Changes in the latter can also affect leaf water content, which can be reflected in sudden changes in water potential. The overall pattern was also very similar to that reported for peaches by Chalmers, Olsson &

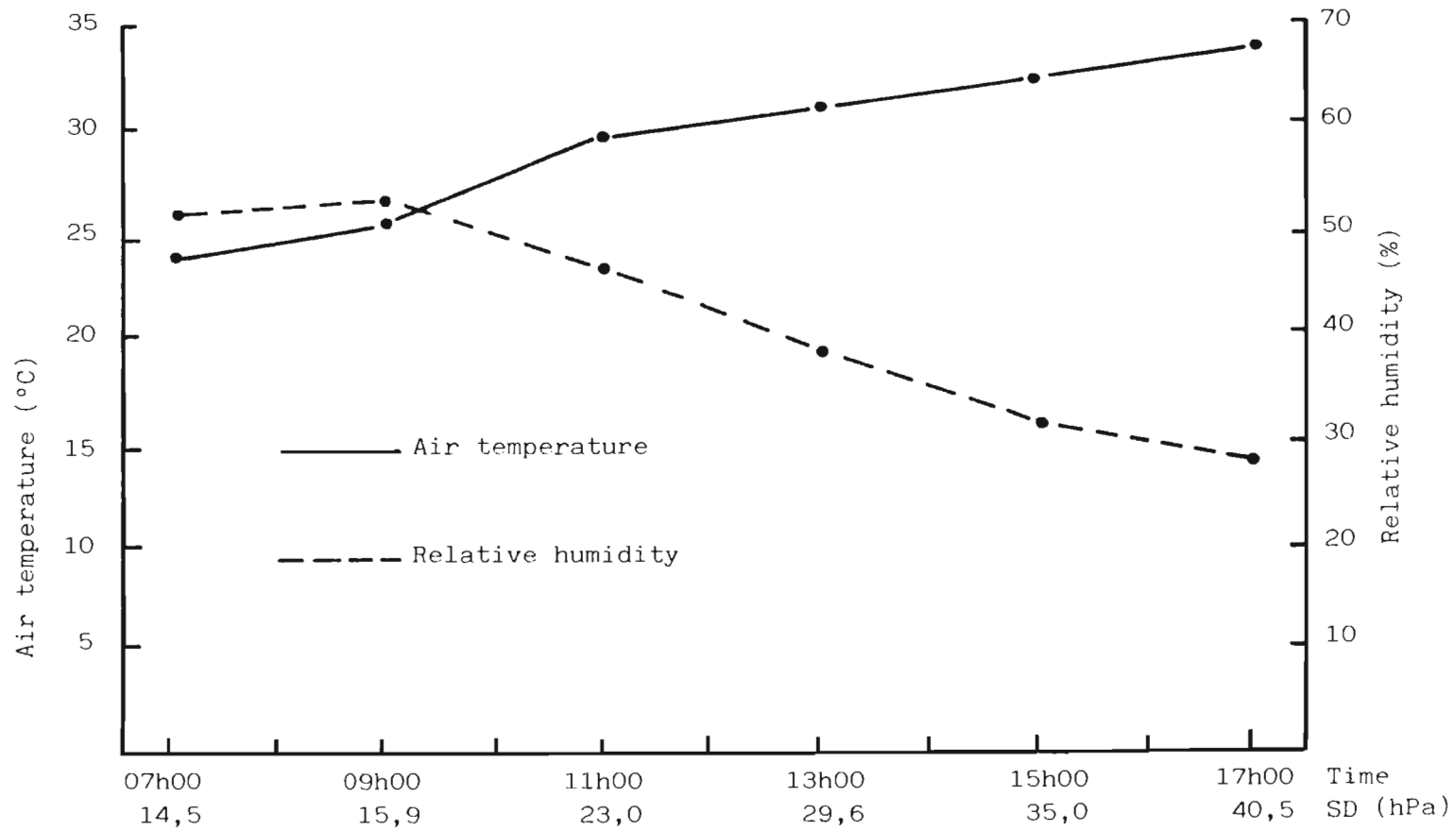


Fig. 6 Air temperature, relative humidity and saturation deficit values during the period 07h00 to 17h00 on January 10, 1984

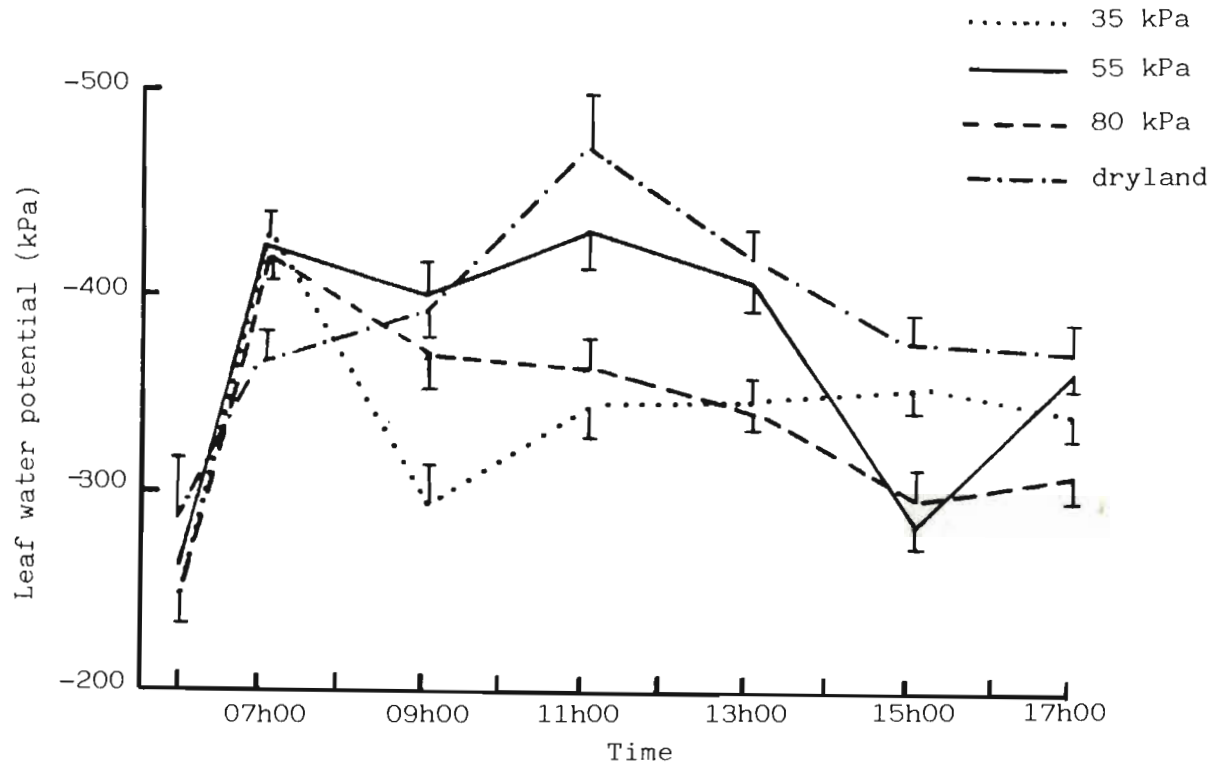


Fig. 7 Diurnal changes in leaf water potential during a summer day (10 January, 1984) for four irrigation regimes. Bars indicate S.E. of means, ten samples per mean. Results before 07h00 are pre-dawn values

Jones (1983), grapes (Smart, 1974) and avocados (Sterne, Kaufmann & Zentmyer, 1977; Scholefield et al., 1980). Water potential did not at any time become as negative as in the work of the latter authors, but was in accordance with previous work of Bower (1978). It is noteworthy that Smart & Barrs (1973) found that environmental parameters accounted for up to 96% of the variation in leaf water potential in the plants on which they worked.

The irrigation treatments also appeared to cause some differences in leaf water potential during the day. The dryland trees showed a slightly more negative water potential, except early in the morning. This will be further discussed later. The 55 kPa treatment showed similar overall results, but the most frequently watered (35 kPa) and infrequently irrigated (80 kPa) trees indicated an interesting trend. Both showed less negative water potentials than the previously mentioned two treatments. Smart (1974) found increased water potentials in poorly irrigated grape vines as opposed to non-stressed plants. This was only partly true in the results presented here, but a further explanation will be possible with reference to the stomatal resistance and transpiration discussed below.

2.1.2.2.1.2 Stomatal resistance and transpiration

The variations in stomatal resistance and transpiration are shown in Figs. 8 and 9 respectively. While interrelated, there is not necessarily a 1:1 ratio between these factors. It is therefore necessary to discuss both.

Stomatal resistance showed an increase between 07h00 and 09h00, followed by a decrease to a fairly stable condition (except dryland trees) until early afternoon, whereafter a general increase towards sunset was noted. For the dryland trees the trend was similar, but delayed. The trend between

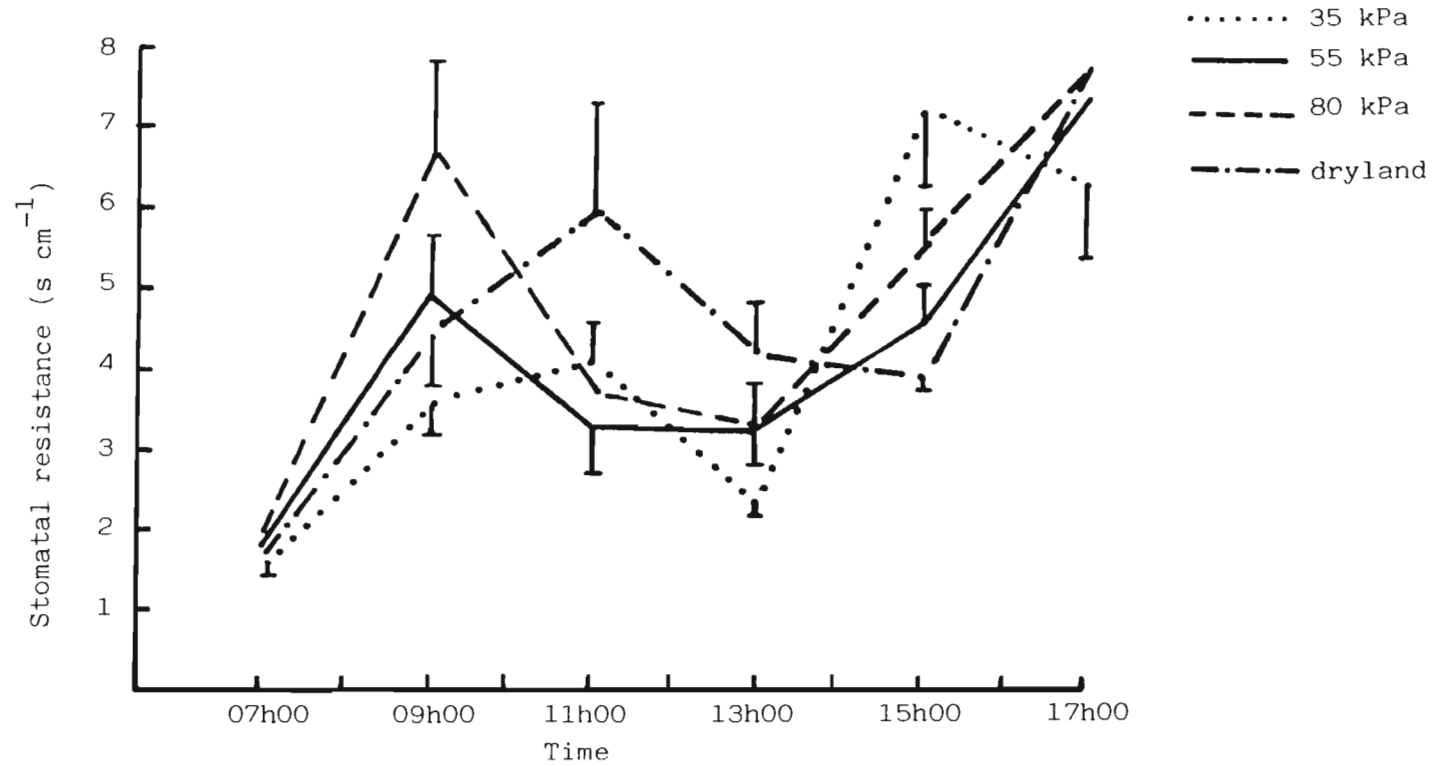


Fig. 8 Diurnal variation in stomatal resistance during a summer day (10 January, 1984) for four irrigation regimes. Bars indicate S.E. of means, ten samples per mean

07h00 and 09h00 was unexpected, as was also the case with leaf water potential. A possible explanation, however, may have been some light, intermittent cloud cover early in the morning which perhaps affected stomatal action. The partial stomatal closure during this period would explain the tendency for water potential to become less negative during this period. It would also explain the general decrease in transpiration between 07h00 and 09h00. The trees receiving the most water showed the least stomatal closure, while the poorly irrigated trees showed the most. There was some evidence of a shift in this early closure tendency in the case of dryland trees, as an early peak in stomatal resistance occurred at approximately 11h00 instead of the 09h00 which was prominent in the 55 kPa and 80 kPa regimes. The actual values of stomatal resistance were in accordance with the results of Scholefield et al. (1980).

Transpiration showed a more consistent trend during the day, and between treatments (Fig. 9) than did stomatal resistance. An initial decline in transpiration mirrored stomatal resistance. Thereafter, a steeply increasing trend from approximately $3 \mu\text{g cm}^{-2} \text{s}^{-1}$ to a maximum at approximately 13h00 of between 6 and $7 \mu\text{g cm}^{-2} \text{s}^{-1}$ occurred, followed by a decline as stomata partially closed during the afternoon. Trees in the 55 kPa, 35 kPa and 80 kPa treatments showed little difference in their pattern or actual transpiration values. Dryland trees, however, showed a less steeply increasing rate of transpiration after 09h00. The early afternoon peak value was also later, occurring at approximately 15h00 and having a lower value of $5,4 \mu\text{g cm}^{-2} \text{s}^{-1}$.

The pattern of transpiration, particularly when the effect of environment is considered, is of note. On the day in question (see Fig. 6), the SD continued to increase throughout the period of measurement, ranging from 14,5 at 07h00 to 40,5 at 17h00. Air temperature increased from $24,4^{\circ}\text{C}$

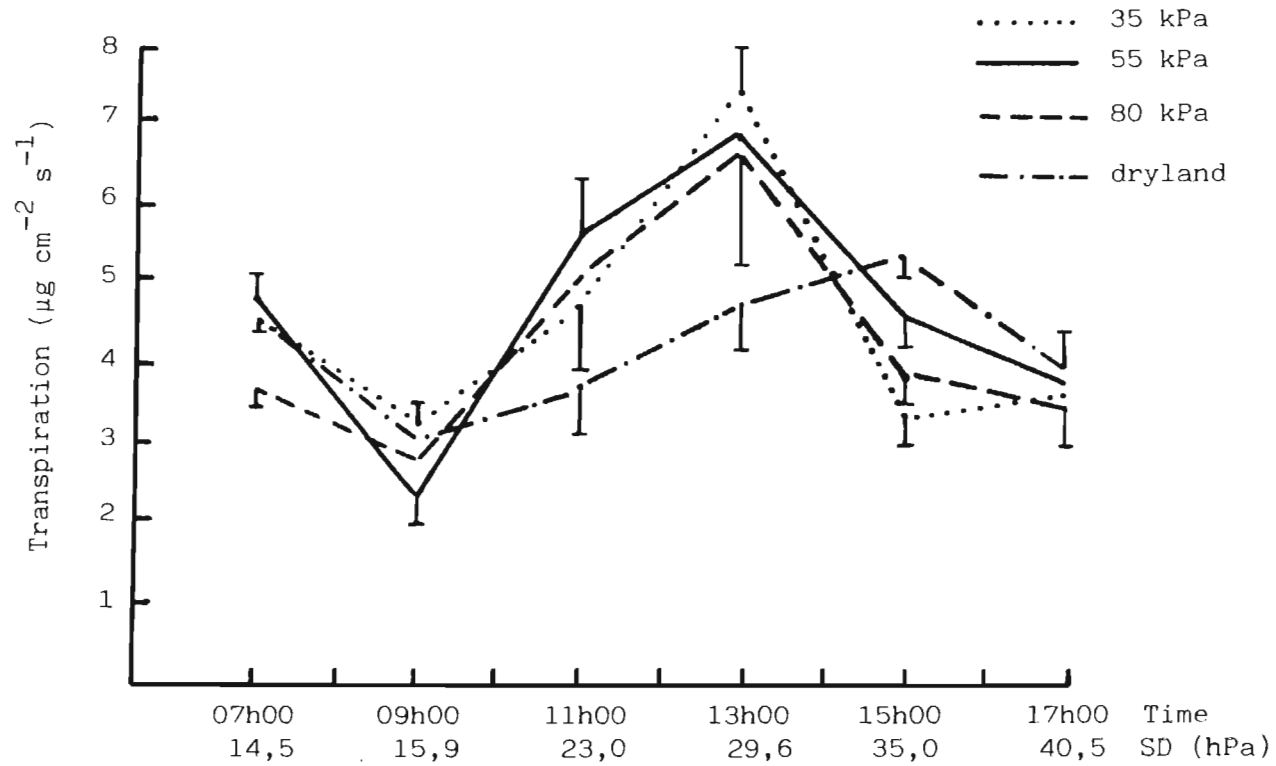


Fig. 9 Diurnal variation in leaf transpiration rate and saturation deficit during a summer day (10 January, 1984) for four irrigation regimes. Bars indicate S.E. of means, ten samples per mean

to 34,7°C and relative humidity at these temperatures decreased from 52,8% to 29,2%. Except for the early morning, the day was cloudless. Conditions could be described as typical of a hot summer day without thunderstorm activity, and it was not noticeably windy.

All the treatments except the dryland showed a similar transpirational response to environmental demand. The most salient feature was that apart from the early morning anomaly, the increasing SD resulted in increased transpiration, until the SD reached approximately 28 hPa, whereafter a decrease in transpiration rate (due to an increase in stomatal resistance as shown in Fig. 8) occurred. Increased variation between readings, particularly in the 80 kPa treatment, implied some stomatal cycling (Barrs, 1971) and therefore temporary stress. This implies that despite satisfactory soil moisture content, the avocado, cv. Fuerte on seedling 'Duke' rootstocks, cannot under normal circumstances maintain transpiration for a prolonged period at a SD of above approximately 28 hPa. At least partial daily stress can therefore be expected when evaporative demand is high, even where irrigation is satisfactory. A decrease in photosynthesis as well as an effect on the many other physiological factors discussed in the Review of Literature section could then be expected.

The dryland trees showed an interesting trend, in that due to a higher stomatal resistance during a portion of the day, the trees exhibited lower transpiration. Although a transpiration increase with increasing SD was evident and similar to other treatments, this occurred at a slower rate than the other treatments, and a peak transpiration rate of lower value occurred at a higher SD (approximately 35 hPa). This may be related to the stress acclimation discussed previously although details of stomatal control are noted by Raschke (1975) to be varied and complex.

2.1.2.2.2 Winter trends

The diurnal trends for stomatal resistance, and transpiration for two warm, very dry winter days are shown in Figs 11 and 12, and the environmental parameters in Fig. 10. Leaves showed considerable differences in terms of transpiration between the northern and southern sides of the trees. This was probably due to the southern side being in almost permanent shade, and incident photosynthetic radiation being below $100 \mu\text{E m}^{-2}\text{s}^{-1}$ for most of the day. Due to the limitation of the radiant flux density on the southern side, stomatal opening was not inhibited. This precludes discussion of the results in terms of water stress. The following discussion therefore refers to northern side leaves. Results should also be seen against a background of the soil moisture status at the time. In 1984, the 35 kPa treatment had a soil moisture tension of 16 kPa at 300 mm depth, having been irrigated five days previously. The 55 kPa treatment showed a tension of 58 kPa and was irrigated the following day, while the 80 kPa treatment had been irrigated 15 days previously and showed a tensiometer reading of 40 kPa. The dryland treatment showed a soil moisture tension of 79 kPa. This is in contrast to 1985, when tensiometer readings were 27 kPa, 50 kPa, 62 kPa and 66 kPa. It should also be noted that environmentally (Fig. 10) the 1984 day was more typical of a summer day, whilst the 1985 day was considerably cooler and more typical of winter.

2.1.2.2.2.1 Stomatal resistance and transpiration

Trends in stomatal resistance and transpiration during the day for the four irrigation regimes (northern sides of the trees) during 1984 are shown in Figs 11a and 12a respectively. Differences between the irrigation regimes and trends through the day were difficult to discern. There was a

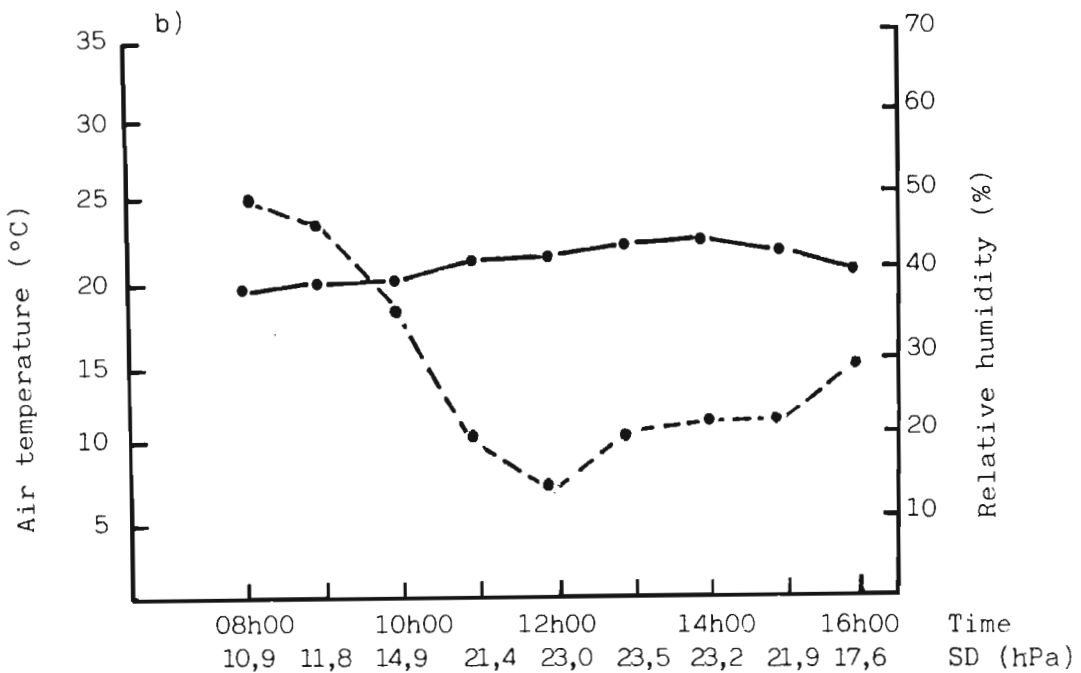
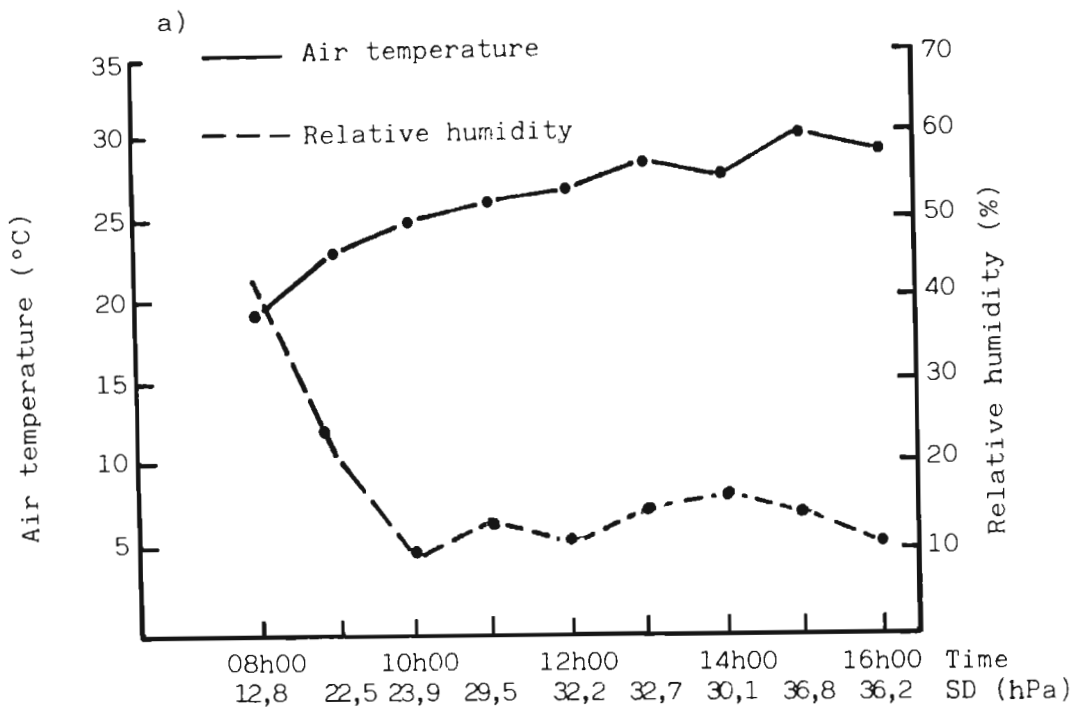


Fig. 10 Air temperature, relative humidity and saturation deficit during the period 08h00 to 16h00 for a) the winter day 1984 and b) the winter day 1985 during which diurnal transpiration and stomatal resistance measurements were made

general trend towards stomatal closure and decreased transpiration, with a fairly rapid change beginning between 12h00 and 14h00.

The lack of distinguishing features is probably due to the SD (Fig. 10a) being in the region of 30 hPa for the greater portion of the day (10h00 to 14h00). Most treatments started showing some degree of stomatal closure during this period, similar to the environmental stress level determined in the January investigation. This is shown not only by higher stomatal resistance values, but also by higher S.E.'s. The maintenance of environmental stress at this level during a large proportion of the day probably caused some stomatal cycling, thus the erratic results and lack of clear trends. It is also possible that the soil moisture levels, with the exception of the dryland trees, were not low enough to cause stress to develop from this source, hence treatment differences were not obvious. It was intended to repeat the work in July, but abnormal rainfall caused high soil moisture levels and thus treatment differences were unlikely. Nevertheless a repeat of the work in July 1985 was possible, and these results are shown in Figs 10b, 11b and 12b.

In the case of stomatal resistance, both the 35 kPa and 55 kPa treatments showed little sign of stomatal closure during the morning, remaining between 3 and 4 $s\ cm^{-1}$. This is similar to the previous year, and is normal for unstressed avocados (Bower, 1979). The temperature was not high, but relative humidity was very low (Fig. 10b). A more important trend occurred in the cases of the 80 kPa and dryland trees. Both showed partial stomatal closure early in the day, presumably indicating an inability to balance environmental water demand with uptake under dry soil conditions. However, the dryland trees showed some recovery between 11h00 and 13h00, before some degree of stomatal closure occurred during the afternoon. In contrast, the 80 kPa treatment continued

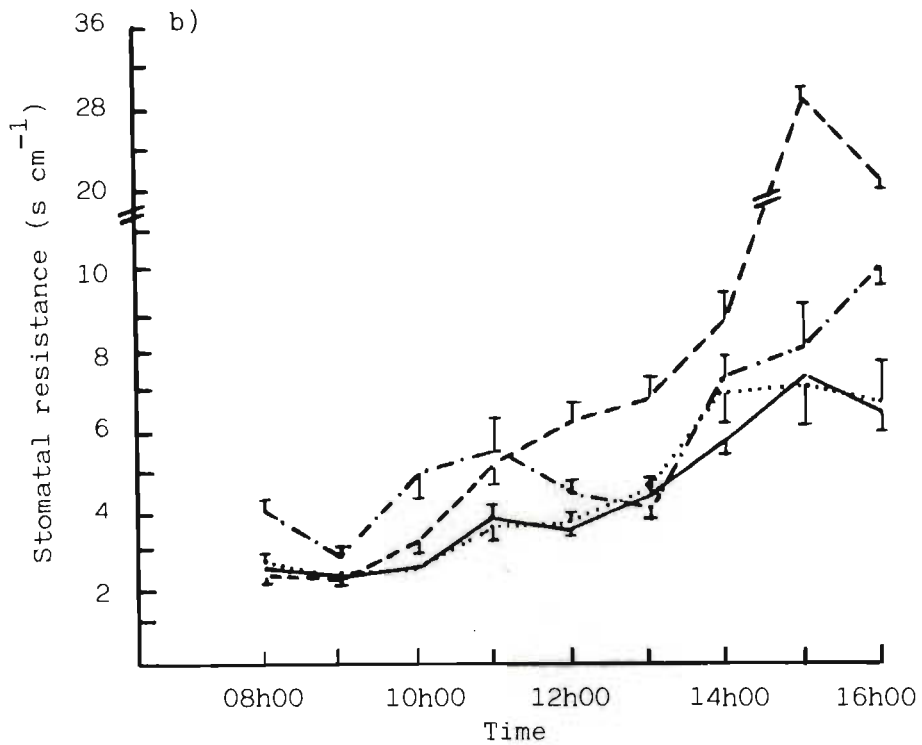
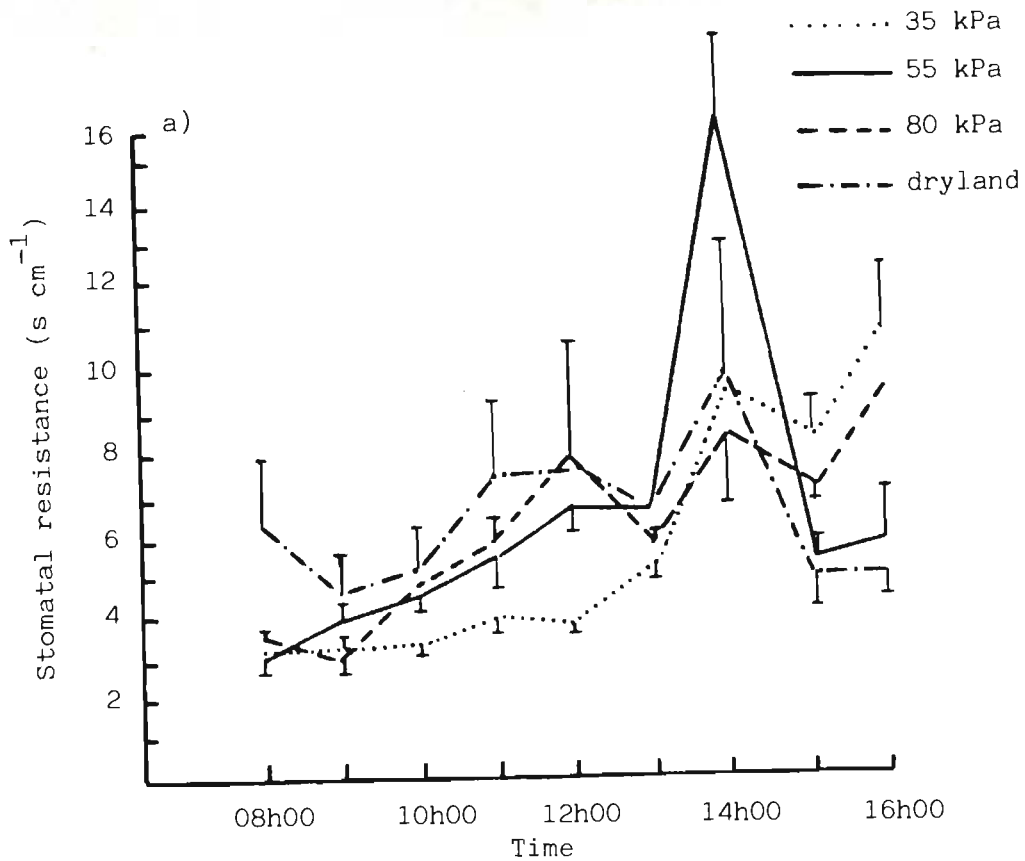


Fig. 11 Diurnal variations in stomatal resistance on the northern side of trees during a winter day in a) 1984 and b) 1985 for four irrigation regimes. Bars indicate S.E. of means, five samples per mean

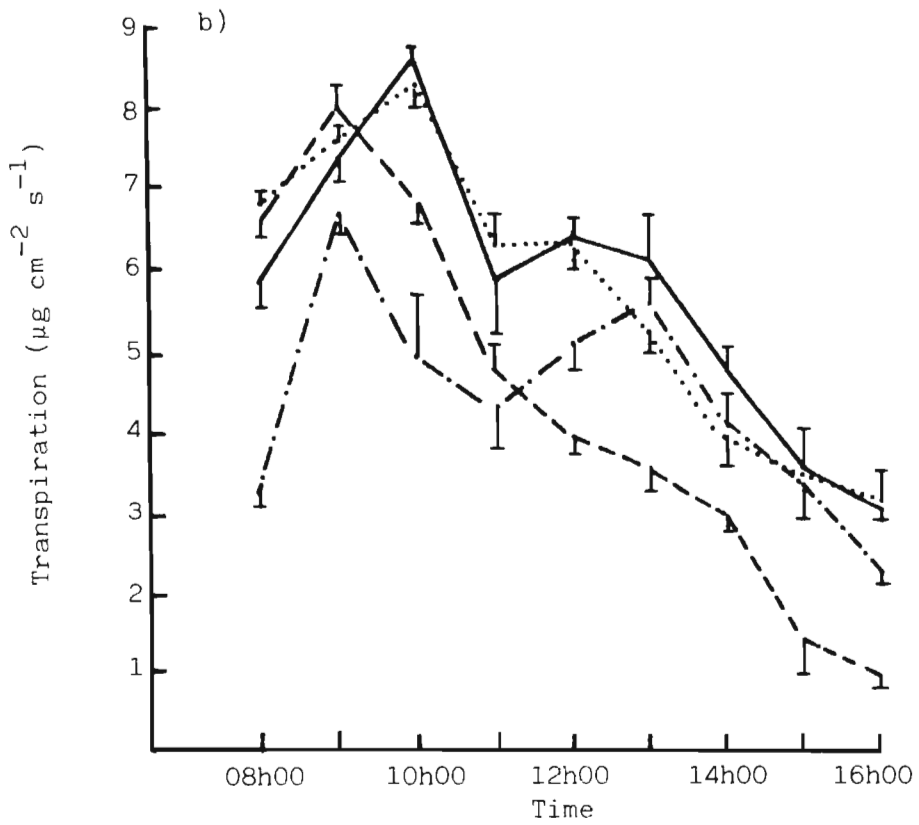
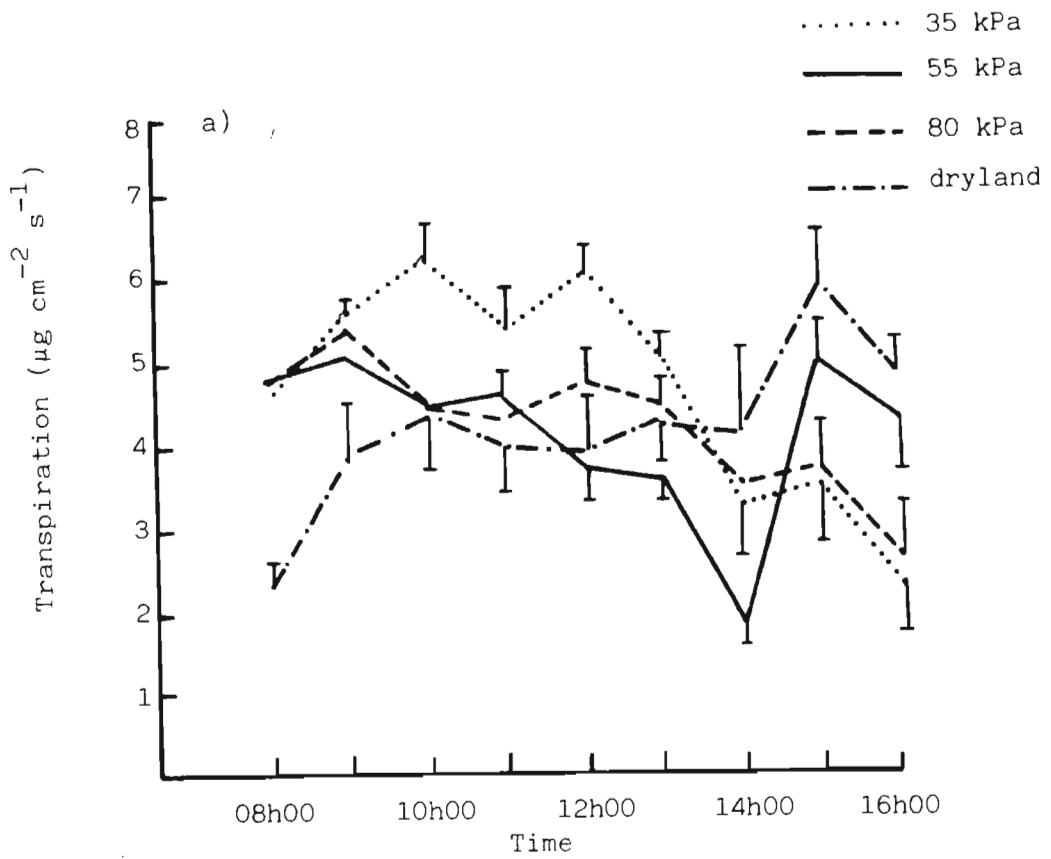


Fig. 12 Diurnal variations in transpiration on the northern side of trees during a winter day in a) 1984 and b) 1985 for four irrigation regimes. Bars indicate S.E. of means, five samples per mean

showing progressive stomatal closure from 09h00 onwards, such that virtually total closure had occurred from 15h00 onwards. There was, therefore, a strong indication that acclimation to water stress was being manifested in the dryland trees, but not in the 80 kPa treatment, thus resulting in an altered reaction to environmental stress.

The pattern of transpiration (Fig. 12b) did not, with the exception of the 80 kPa trees, show conclusive trends. This can probably be ascribed to the moderate temperatures throughout the day, with low relative humidity for the major portion of the day.

Detailed direct comparisons between the 1984 and 1985 results could not be made. Environmental conditions differed, particularly in respect to temperature and the rate at which environmental demand changed through the day.

Further investigation will be necessary to indicate the individual effects of temperature and relative humidity, which may result in a modified and more accurate index of the environmental demand producing stress in avocados, than at present possible with the measurement of saturation deficit.

2.1.2.2.3 Spring trends

Results of changes in stomatal resistance, transpiration and leaf water potential between 07h00 and 17h00 are shown in Fig. 13a, b and c.

At the time of measurement, soil moisture tensions at 300 mm depth were 17 kPa, 64 kPa and 78 kPa for the 55 kPa, 80 kPa and dryland treatments respectively.

Stomatal resistance (Fig. 13a) remained fairly constant with little difference between treatments from 07h00 until 11h00.

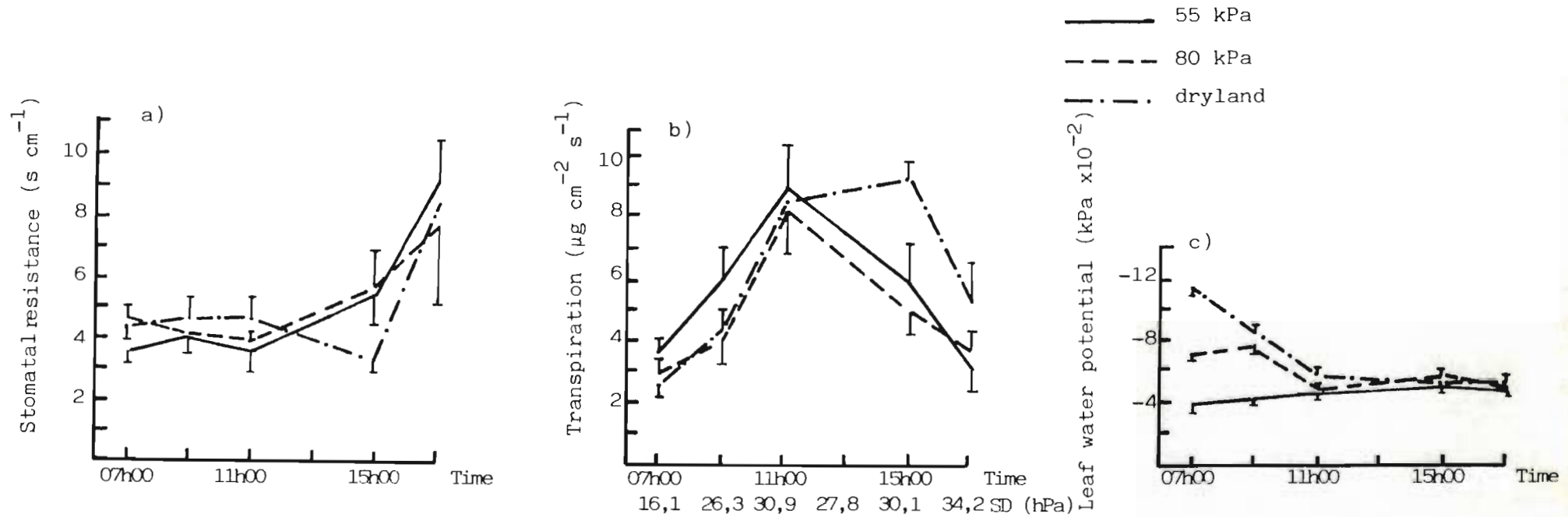


Fig. 13 Diurnal variations in a) stomatal resistance b) transpiration and c) leaf water potential, and saturation deficit during a spring day (October, 1985) for three irrigation regimes. Bars indicate S.E. of means, five samples per mean

At 13h00 cloud was present, and this resulted in considerable stomatal closure in all treatments. The cloud had begun clearing by 14h00, and normal readings could once again be taken by 15h00. At this stage, the 55 kPa and 80 kPa treatments showed increased stomatal closure as compared with earlier in the day, and this trend continued towards evening. The dryland treatment showed a similar, but delayed pattern, stomatal closure only beginning after 15h00.

Transpiration (Fig. 13b) showed a rapid increase in all treatments with increasing SD during the morning. After 11h00 the 55 kPa and 80 kPa trees showed a steady decline in transpiration towards evening. The SD value between 11h00 and 15h00 was between 28 and 30 hPa, previously found in the summer study to be a critical level of environmental stress for these treatments. With the exception of the cloudy period, the dryland trees did not show a decline in transpiration until after 15h00, when the SD increased from 30 to 34 hPa, again previously found to be critical for this treatment. These results confirm the possibility of acclimation to water stress in dryland trees during winter, but not in those receiving supplementary irrigation.

Leaf water potential was almost constant throughout the day for the 55 kPa treatment. The dryland and 80 kPa treatments were less negative early in the day, but after 11h00 closely followed that of the 55 kPa treatment. This parameter does not react as dynamically to environmental conditions as stomatal resistance and transpiration.

2.2 FRUIT WATER RELATIONS

2.2.1 MATERIALS AND METHODS

In order to gauge the fruit water status at the time of picking, it was decided to measure fruit water potential. A destructive technique could not be contemplated. Since fruit had to be used later for physiological studies, a pressure chamber method was therefore impossible. Removal of tissue samples from the fruit for psychrometric testing was also not possible. A new non-destructive technique for estimation of water potential in avocado fruit using a psychrometric method was accordingly developed.

The normal abscission zone of avocado fruit occurs on the pedicel approximately 10 to 20 mm from the fruit. The pedicel immediately adjacent to the fruit could therefore be considered a part of the fruit, and this hypothesis formed the basis of the technique. A "Wescor L51" leaf psychrometer connected to a "Wescor HR 33T" micro voltmeter was used. Calibration of the psychrometer was necessary before experimental results could be obtained. As the measurement technique was similar to that of the calibration, the latter will be described first.

The calibration technique of Savage, de Jager, & Cass (1979) and of Savage, Cass & de Jager (1981) was used. An important source of error in thermocouple psychrometry is temperature fluctuation within the hygrometer. In order to eliminate possible temperature gradients (Campbell, 1979) and validly assume that the air temperature in the chamber and other parts of the hygrometer was equal, considerable insulation was necessary. The sample chamber was placed in the centre of a 300 mm deep styrofoam box filled with styrofoam chips.

Calibration was achieved by using small filter paper discs

placed in an aluminium envelope, with a small hole cut in the envelope to expose the discs. Solutions of varying molarities of NaCl were placed on similar filter papers for measurement. The water potentials of the solutions were known, using data of Lang (1967), and multiplying by density to obtain volumetric water potential. The foil envelope was placed in the slit of the "L51" and the cylinder containing the thermocouple pressed onto it and secured with the retaining screw. To ensure a good vapour seal, a thin layer of petroleum jelly was applied to the rim of the cylinder. The entire assembly was placed in the styrofoam box and left to achieve thermal and vapour equilibrium. Thermal equilibrium was checked before each reading by switching the function switch on the micro voltmeter from short to read. When no difference was recorded, thermal equilibrium was assumed.

The thermocouple was cooled using a constant cooling time of 20s, as recommended by Savage et al. (1979). The voltage output was then observed, and the point of inflection (the plateau region or point where a zero change in voltage with time occurs) estimated by eye. It is conceded that a chart recorder such as used by Bristow & de Jager (1980) is more desirable, but it was not available. Once the voltage at the inflection point had been obtained, the results were adjusted for temperature using 25°C as a standard. The formula of Wiebe, Brown, Daniel & Campbell (1970) was used, where:
adjusted voltage = recorded voltage / (0,325 + 0,027T), and T is the recorded temperature in °C. The calibration curve obtained is shown in Fig. 14.

Fruit water potential was measured as soon after picking as possible. A small section of the pedicel immediately adjacent to the fruit was removed, the xylem excised and crushed onto the filter paper disc. Thereafter, the procedure followed that of the calibration. It was necessary to know the time

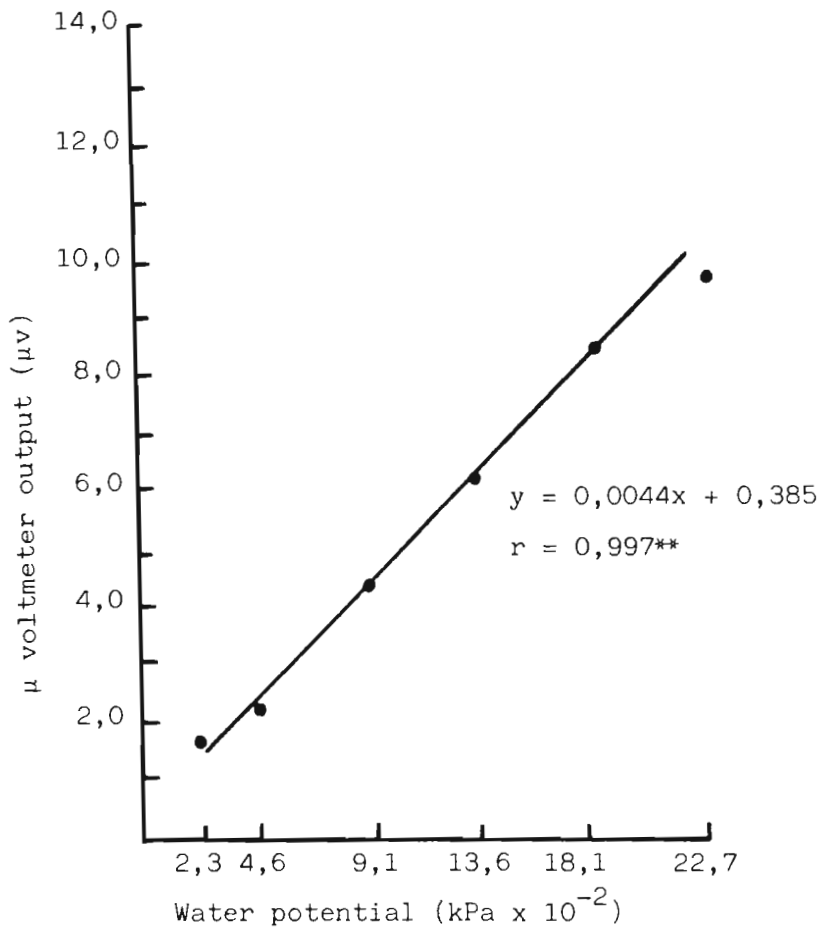


Fig. 14 Leaf chamber calibration curve

required for vapour equilibrium, as samples had to be processed as rapidly as possible. Results of a trial in which the water potential of a sample was measured at regular intervals are shown in Fig. 15. On the basis of these results, it was decided that a 15 min equilibration period was adequate.

In order to check that the results did indeed represent an estimate of water potential, comparison with the pressure chamber method of water potential (long considered a standard instrument) was made. A portion of fruit pedicel was removed for the psychrometric technique such that sufficient remained attached to the fruit to allow insertion through a large aperture in the top of a "PMS" pressure chamber. Sealing was effected by means of "Prestik". The work was done early enough in the season that small fruit were still (in most cases) able to fit in the pressure chamber. Where fruit were too large, they were trimmed, and immediately sealed in the pressure chamber. Pressure was increased until the point of first sap flow at the cut xylem surface became visible in accordance with Scholander, Hammel, Bradstreet & Hemmingsen (1966). The pressure was then taken as the fruit water potential. The psychrometric technique was carried out as described. The relationship between the psychrometric method and the pressure chamber is shown in Fig. 16. A fairly good linear correlation of 0,88 ($P < 0,01$) was obtained within the range measured. The psychrometric method did tend to over-estimate water potential by a mean of 132 kPa. However, this compares fairly well with the results of Campbell & Campbell (1974), who found a 100 kPa depression of water potential reading with a pressure chamber as compared with an in situ thermocouple hygrometer. The method was thus considered acceptable for measurement of fruit water potentials at harvest.

In 1982, fruit water potentials were measured for each of the

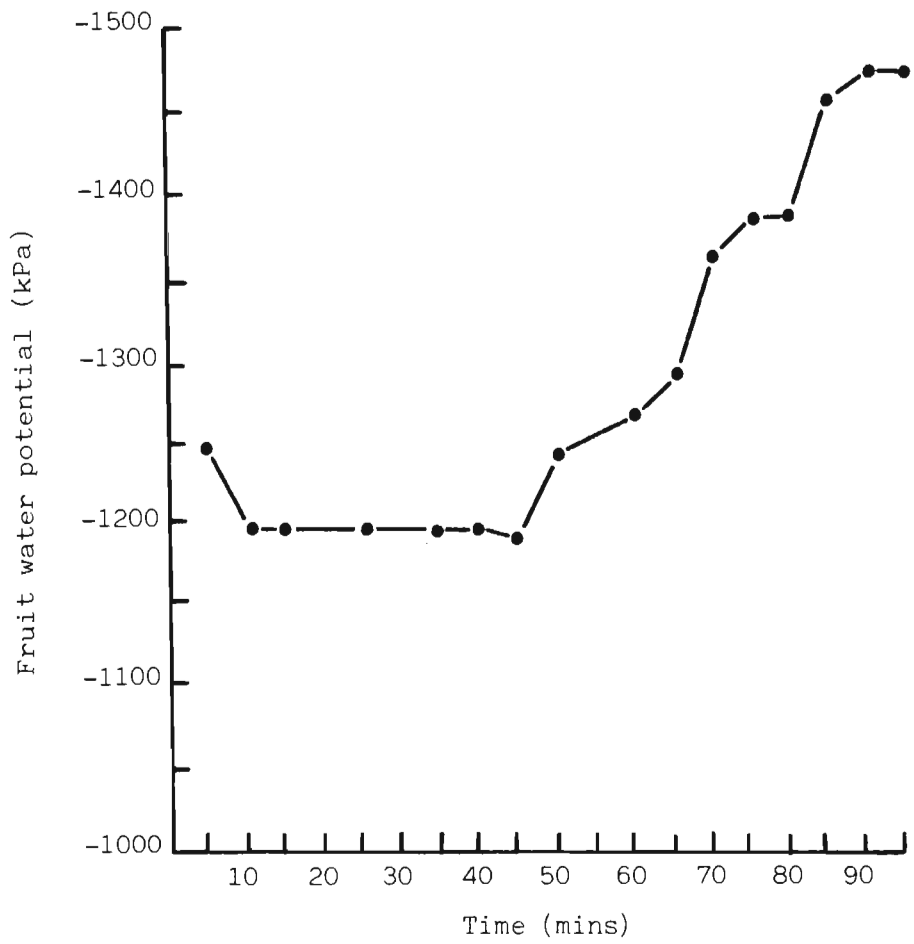


Fig. 15 Effect of equilibration time on fruit water potential

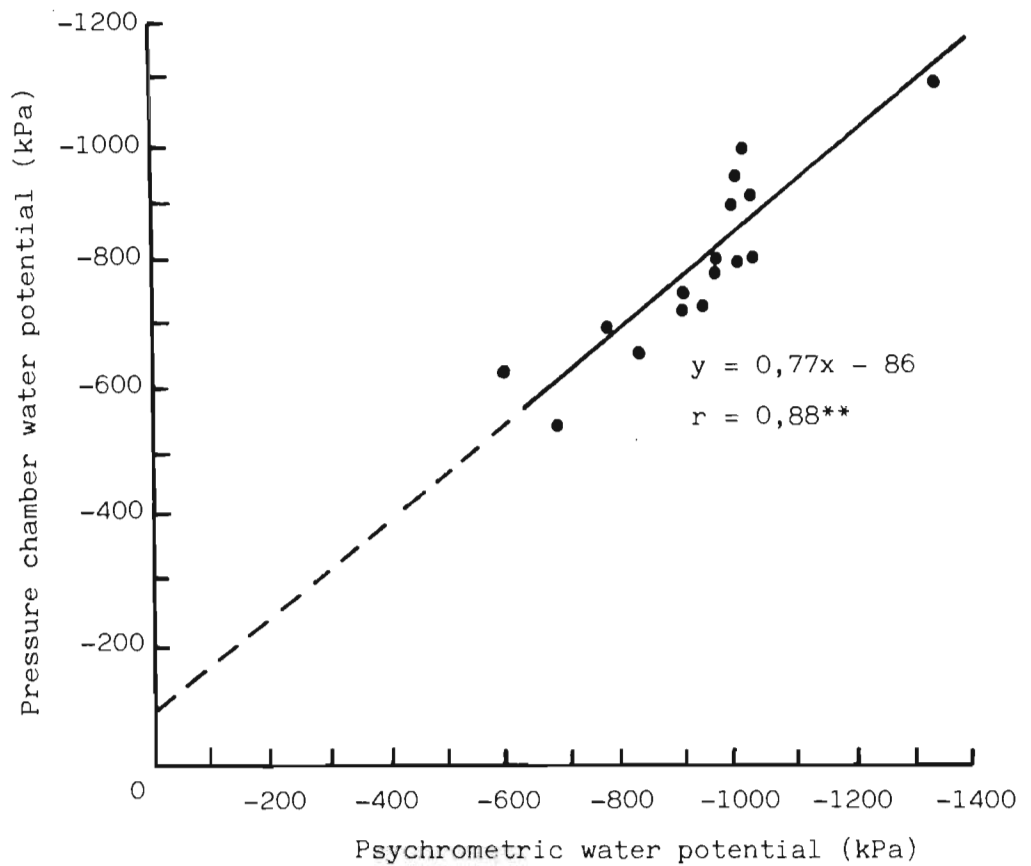


Fig. 16 Relationship between the estimate of avocado fruit water potential as measured by psychrometric and pressure chamber methods

irrigation regimes and at six different dates during the season. Five fruits were selected randomly from the five data trees for each treatment. In 1983, three dates were tested for each of three irrigation regimes (dryland excluded due to lack of fruit). Ten fruits were selected from the data trees as previously described. These fruits were among those later used for enzyme analysis and abscisic acid determinations, as discussed in Chapter 3. During 1984, 10 fruits from each irrigation regime were tested for a single picking date in early May.

It was not always possible to complete measurements on a single day, and therefore picking and measurement was done on consecutive days. No rain occurred, nor was any irrigation applied on any picking date. The dates indicated in the results are in each case those of the first day of picking.

2.2.2 RESULTS AND DISCUSSION

The results of fruit water potential at the time of picking during the 1982 season are indicated in Table 1.

Table 1 Changes in avocado fruit water potential (kPa) at picking, for four irrigation regimes, during the 1982 picking season. Significance levels are indicated by * for P=0,05 and ** for P=0,01. S.E. of means are shown in brackets

Picking date	Fruit water potential (kPa) for irrigation regime				LSD	cv%
	35 kPa	55 kPa	80 kPa	Dryland		
03/17	-1168,5 (+27,2)	-1229,9 (+37,8)	-1150,5 (+30,2)	-1224,9 (+28,1)	N.S.	
03/31	-1307,3 (+57,0)	-1207,1 (+48,2)	-1207,0 (+42,5)	-1181,6 (+53,3)	N.S.	
04/19	-1208,2 (+28,6)	-1319,4 (+48,2)	-1421,2 (+81,9)	-1296,9 (+64,6)	*	160,4 197,4 9,1
05/05	-1293,8 (+30,9)	-1264,5 (+28,6)	-1221,6 (+38,7)	-1347,8 (+27,5)	**	63,5 86,0 3,6
05/17	-1342,1 (+24,9)	-1242,3 (+59,9)	-1583,6 (+88,7)	-1384,8 (+53,2)	*	199,0 279,1 10,4
06/08	-1356,4 (+28,5)	-1326,0 (+54,2)	-1560,0 (+69,9)	-1442,1 (+77,0)	N.S.	
	**	N.S.	**	*		
LSD P=0,05	109,6		169,7	152,4		
P=0,01	149,5		231,4	207,9		
CV%	6,5		9,4	8,8		

For each irrigation regime, there was an indication of increasing stress as the season progressed. However, for the 55 kPa soil moisture tension treatment there was no significant change in water potential during the season, and only a slight increase (more negative) by the end of the picking period. The greatest increase in water potential through the season occurred in the 80 kPa soil moisture treatment. The fact that the dryland treatment showed less apparent stress than the 80 kPa treatment may be further evidence of stress acclimation by the dryland trees compared to those which received occasional supplementary irrigation.

Examining the results in terms of each picking date, it is evident that no differences in stress occurred early in the season, and only by mid-April were significant differences noticed between the treatments. There were also significant differences between treatments on 05/05 and 05/17 but not at the end of the season due to a general increase in water potential in all treatments. The results do imply, however, that fruit water stress is unlikely to be a problem if fruit is picked early in the season especially if rainfall between January and April is adequate or if suitable irrigation is practised.

The 1983 results are presented in Table 2 .

Table 2 Changes avocado in fruit water potential (kPa) at picking for three irrigation regimes, during the 1983 picking season. Significance levels are indicated by * for P=0,05 and ** for P=0,01. S.E. of means are shown in brackets

Picking date	Fruit water potential (kPa) for irrigation regime				LSD		CV%
	35 kPa	55 kPa	80 kPa		P=0,05	P=0,01	
04/13	-1156,4 (+14,6)	-1144,8 (+13,3)	-1282,2 (+12,9)	*	37,4	51,3	3,3
06/08	-1278,9 (+27,1)	-1139,6 (+20,2)	-1352,4 (+32,8)	*	76,9	105,3	6,5
07/12	-1388,6 (+30,5)	-1348,0 (+18,0)	-1575,8 (+23,2)	*	74,7	102,3	5,5
	**	**	**				
LSD P=0,05	85,23	38,0	76,8				
P=0,01	116,76	52,0	105,3				
CV%	7,1	3,3	5,8				

In terms of irrigation regime, significant changes occurred as the season progressed. This was noticeable in the case of the 80 kPa regime. Due to the very small crop, comparison with dryland fruit could not be made. The fruit water potential of 35 kPa and 55 kPa treatments increased less than that of the 80 kPa treatment. It may be argued that the size of the crop could contribute to these results. However, this can be countered by the fact that mean yield tree⁻¹ for the 35 and 55 kPa treatments was similar at 34 and 37kg respectively, while for the 80 kPa regime it was only 20 kg.

From a physiological point of view, the differences in stress between irrigation regimes on a particular date are probably of more interest. Statistically, some significance did exist in all cases. However, the results must be carefully interpreted, as statistically significant results may not

constitute a physiologically significant difference.

Results for 1984 are given in Table 3.

Table 3 Changes avocado in fruit water potential (kPa) at picking for four irrigation regimes, during early May, 1984. S.E. of means are shown in brackets

Fruit water potential (kPa) for irrigation regime

Picking date	35 kPa	55kPa	80 kPa	Dryland	Significance
15/05	-1146,3 (+26,2)	-1168,8 (+18,2)	-1126,8 (+36,3)	-1121,1 (+51,0)	N.S.

No significant differences in water potential were found between any of the treatments for fruit picked in early May. Somewhat heavier and later rainfall occurred in 1984 as compared with previous years, and this would explain the lack of differences in water potential later in the season.

Overall, fruit water potential did become more negative (higher stress) as the picking season progressed, being particularly noticeable with under-irrigation. Fruit picked early in the season showed no or very small differences in inherent water stress, while irrigation became important in the prevention of stress later in the season, once normal rainfall had declined. It was also recorded that the most frequent irrigation (35 kPa soil moisture tension) usually showed a higher stress than the 55 kPa treatment.

These results assume greater relevance when interpreting the results of the fruit physiology investigations described in Chapter 3.

2.3 GENERAL DISCUSSION

Seasonal changes in tree water status during the entire fruit growth period, to form a background to the fruit physiology results in Chapter 3, have been shown. Tromp (1984) highlighted much evidence to link the fruit stress status of apples with that of the whole tree. An important aspect to emerge from these studies is that not only can moisture stress result from poor irrigation practices, but that under certain circumstances considerable adaptation can occur.

From a practical point of view, adaptation to moisture stress would at first seem an advantage, in that it could allow trees to be grown without supplementary irrigation in most of the traditional South African avocado areas. Stress would increase during the winter months, and by the time of fruit set and early growth during August to November (according to the results obtained), acclimation could, to a certain extent, protect the trees from a lack of water. This may have a positive effect on early fruit growth, and help in combatting excess tree vigour. Summer rains would normally then be sufficient to supply water for further fruit growth and development.

In order to adapt to stress, however, the latter first has to occur. The likely increased stomatal resistance will cause a subsequent decrease in photosynthesis (Bower et al., 1979; Lakso, 1979). Lakso (1979) found that in apples, biochemical suppression of photosynthesis only occurred at extremely low water contents, and thus concluded that stomatal conductance was the most important controlling factor for photosynthetic activity in the absence of radiant flux density or temperature stress. One could therefore conclude that productivity may be seriously affected in late winter-early spring.

The low levels of incident photosynthetically-active radiation (PAR) on the southern sides of the trees in winter could be limiting. Scholefield et al. (1980) found PAR compensation point of individual avocado leaves to be $63 \mu\text{E cm}^{-2} \text{ s}^{-1}$, with saturation at $500 \mu\text{E cm}^{-2} \text{ s}^{-1}$ in field grown avocado trees. As there was an incident PAR of less than $100 \mu\text{E cm}^{-2} \text{ s}^{-1}$ recorded for most of the day, the trees were, under the best environmental conditions, liable to a decrease in photosynthetic productivity. Water stress would decrease this reduced productivity even further, which may, in the long term, have an effect on yield and alternate bearing, particularly as the avocado has a high energy requirement for fruiting, but a relatively low photosynthetic capacity (Wolstenholme, 1985). These aspects may be better illustrated by reference to mean per tree yields for each irrigation regime (Table 4).

Table 4 Mean tree yields (kg) for four irrigation regimes, as during the 1982/1983 and 1983/1984 seasons

Irrigation regime	Yield (kg tree ⁻¹)		Accumulative total
	1982/1983	1983/1984	
35 kPa	33,9	17,2	51,1
55 kPa	37,6	17,8	55,4
80 kPa	20,4	13,9	34,3
Dryland	1,1	25,3	26,4

Whether or not photosynthesis acclimates at the same time as the trees may acclimate to low water contents is unknown, but Matthews & Boyer (1984) demonstrated that this could occur in sunflower. The yields in Table 4 do not indicate that meaningful compensation occurred under the conditions studied, and that severe alternate bearing may take place.

The danger of dryland avocado farming, however, is that acclimation can rapidly be reversed in response to rainfall. Thus, unseasonal rainfall would cause considerable problems if followed by a dry period. Where some supplementary irrigation is provided, such that acclimation does not occur, the safeguards of acclimation will not be present.

The environmental conditions causing moisture stress would appear critical in all plants which have not acclimated. The results obtained during spring and summer were similar, and not disproved during winter, suggesting no major change in this critical point with season. While not of direct applicability to the results in Chapter 3, this finding is nevertheless useful, in that ecologically suitable zones can be more easily identified. If at least the SD values at 14h00 were to be computed, an indication of the stress potential of the region is possible. The spring (flowering and fruit set) period before commencement of normal rainfall, is perhaps the most critical. It is important that Leopold & Kriedemann (1975) quote work on cotton, which showed that increased ABA levels occurred during the period of early fruit drop. As this growth regulator is generally accepted to be stress-related, any increase in stress at this time could be expected to increase the chance of fruit drop and consequently reduce yield.

Knowledge of the critical SD may also help in modifying irrigation scheduling practices. Experience of the author has shown the class A evaporation pan to be unreliable for avocado irrigation scheduling. Sensitivity to the summer environment may be a reason. Partial stomatal closure would result in a lower water usage than expected, requiring a change in crop factor. This also emphasises the need for flexible irrigation systems, by showing the unsuitability of fixed scheduling based on long-term evaporation data and a

constant evapotranspiration relationship. The results further confirm the overall sensitivity of the 'Fuerte' avocado to environmental stress, and indicate that cool subtropical regions, if possible with high humidity in summer (Sterne et al., 1977) should be chosen for 'Fuerte' avocado production. A table of temperature and relative humidity combinations with resultant SD values would be useful. The critical SD may also be useful in future work, as a stress hour or, by integration, stress day can be computed. The relationship between length and severity of stress period and various physiological reactions can then be examined, which could lead to a better understanding of, for example, fruit drop and fruit quality differences from season to season. It may even be possible to predict reactions later in the season once a stress pattern is known. Before firmer recommendations can be made, however, it would be advisable to examine the environmental stress relationship during different seasons, and over a number of years.

Growers often have a tendency to believe irrigation to be unnecessary. However, if all factors are taken into account, irrigation would seem essential. Dryland farming can be practised, but only with knowledge that a calculated risk is being taken. In addition, fruit would have to be picked as early as possible in order to avoid excessive fruit water stress as the dry winter season progresses (see Tables 1 and 2). Table 4 shows likely yield disadvantages. On the other hand, if irrigation is practised, application should be sufficient to avoid stress, particularly during the critical flowering and fruit set stages, otherwise little advantage will be gained. Care should also be taken to ensure that over-irrigation does not occur.

The results of the 35 kPa treatment were at times anomalous. Some discussion of the possible reasons is therefore in order. Apart from the well-known sensitivity of avocados to

water-logged soils, which the 450 mm deep tensiometer readings showed to be a possibility in that they seldom reached 35 kPa even during dry periods, and those with low oxygen contents (Curtis, 1949), there is also the increased danger of Phytophthora cinnamomi infection. There is sufficient evidence to indicate a considerable disturbance in water relations (Sterne, Kaufmann & Zentmyer, 1978; Bower, 1979) where this fungus is present. Further, Sterne et al. (1978) found this disruption in water relations in P. cinnamomi infected trees to be manifested in the same way as water stress. The trees in this study are still visually healthy, and no obvious differences can be detected between any of the treatments. The P. cinnamomi status of all the treatments was recently checked, and although all had an apparently large number of healthy feeder roots, the disease was present. It may be postulated that the more favourable root disease environment created under Burgershall conditions (with a fairly textured soil) by the longer periods of wetter soil in the 35 kPa treatment as opposed to the others, could have resulted in a marginally higher level of the disease and thus some stress, although this was not confirmed.

Dawson & Weste (1982) found that root infection in forest trees caused alterations in stomatal conductance, which were not necessarily water induced, and suggested, on the strength of other work, that root damage may cause plant growth regulator changes which affect stomatal responses. As there was no obvious advantage in irrigating as frequently as in the 35 kPa treatment, and bearing in mind the known P. cinnamomi risk on heavy soils in South Africa, a less frequent irrigation schedule would be advisable.

CHAPTER 3

INFLUENCE OF WATER RELATIONS ON FRUIT PHYSIOLOGY

As discussed in the introduction, physiological disorders, particularly fruit browning, are of considerable importance to the South African avocado industry. Abnormal ripening is a lesser, but nevertheless noteworthy problem. With this in mind, polyphenol oxidase (PPO) and phenolics are discussed first, followed by fruit ripening and the ethylene evolution pattern. This is followed by the work on calcium and abscisic acid (ABA), both of which may be involved in physiological disorders and fruit ripening.

3.1 POLYPHENOL OXIDASE AND PHENOLICS

3.1.1 MATERIALS AND METHODS

The first batch of 'Fuerte' fruit from the field trials at Burgershall Research station was picked for PPO analysis during the first week of April, 1983, when legal maturity (moisture content less than 80%) had been reached. Sixty fruits were picked separately from the north and south sides of five randomly-selected data trees from each irrigation regime, to investigate the effects of microclimate or fruit position on quality. No fruit was picked from the dryland treatment due to a very poor fruit set and yield (see Table 4), with some data trees having no fruit. Oil was measured indirectly by the dry matter method of Swarts (1978). After measurement of fruit water potential (see section 2.2), half the fruits were placed in a cold store at 5,5° C for 30 days, simulating shipping for export. The remaining fruit sample was divided into two, half of the fruits being allowed to ripen to a firmometer reading of 100 (Swarts, 1981) while the other half was immediately prepared for storage at -18°C prior to analysis. Five fruits of each category for each

treatment were analysed. Fruits were peeled, cut longitudinally, the seed removed, and the fruits cut into proximal and distal halves before being grated in a kitchen vegetable grater. The soft fruits were similarly treated, except that it was possible to pulp instead of grate the samples. The same procedure was followed for stored fruit after 30 days in storage.

A second batch of fruit was picked during the first week of July 1983, and treated in the same way.

In 1984, the possible interaction between moisture stress in the field and restricted ventilation of containers was investigated. Fruits were picked in early May, from the 55 kPa and 80 kPa soil moisture tension treatments, these having been the two extremes (in respect of PPO activity) in the 1983 study. Two cartons of 10 fruit each were packed for both treatments, and immediately stored at 5,5°C for 30 days. On removal from the cold room, one carton of fruit from each treatment (control) was allowed to ripen at 22°C, while fruits from the remaining two were placed in large glass jars for 48 h at 22°C, as was used by van Lelyveld & Bower (1984). Thereafter, the fruits were allowed to soften in a normal atmosphere. When soft as determined by the firmometer (Swarts, 1981), five fruits from each treatment were randomly selected, cut as previously described, pulped and stored at -18°C until analysed for PPO activity. The distal halves of the fruits were analysed, as it is this portion of the fruit which shows the most intense browning reaction.

In 1985, a total of 10 fruits were picked in early April from each irrigation regime, two fruits per data tree, in order to investigate whether similar trends in PPO activity to those obtained in 1983 existed, even though water stress conditions were considerably different. Dryland fruit were also

available to be included as a treatment. Fruits were stored for 30 days at 5,5°C to simulate sea shipment, whereafter five fruits of each treatment were ripened at 22°C before cutting and analysis for soluble and total PPO activity as described for the 1983 fruits.

3.1.1.1 Extraction of PPO

Most literature describes PPO extraction by a preparation of acetone powder. However, Mayer & Harel (1979) pointed out that enzyme modification may occur, resulting in a changed activity. Benjamin & Montgomery (1973) found changes in isoenzyme pattern after acetone precipitation. It was therefore decided to use a modification of the method used by Golan, Kahn & Sadovsky (1977), as described by van Lelyveld & Bower (1984), for the extraction of soluble PPO. A 2,5g fruit sample was ground in a mortar and pestle for 1 min at room temperature (Kahn, 1977b) in 5 ml cold 10 mM acetate buffer, pH 5,0.

There is some disagreement over the optimal pH for avocado PPO extraction in the literature. Golan, Kahn & Sadovski (1977) used a buffer pH 6,5, while Kahn (1975) found a pH optimum of 5,3 to 6,7. On the other hand, van Lelyveld & Pretorius (1973) found the optimum to be pH 5,0 within the range tested of 4,7 to 6,7. Mayer & Harel (1979) noted the same anomaly in literature they surveyed, and indicated a wide pH optimum which could vary depending on fruit origin. The pH 5,0 buffer has been successfully used by van Lelyveld (pers. commun.) in numerous extractions and assays for PPO and thus it was decided to use it in this study.

Gentle grinding for a constant time of 1 min is important. Sharon & Kahn (1979a) found a considerable release of enzyme from the particulate fraction (due perhaps to the destruction of organelles and membranes) when tissue was homogenised in a

Waring blender. With unripe (hard) fruit, grinding was facilitated by the use of a small amount of chemically pure sand. In all cases, insoluble polyvinyl pyrrolidone (PVP) or "Polyclar AT" was added (Loomis 1973), to remove activators and inhibitors. Thereafter, the homogenate was squeezed through a gauze cloth and centrifuged at 18 000 x g for 45 min at a temperature of 0 to 4°C in a "Sorval Superspeed RC-2" centrifuge. The supernatant (excluding oil droplets) was kept for enzyme assay, and the residue discarded. In order to assay total PPO activity (soluble plus bound plus latent), 0,1% sodium dodecylsulphate (SDS) (Roberts, 1974; Kahn, 1977b) was added to the buffer during extraction.

3.1.1.2 Assay of PPO

Mayer & Harel (1979) outlined a number of methods for the assay of PPO activity. The most commonly used is a spectrophotometric method. PPO catalyses the oxidation of phenols to o-quinones, and the further irreversible oxidation to coloured compounds. The initial rate of colour formation is followed. It is important that only the initial rate is recorded, as the enzyme rapidly becomes inactivated (Mayer & Harel, 1979). A modification of the method described by Kahn (1975) was followed.

The reaction mixture consisted of 3 ml pH 5,0 acetate buffer plus 3 ml 0,02M 4-methyl catechol. To this the crude enzyme extract was added. For the fruit picked early in the season 50 µl was used, while for the later picking date it was necessary to decrease this to 10 µl. The initial rate of colour formation was recorded at 420 nm on a "Pye-Unicam SP8 200" UV VIS spectrophotometer, at 25°C. This was coupled to an "Apple II Plus" microcomputer, programmed to record the optical density change (ΔOD_{420}) during a 6s period and express the change over 60 s. This was to ensure that only the linear portion of the reaction curve was recorded.

Regular checks were made graphically to ensure this. The results of PPO are expressed as specific activity, i.e. ($\Delta OD_{420} \text{min}^{-1} \text{mg protein}^{-1}$). Dilution by the reaction mixture was also taken into account.

3.1.1.3 Protein determinations

Protein determinations were made in accordance with the widely used method of Lowry, Rosebrough, Farr & Randall (1951), as modified by Leggett-Bailey (1962). The crude protein extract (0,5 ml) was precipitated in an equal volume of 10% tri-chloro-acetic acid and left for 15min. before centrifuging at 18000 x g for 45 min. The residue was redissolved in 100 μl 3% NaOH and after vigorous shaking, 1 ml water added. To this was added 2 ml Folin A + B in the ratio of 1:30 v.v. Folin A consisted of 0,5% $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in 5% Na-citrate. Folin B was made up of 2% Na_2CO_3 in 0,1 N NaOH. After 10 min., 100 μl Folin Ciocalteu reagent diluted 1:1 with water was added, and the mixture allowed to stand for 15 min. Absorbance at 750 nm was read, and compared with a standard curve obtained using bovine serum albumin (BSA), and programmed onto the previously-mentioned microcomputer. This curve is shown in Fig.17. Each unknown enzyme activity and protein determination was assayed twice and the mean determined.

3.1.1.4 Extraction of total phenols

Determination of total phenols was done according to the method of Weurman & Swain (1955). Extraction was done using 5 g of fruit tissue ground in 10 ml 70% methanol, in a mortar and pestle. The homogenate was squeezed through gauze cloth and centrifuged at 18 000 x g for 45 min. The supernatant was used for the assay.

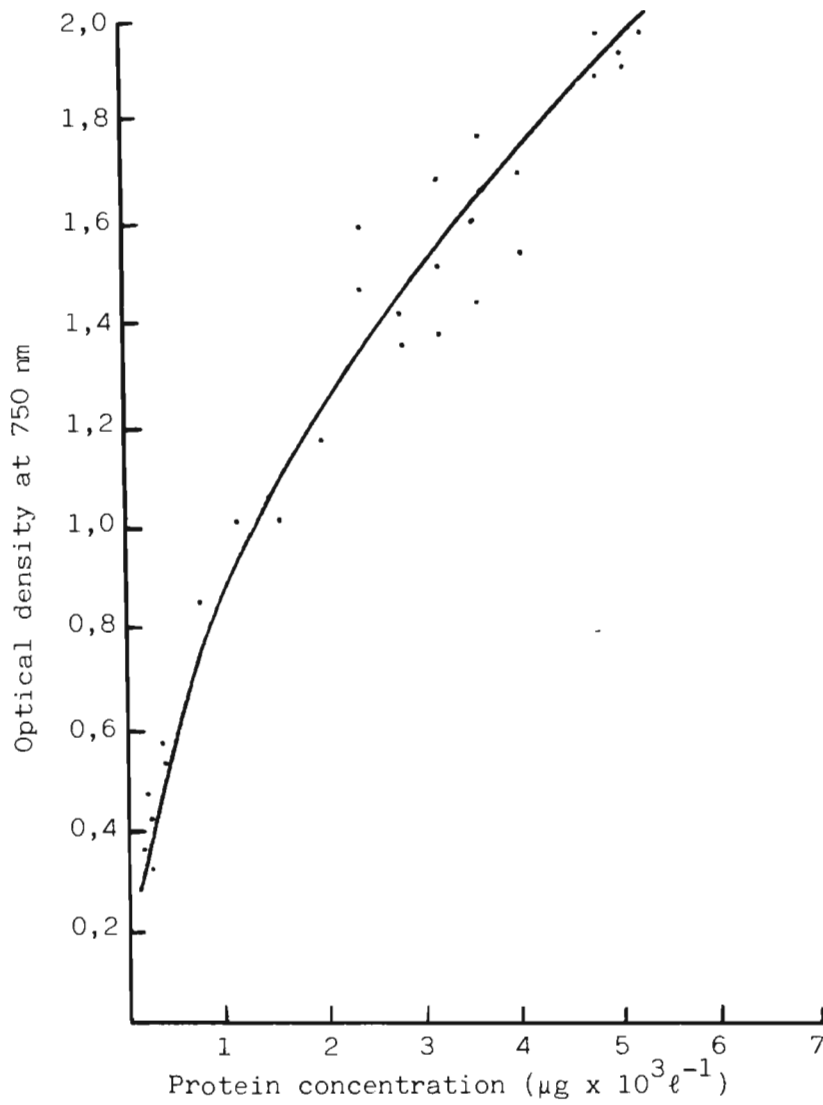


Fig. 17 Calibration curve as used for protein determinations

3.1.1.5 Assay of total phenols

To 1 ml of supernatant was added 10 ml water and 0,2 ml Folin Denis reagent. After standing for 5 min, 1 ml saturated Na_2CO_3 was added, and optical density at 700 nm read after 1 h. A standard of $25 \mu\text{g ml}^{-1}$ catechol was used. This was in accordance with the method of Weurman & Swain (1955).

3.1.2 RESULTS AND DISCUSSION

3.1.2.1 Polyphenol Oxidase

3.1.2.1.1 First picking date

Considering the known correlation between browning and PPO activity (Luh & Phithakpol, 1972; Kahn, 1975), and also the fact that browning is first visible or more severe in the distal flesh, it was decided to report fully only on PPO activity in the distal end of the fruit flesh. While fruit had originally been picked from both north and south sides of trees and analysis results recorded separately, no significant difference was found. It was therefore decided to combine these data.

3.1.2.1.1.1 Soluble PPO activity

The influence of irrigation regime interacting with storage and fruit softening, on the activity of soluble PPO, is indicated in Fig. 18. These results would indicate the immediate browning potential of a cut fruit. A trend appeared, showing the 35 kPa soil moisture tension to have results intermediate to those of the other treatments.

3.1.2.1.1.1.1 Unstored fruit

In the case of unstored soft fruit (Fig.18), irrigation resulted in highly significant ($P < 0,01$) differences in PPO values. Overall, irrigation at 55 kPa soil moisture tension resulted in the lowest PPO activity, with under-irrigation significantly higher ($P < 0,01$). Irrigation at 35 kPa resulted in a PPO (and thus browning potential) approximately midway between the other treatments, lower ($P < 0,05$) than the 80 kPa, and higher ($P < 0,01$) than the 55 kPa treatment, respectively.

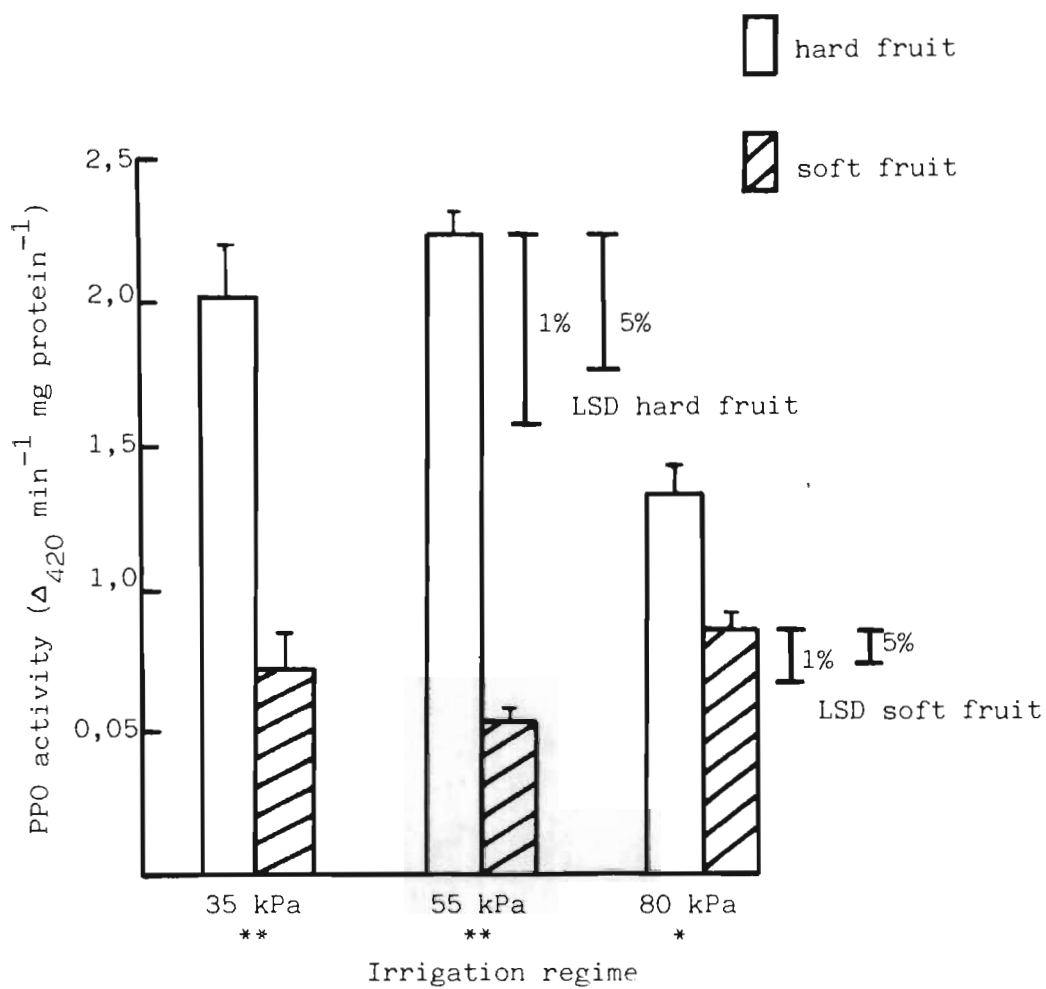


Fig. 18 Influence of irrigation regime on soluble PPO activity in unstored hard and soft 'Fuerte' fruit for April 1983 harvest. Significance levels between hard and soft fruit are indicated by * for P=0,05 and ** for P=0,01. Bars indicate S.E. of means, ten samples per mean

In the case of hard fruit (Fig. 18) irrigation again had a highly significant ($P < 0,01$) effect on PPO level. However, the trends were the reverse of soft fruit. Fruit from trees receiving least water had the lowest PPO activity, and those from the 55 kPa treatment the highest ($P < 0,01$), with the 35 kPa treatment intermediate. The latter was, however, not significantly different to the 55 kPa treatment.

The process of fruit softening appears important in PPO metabolism, and the long-term irrigation history does seem to play a vital role in the expression of the changes which take place (Fig. 18). In all experiments, PPO activity decreased during fruit softening which is consistent with the work of Sharon & Kahn (1979 b). Thus a 64% decrease in soluble enzyme activity was recorded in fruit from trees irrigated at 35 kPa soil moisture tension, 76% for the 55 kPa treatment and only a decrease of 37% in fruit from under-watered (80 kPa) trees. This decrease in PPO activity with softening was significant ($P < 0,01$) for the 35 kPa and 55 kPa treatments, and ($P < 0,05$) for the 80 kPa treatment. Possible reasons for this phenomenon are varied, and should be evaluated in terms of the theories of latency in PPO. There is no current evidence to suggest which factor may be important in this case. Conformational changes, as shown in grapes by Lerner, Mayer & Harel (1972) can be reversible, and aggregation or dissolution of sub-units was shown by the work of van Lelyveld *et al.* (1984) to be a possibility. A considerable amount of basic research is needed to elucidate the actual mechanism, and the method by which long-term irrigation may affect this process.

3.1.2.1.1.1.2 Stored fruit

Only the results for soft (eating ripe) fruits are given. The previous section indicated the extent to which PPO activity decreases during softening. Low temperature storage will

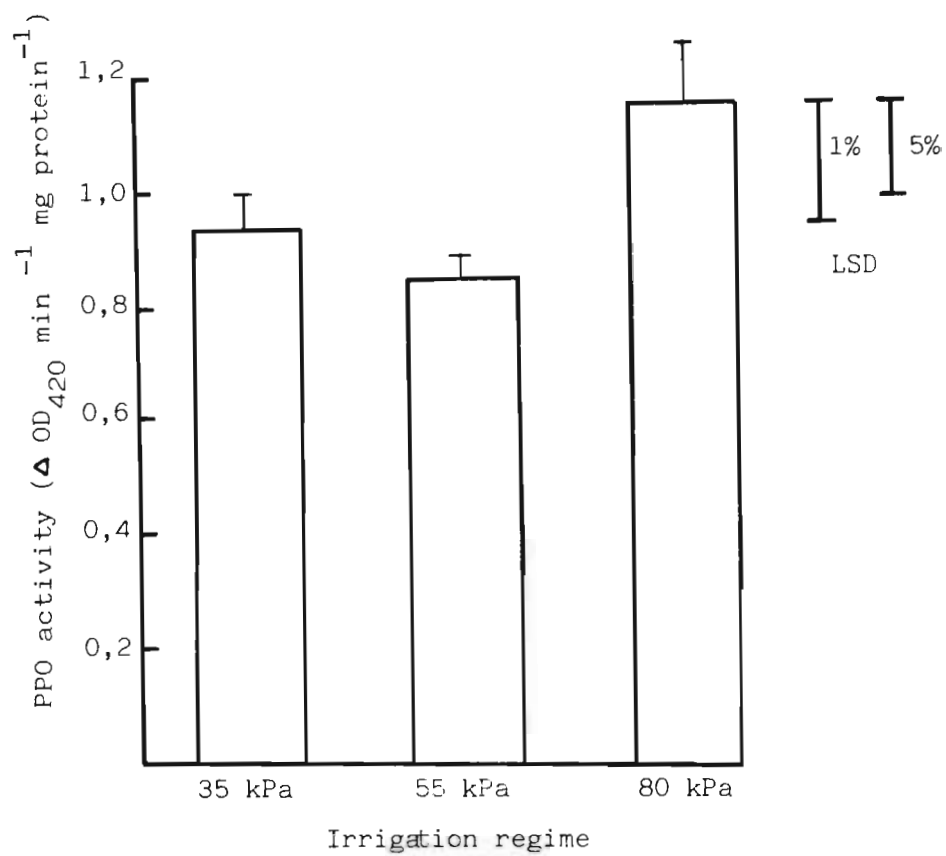


Fig. 19 Influence of irrigation regime on soluble PPO activity in stored, soft 'Fuerte' fruit for April 1983 harvest. Bars indicate S.E. of means, ten samples per mean

retard but not prevent the softening process. Firmometer readings of "hard" fruit before and after storage confirmed this. Due to differing fruit ripening rates between treatments, results for hard, stored fruit can not be compared and are therefore omitted.

Soft, stored fruit (Fig. 19) showed the same highly significant influence of irrigation regime as did unstored fruit (Fig. 18). The under-watered treatment had the highest soluble PPO activity, which was significantly higher than at the 55 and 35 kPa soil moisture tensions ($P < 0,01$ and $P < 0,05$ respectively).

There was a general tendency for PPO to increase with storage. All the irrigation regimes had higher ($P < 0,01$) PPO activity after storage.

3.1.2.1.1.2 Total PPO activity

3.1.2.1.1.2.1 Unstored fruit

The activity of total PPO will indicate the potential severity of browning, should all PPO be released or activated by adverse ripening conditions (van Lelyveld et al., 1984). The irrigation history again had a highly significant ($P < 0,01$) effect on PPO activity of soft fruit (Fig. 20), with fruit from the 55 kPa soil moisture tension treatment having the lowest activity and from the 80 kPa treatment the highest ($P < 0,01$). The 35 kPa treatment was significantly higher ($P < 0,05$) than the 55 kPa treatment and lower ($P < 0,01$) than the 80 kPa regime fruit.

Softening had a similar effect to that encountered with soluble PPO (Fig. 18), where a considerable decrease occurred. The fruit PPO activity of the 35 kPa treatment decreased by 69% ($P < 0,01$) the 55 kPa fruit by 74% ($P < 0,01$)

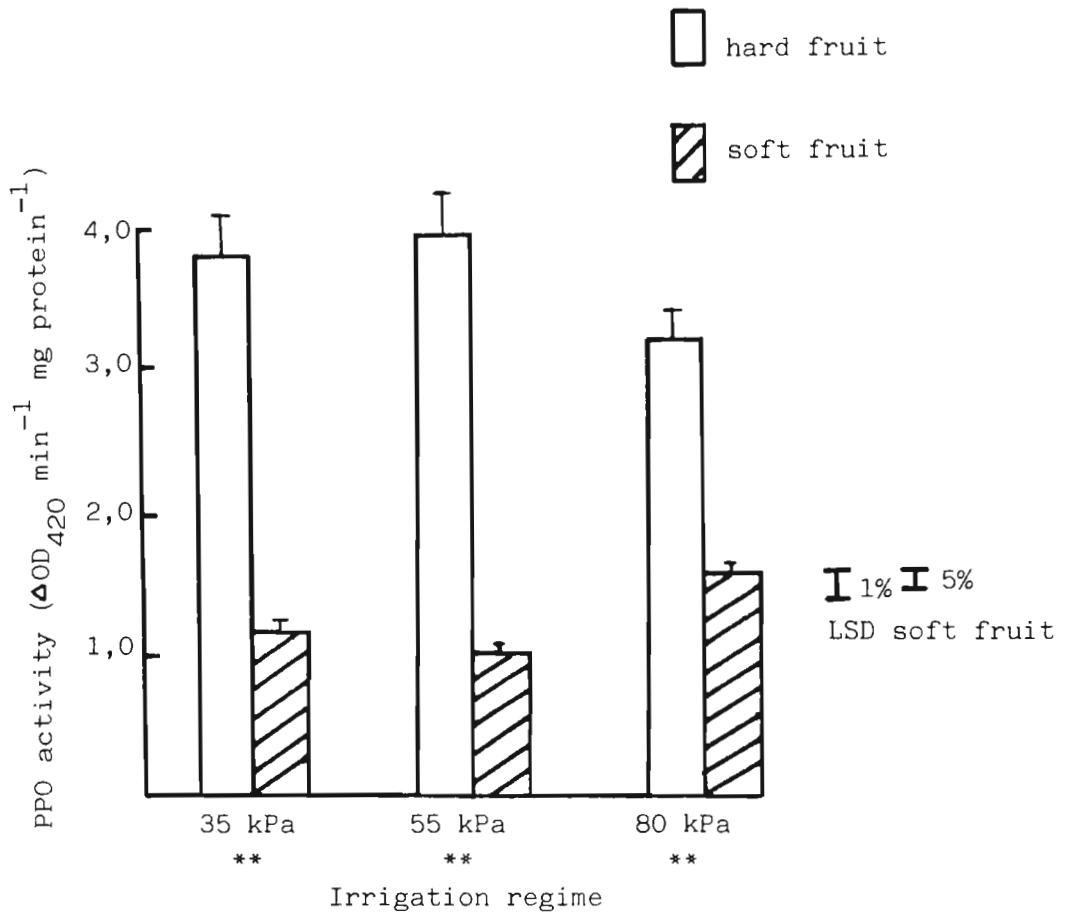


Fig. 20 Influence of irrigation regime on total PPO activity in hard and soft unstored 'Fuerte' fruit for April 1983 harvest. Significance levels between hard and soft fruits are indicated by ** for P=0,01. Bars indicate S.E. of means, ten samples per mean

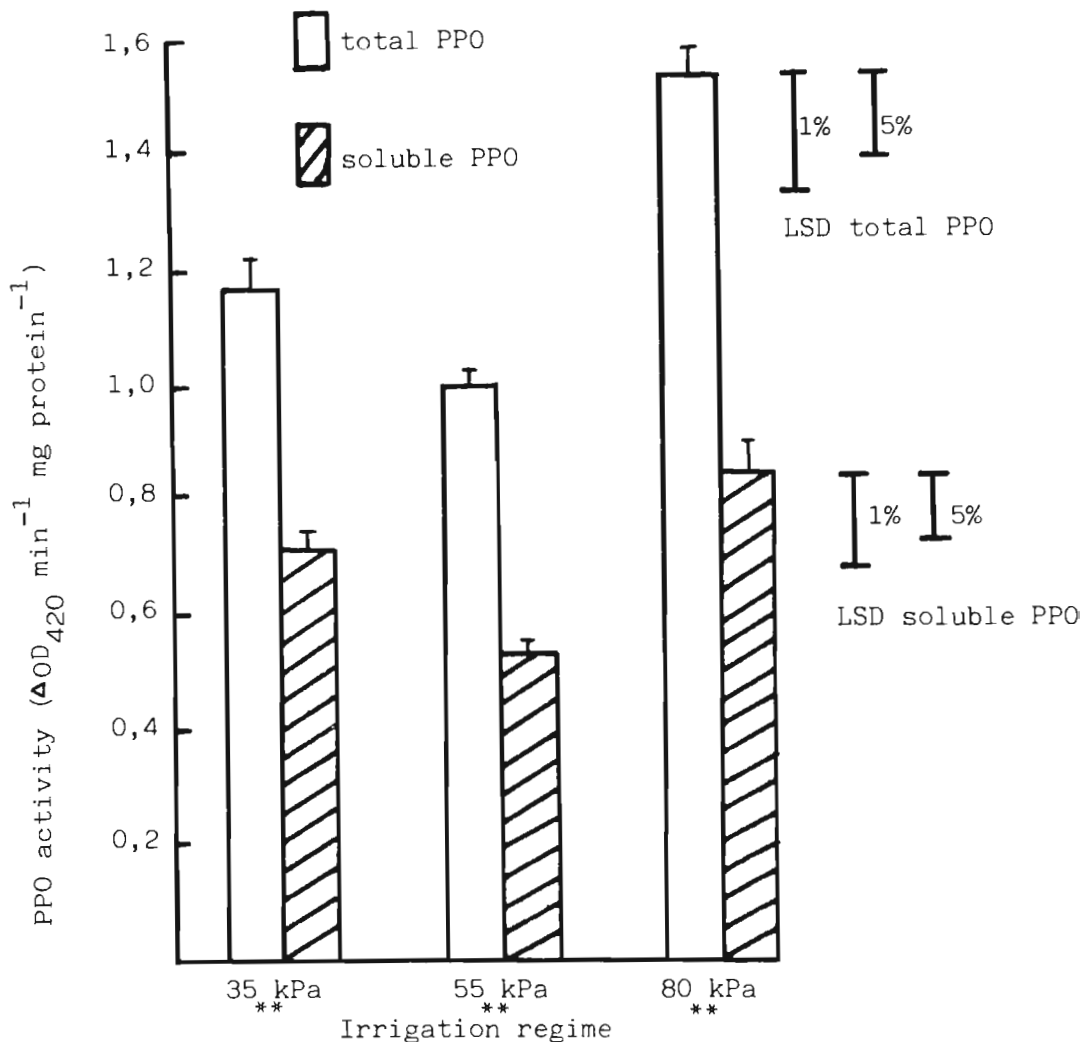


Fig. 21 Relationship between total and soluble PPO activity as influenced by irrigation regime in soft, unstored 'Fuerte' fruit for April 1983 harvest. Significance levels between total and soluble PPO activity are indicated by ** for P=0,01. Bars indicate S.E. of means, ten samples per mean.

and the 80 kPa fruit by 52% ($P < 0,01$), during softening.

The relationship between soluble and total PPO activity for soft fruit (Fig. 21), showed a greater amount of PPO activity still inactive in the 80 kPa soil moisture treatment than in either of the others, even though there was a high activity in the soluble fraction. There is therefore a higher total browning potential in soft fruit where water stress has occurred, although the difference between the total and soluble PPO activity was significant in all cases ($P < 0,01$).

Hard fruit showed no significant differences in total PPO activity, although the same trend as for soluble PPO activity was evident.

3.1.2.1.1.2.2 Stored fruit

The results of the total PPO activity for stored soft fruit are more difficult to interpret. Although there was a significant difference between irrigation regime and PPO activity, the highest value was obtained for the 35 kPa soil moisture tension treatment. This differed ($P < 0,05$) from both the other two treatments, which did not differ significantly from each other. There were large increases in total PPO activity ($P < 0,01$) between the unstored and stored fruit for the 35 and 55 kPa soil moisture tension treatments, but not in the 80 kPa soil moisture tension treatment (Fig. 22). Thus, as with soluble PPO activity, storage does seem to result in some increase in activity. The biochemistry of these changes requires further research.

3.1.2.1.2 Second picking date

The results for the second picking date (July) are presented in Figs 23 and 24.

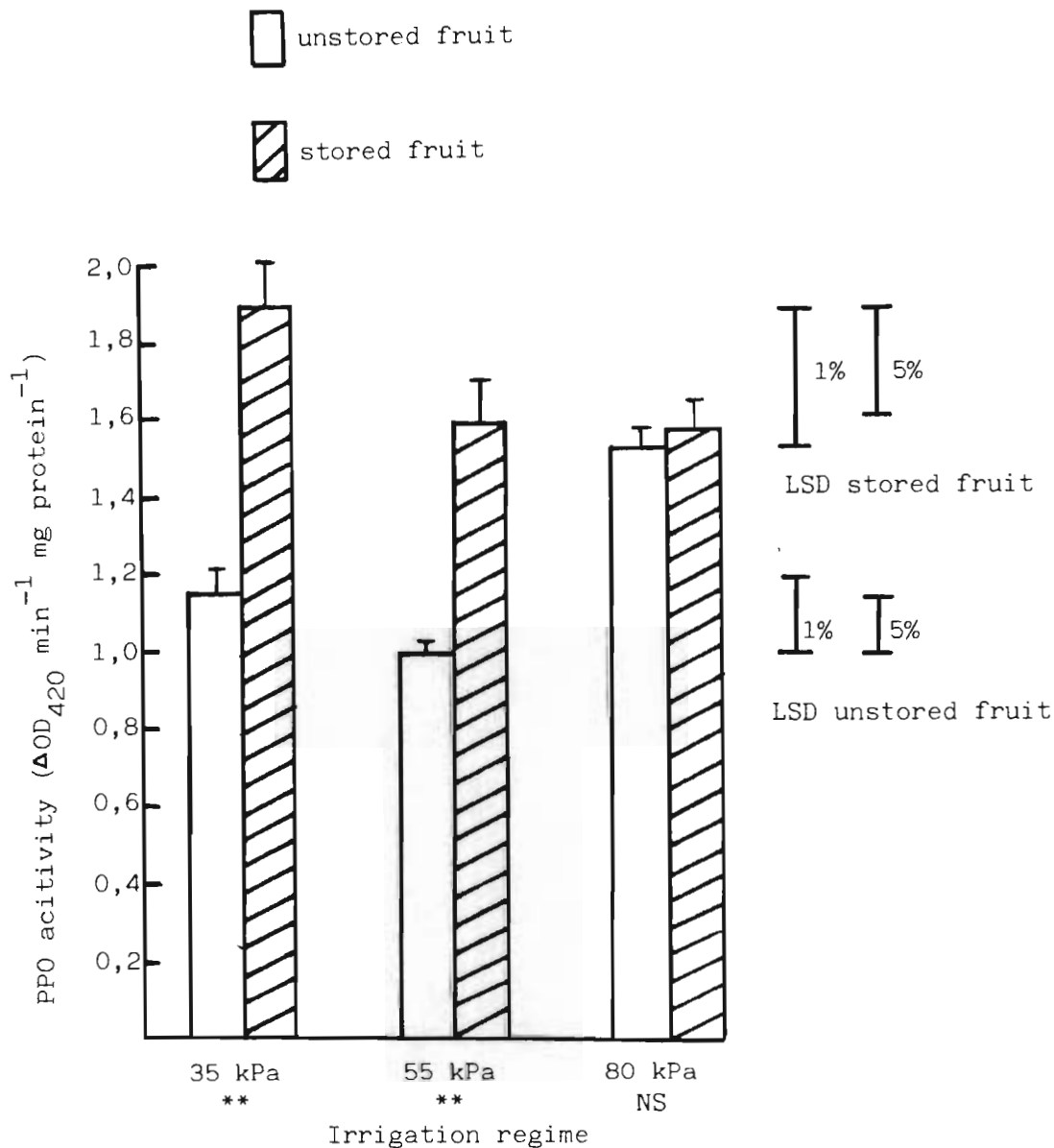


Fig. 22 Influence of irrigation regime on total PPO activity in soft stored and unstored 'Fuerte' fruit for April 1983 harvest. Significance levels between unstored and stored fruit are indicated by ** for $P=0,01$. Bars indicate S.E. of means, ten samples per mean

3.1.2.1.2.1 Soluble PPO activity

3.1.2.1.2.1.1 Unstored fruit

Soft, unstored fruit showed the same trends as for fruit picked early in the season. A highly significant ($P < 0,01$) difference in PPO activity resulting from irrigation regime was found (Fig. 23). The 35 kPa and 55 kPa treatments differed significantly ($P < 0,05$), while the 80 kPa regime was significantly higher ($P < 0,01$) than the 55 kPa treatment. The most frequently (35 kPa) and least frequently irrigated (80 kPa) treatments did not differ significantly.

Hard fruit did not show any significant differences as a result of irrigation (Fig. 23), but there was some decrease in PPO activity with fruit softening. The 35 kPa treatment showed a 22,1% decrease in activity, the 55 kPa a 40,6% decrease and in the case of the 80 kPa soil moisture regime only a 7,4% decrease in PPO activity. Only the 55 kPa treatment was significantly different ($P < 0,01$) as a result of softening.

3.1.2.1.2.1.2 Stored fruit

For the same reasons as previously outlined, only soft fruit should be considered after storage.

The 55 kPa soil moisture treatment indicated the lowest soluble PPO activity followed by the 35 kPa and 80 kPa regimes (Fig. 24). The differences were, however, a little less marked than in the case of unstored fruit, with only the 55 kPa and 80 kPa treatments differing statistically ($P < 0,01$).

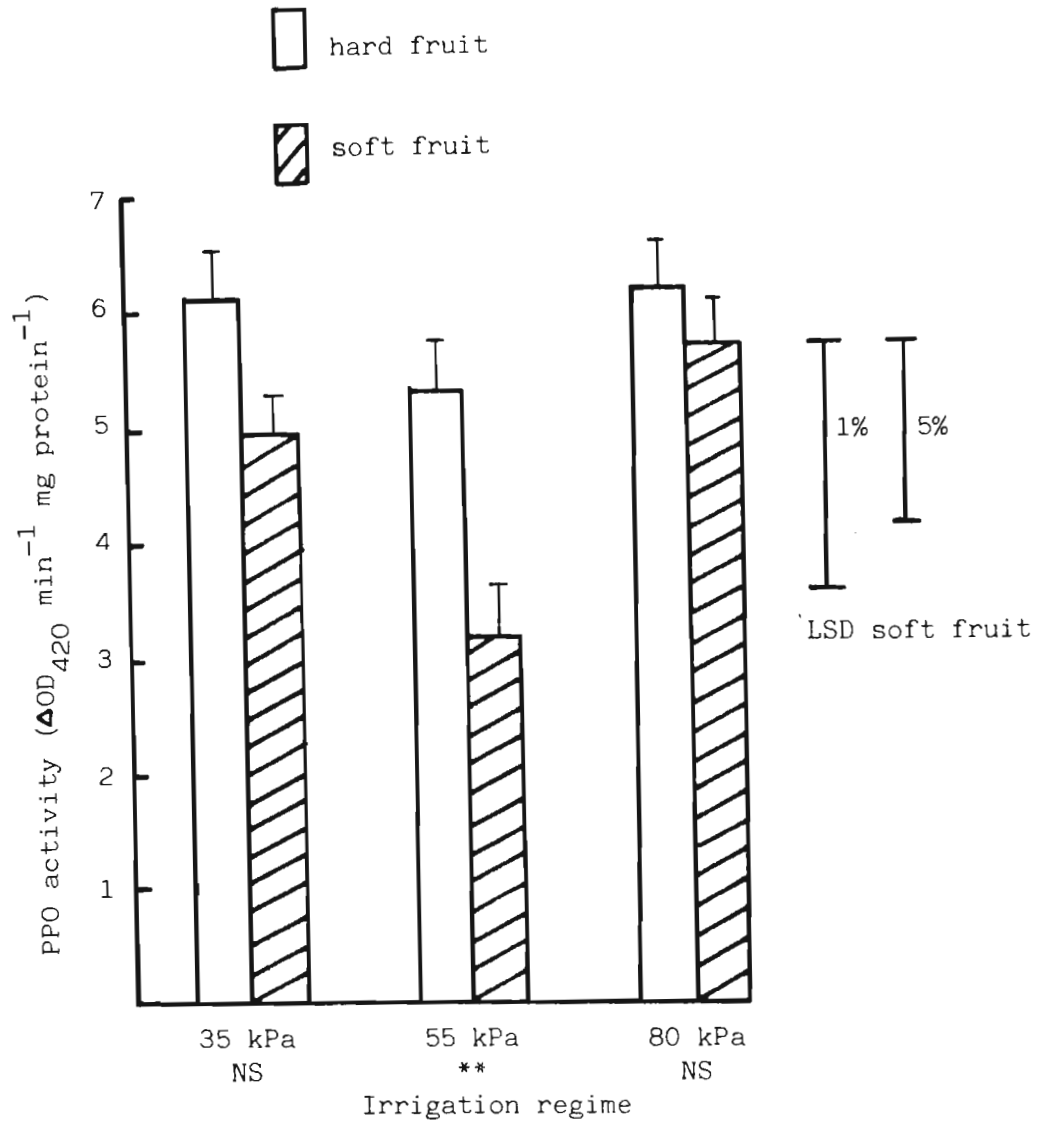


Fig. 23 Influence of irrigation regime on soluble PPO activity in unstored hard and soft 'Fuerte' fruit for July 1983 harvest. Significance levels between hard and soft fruits are indicated by ** for P=0,01. Bars indicate S.E. of means, ten samples per mean

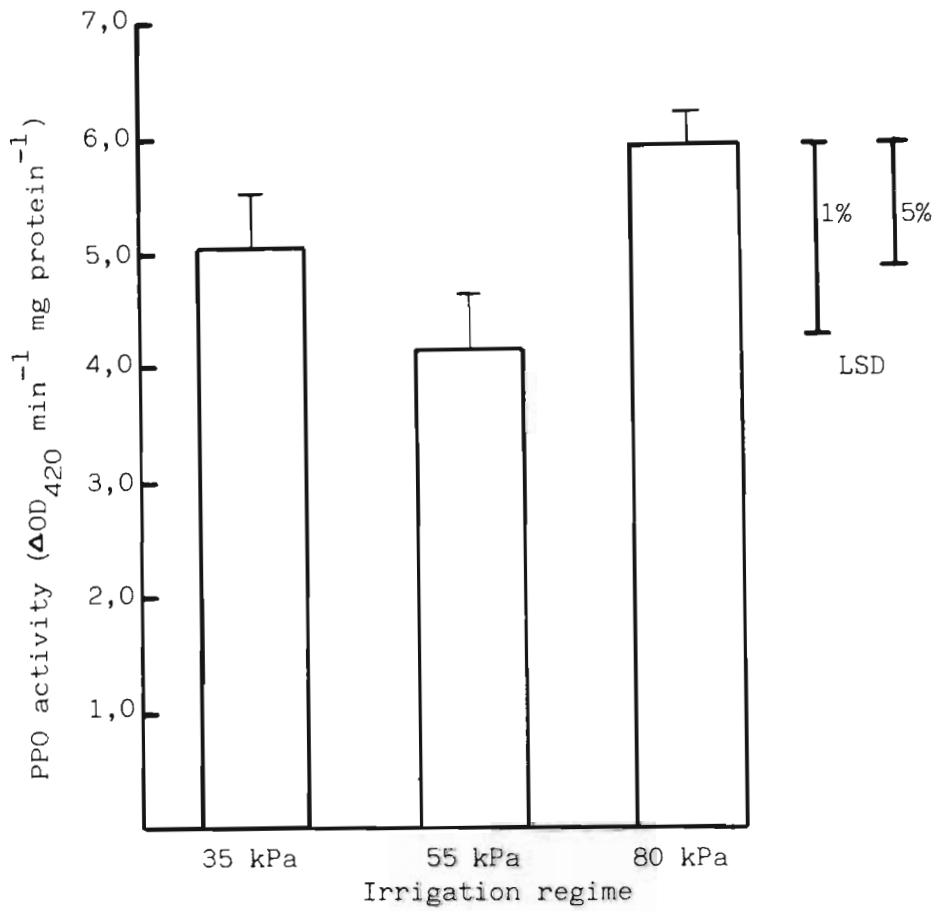


Fig. 24 Influence of irrigation regime on soluble PPO activity in stored, soft 'Fuerte' fruit for July 1983 harvest. Bars indicate S.E. of means, ten samples per mean

3.1.2.1.2.2 Total PPO activity

There were no significant differences due to irrigation in any of the groups of total PPO analysed.

A noteworthy factor as far as soft fruit is concerned, was the somewhat similar values of total and soluble PPO activity obtained. This was especially so in the case of stored fruit, where the total values were lower than soluble activity (Table 5).

Table 5 Total and soluble PPO activity ($\Delta OD_{420} \text{min}^{-1} \text{mg protein}^{-1}$) in soft, stored 'Fuerte' fruit for July picking date as influenced by irrigation regime. Significance at $P=0,05$ is indicated by *. S.E. of means are shown in brackets

Irrigation regime	PPO activity ($\Delta OD_{420} \text{min}^{-1} \text{mg protein}^{-1}$)	
	Total PPO	Soluble PPO
35 kPa	3,90 (+0,46)	5,08 (+0,42)
55 kPa	4,10 (+0,51)	4,19 (+0,47)
80 kPa	4,65 (+0,33)	5,91 (+0,26)
LSD $P=0,05$ $P=0,01$	N.S.	* 1,14 1,56

This is an apparent anomaly, but may perhaps be explained by the results of Maurer (1971) who indicated that SDS can, to some extent, affect protein conformation. If all or almost all PPO has been released and is active, the addition of SDS could, after complexing with the proteins result in some loss of activity. An apparently lower total than soluble PPO activity would then result. The same thing occurred for the most stressed (80 kPa soil moisture tension) unstored

treatment. Most, if not all, PPO had apparently been activated by a combination of water stress and other factors to be discussed later.

3.1.2.1.3 Effect of irrigation regime on PPO activity in the 1984/1985 season

The trends in PPO activity for fruits picked during April, 1985 are shown in Table 6.

Table 6 Soluble and total PPO activity ($\Delta OD_{420} \text{min}^{-1} \text{mg protein}^{-1}$) in soft, stored 'Fuerte' fruit picked in April, 1985 as influenced by irrigation regime. The S.E. of means are shown in brackets

Irrigation regime	PPO activity ($\Delta OD_{420} \text{min}^{-1} \text{mg protein}^{-1}$)	
	Soluble PPO	Total PPO
35 kPa	2,09 (+0,35)	2,39 (+0,24)
55 kPa	1,87 (+0,20)	2,36 (+0,35)
80 kPa	2,35 (+0,36)	2,95 (+0,46)
Dryland	2,21 (+0,24)	2,61 (+0,22)

While the differences between treatments were not significant, the same trend as found in 1983 was evident. The results should be evaluated in terms of the rainfall during the fruit growth period, which determines the potential for the development of water stress. Monthly rainfall from July 1984 through March 1985 is shown in Table 7.

Table 7 Monthly rainfall at Burgershall for the period July 1984 through March 1985

Month	Rainfall (mm)
July	123
August	12
September	68
October	119
November	146
December	110
January	100
February	407
March	102

The rainfall, particularly between September and December was both higher and better distributed than the 1982/1983 season (Fig. 2). During the early flowering and fruit development period some irrigation was necessary. Between August and December, the 35 kPa treatment received three, the 55 kPa two and 80 kPa one irrigation. The majority of the irrigations were in August and September. The potential for the development of fruit water stress was therefore low, although some differences could be expected due to the need for irrigations. This could explain the similar trends in PPO activity obtained during the two seasons investigated. The non-significant differences during 1984/1985 may be attributed to the lack of substantial water stress in a season of above average rainfall.

3.1.2.1.4 Effect of irrigation regime and restricted ventilation on PPO activity

3.1.2.1.4.1 Soluble PPO activity

Considerable differences in soluble PPO activity were evident, as shown in Fig. 25. Fruit ripened with ventilation showed the lowest PPO activity, and in this case there was also no significant difference between the 55 kPa and 80 kPa irrigation treatments. This differed somewhat to results from the previous year, as did the absolute values of PPO activity. The time of picking was, however, different, falling between that of the 1983 first and second pickings. This may be important, as a considerable increase in PPO activity occurred between these two dates. It is not known whether the fruit PPO activity changes at different rates during maturation, dependent on previous history.

While restricted ventilation did not increase the PPO activity significantly in the case of the 55 kPa treatment, the increase was highly significant ($P < 0,01$) for the 80kPa irrigation regime. Further, the interaction between irrigation and ventilation was significant ($P < 0,05$). The restricted ventilation which may occur in containers after discharge at European ports, could enhance the likelihood of flesh (pulp) discoloration (Van Lelyveld & Bower, 1984), but the symptoms will be more severe where the fruit has a history of long-term stress.

3.1.2.1.4.2 Total PPO activity

Total PPO activity showed the same pattern as the soluble, with no statistical increase due to restricted ventilation in the 55 kPa treatment. The 80 kPa soil moisture tension treatment, was higher ($P < 0,01$) after "suffocation" (enclosed under restricted ventilation). This indicates that the

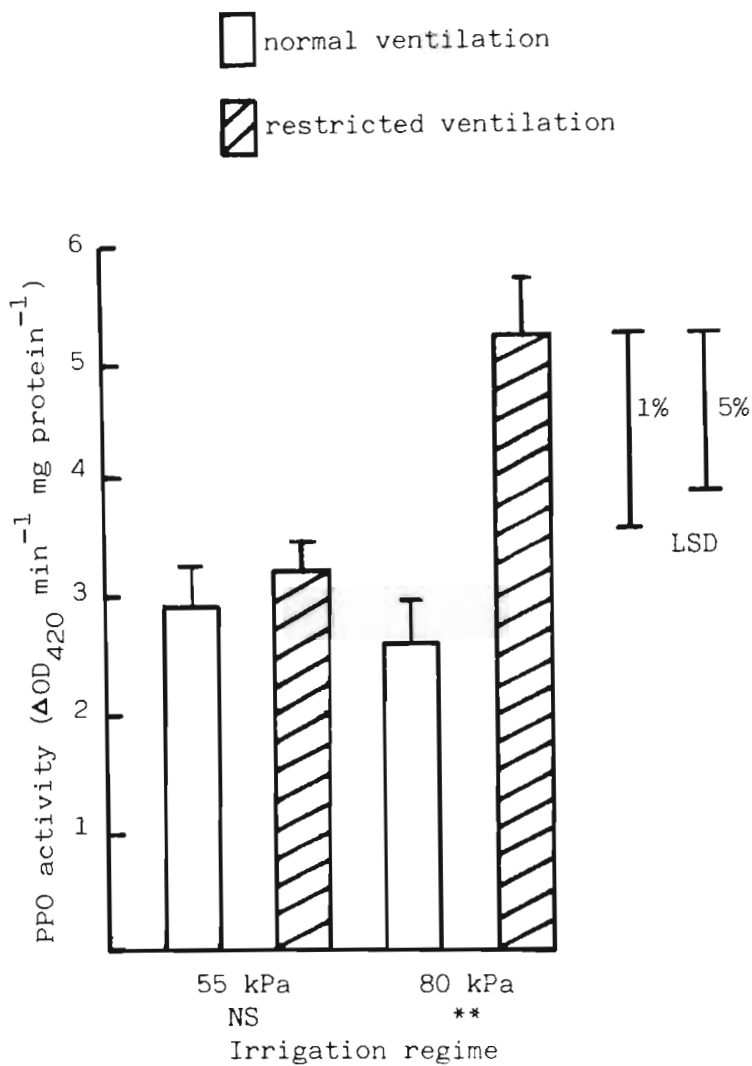


Fig. 25 Effect of irrigation regime and container ventilation on soluble PPO activity in soft, stored 'Fuerte' fruit May 1985 harvest. Significance levels between normal and restricted ventilation are indicated by ** for P=0,01. Bars indicate S.E. of means, five samples per mean

mechanism by which PPO becomes activated under such environmental conditions also involves activation of latent PPO, and not purely the release of bound PPO from membrane surfaces. If the soluble PPO activity differences had been due only to release of bound PPO, the total PPO values for the 80kPa treatment should not have increased after "suffocation". Some release cannot be discounted, as in the case of the 55 kPa treatment total PPO increased by 5,4% due to restricted ventilation, while soluble PPO increased by 8,6%. In the case of the 80 kPa treatment, total PPO activity increased by 64% as a result of restricted ventilation, but by 93% in the case of soluble PPO. The increase in total PPO was thus probably due to activation of latent PPO, while in the case of soluble PPO, a part was the result of activation, while the rest could have been due to the release of bound PPO.

Where fruit have a history of moisture stress, structural weaknesses in membranes may occur. Lyons, Wheaton & Pratt (1964) reported that membranes with more unsaturated fatty acids are more resistant to cold damage. It is not yet known whether water stress during the formation of the membranes in the fruit results in a different lipid composition, although this is a possibility. If for this or other reasons, membranes are less stable, then the stress of low O₂ in containers could cause membrane breakdown with resultant release of PPO, which would explain the influence of stress on PPO activity in conjunction with restricted ventilation. Further in-depth research is required to clarify these aspects.

Of importance, is the threshold level of PPO activity at which browning symptoms can be expected soon after the fruit is cut. Observations of the degree of browning in fruit used in sections 3.1.2.1.2 and 3.1.2.1.3 indicated a specific PPO activity of between 4 and 5.

3.1.2.2 Total phenols

There was no significant difference due to irrigation for the total phenols present. The total quantity of phenols does not appear to be an important factor as far as stress history-related browning potential is concerned. The mean results obtained, in μg equivalents catechol g fresh mass⁻¹, were:

118 (+7,4) for the 35 kPa treatment,
149 (+9,5) for the 55 kPa treatment and
132 (+4,5) for the 80 kPa treatment.

3.2 FRUIT RIPENING

3.2.1 MATERIALS AND METHODS

3.2.1.1 Fruit softening

The effect of long-term irrigation treatment, as well as actual fruit water potential at the time of picking, on the rate of ripening (and thus the export soft fruit problem) was investigated during the 1982, 1983 and 1984 picking seasons. In 1982, the rate of softening was followed on six occasions through the normal export season, in fruit stored for 30 days at 5,5°C, followed by ripening at 22°C. All four irrigation treatments were used. In 1983, both stored and non-stored fruit were used, fruit being picked on three occasions during the season, viz. early April, early June and early July. Due to the lack of fruit in the dryland treatment during that year, as already outlined, only the treatments irrigated at 35 kPa, 55kPa and 80 kPa soil moisture tension were evaluated. In 1984, non-stored fruit picked in early May were studied. In many cases, the fruit for softness evaluations were also used for other purposes, such as PPO analysis, trends in ethylene production or ABA analysis. As a result, considerable additional and possibly valuable data could be compared.

Fruit softness was evaluated daily using the firmometer method of Swarts (1981). Each daily percentage softness reading was a mean of four readings, taken around the circumference of the fruit, approximately equidistant from the proximal and distal ends. Fruit was considered ripe when a mean reading of 100 was obtained.

Fruit water potential (as a measure of stress) at the time of picking, was estimated psychrometrically using the method described in Chapter 2, immediately after picking.

3.2.1.2 Ethylene evolution

Ethylene evolution during the ripening phase was studied in non-stored fruit picked in June, 1983 and May, 1984. Non-stored fruit was used, so as to investigate ripening physiology as linked to irrigation and water stress, without the complication of long, low-temperature storage times. In view of the important role of ethylene in avocado fruit ripening, as indicated in the Literature Review, as opposed to carbon dioxide evolution which is probably a result rather than a cause of ripening, it was decided to concentrate on ethylene.

Fruits were picked, and after water potential and mass determinations, were packed in export-type cartons and allowed to ripen at 22°C. The softness, as indicated by firmometer, was determined daily as previously indicated, followed by ethylene evolution. Ten fruits for each treatment were evaluated. Each fruit was placed in a 1,5 l glass container, the open (bottom) end sealed by standing in water. Nelson, Isebrands & Rietveld (1980) recommended a saturated solution of ammonium sulphate, but this is probably only necessary where carbon dioxide is to be measured, as water avidly absorbs carbon dioxide. Each fruit was placed in a small aluminium dish inside the container. After 1 h, a 1 ml gas sample was withdrawn from the top of the container through a hole drilled in the bottle lid, which was sealed with masking tape.

Ethylene concentrations were determined using a "Carlo Erba 4 200" gas chromatograph fitted with a 2 m long activated alumina column with a flame ionization detector. The injection temperature was 160°C, oven temperature 125°C and the detection temperature 160°C. Gas flow was: air 300 ml min⁻¹, helium (the carrier gas) 40 ml min⁻¹ and hydrogen at

50 ml min⁻¹. Calibration was done using a 5% ethylene in nitrogen standard gas. The chromatograph was coupled to a "Spectra Physics 4 100" integrator recorder. The calibration resulted in a linear equation, which was then used by the integrator to determine, after peak area integration, the ethylene in the 1 ml sample injected. This result was used to calculate fruit ethylene evolution, expressed in $\mu\text{l kg fruit}^{-1} \text{ h}^{-1}$. The volume of air in the container surrounding the fruit is necessary for the calculation, and thus the volume of each fruit was required. To avoid having to determine this experimentally, a relationship between fruit mass and volume at picking was investigated. Fruit volume was determined by the volumetric displacement of water, and the relationship with fruit mass is shown in Fig. 26. A linear regression with a correlation coefficient of 0,992 was obtained. The regression equation was then used to estimate fruit volume, from mass at picking for cv. Fuerte.

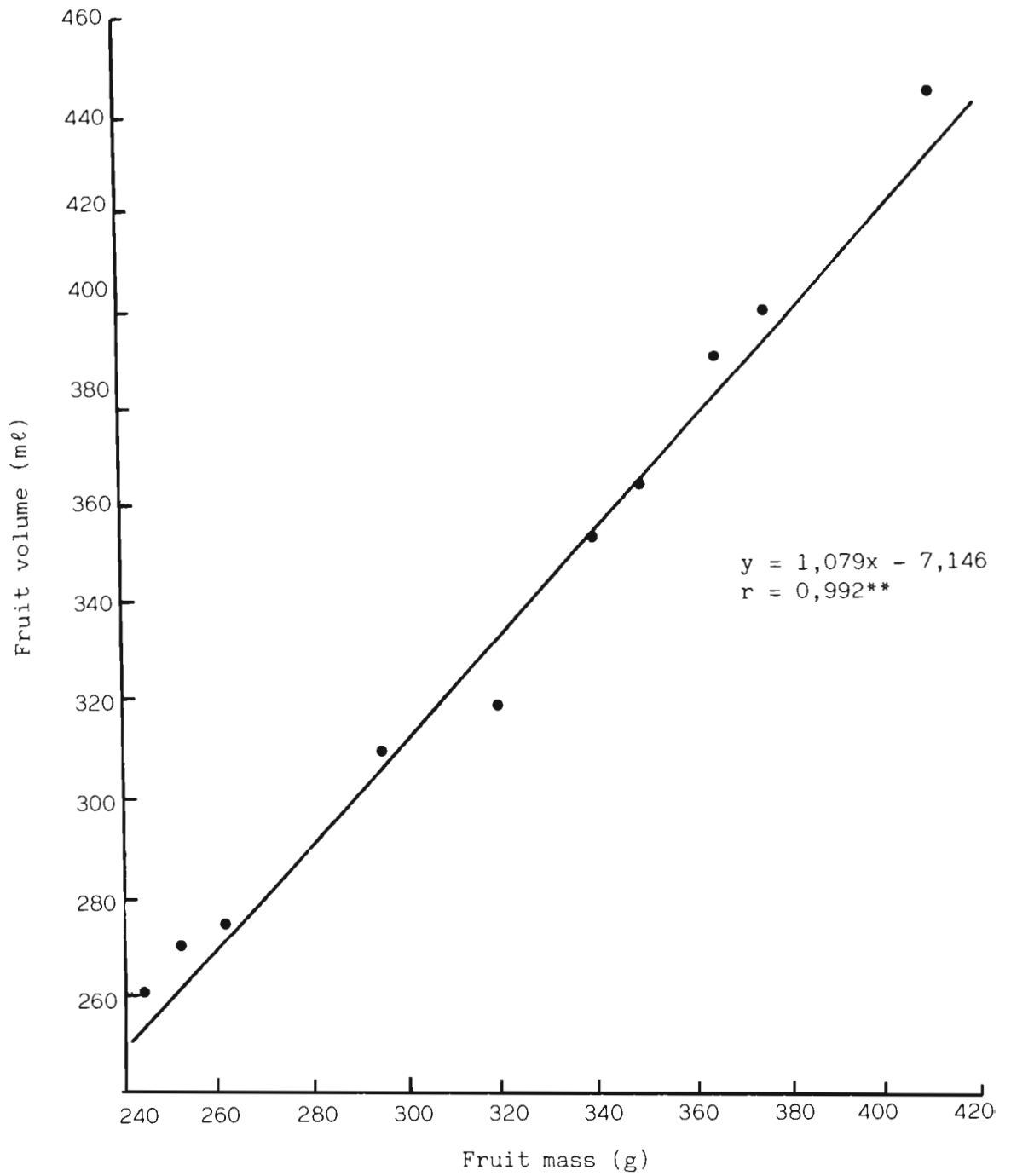


FIG. 26 Relationship between 'Fuerte' avocado fruit mass and volume

3.2.2 RESULTS AND DISCUSSION

3.2.2.1 Fruit softening

3.2.2.1.1 Harvest 1982

Fruit softening results for 1982 are shown in Table 6.

Table 6 Effect of irrigation regime on 'Fuerte' fruit softening after 30 days storage at 5,5°C during the 1982 picking season. Significance levels are indicated by * for P=0,05 and ** for P=0,01. S.E. of means are shown in brackets

Days to fruit softening for irrigation regime					
Picking date	35 kPa	55 kPa	80 kPa	Dryland	Significance
03/17	8,6 (+0,7)	9,4 (+0,2)	8,6 (+0,8)	7,4 (+0,7)	N.S.
03/31	6,4 (+0,8)	6,0 (+0,7)	6,0 (+0,8)	5,0 (+0,7)	N.S.
04/19	4,2 (+0,2)	4,0 (+0,0)	3,8 (+0,6)	5,4 (+1,2)	N.S.
05/05	5,0 (+0,3)	4,8 (+0,4)	6,0 (+0,9)	4,6 (+0,2)	N.S.
05/17	5,4 (+0,4)	4,8 (+0,6)	3,8 (+0,2)	4,0 (+0,3)	N.S.
06/08	3,0 (+0,4)	3,0 (+0,4)	3,0 (+0,3)	4,0 (+0,4)	N.S.
	**	**	**	*	
LSD P=0.05	1,6	1,1	1,8	2,0	
P=0,01	2,1	1,5	2,4	2,7	
CV%	21,9	16,1	25,9	19,9	

In the case of all irrigation treatments, a general decrease in ripening times occurred with increasing fruit maturity. From the work of Wang & Schiffman-Nadel (1972) and Eaks (1980), this trend can be considered normal. Further, as

fruit water potential became more negative during the season, (section 2.2.2) there was a relationship between fruit water potential and ripening rate. Linear correlations of -0,59; -0,71; -0,91 and -0,69 for the 35 kPa, 55 kPa, 80 kPa and dryland treatments respectively, were obtained. These were not particularly good except in the case of the 80kPa treatment, which was significant ($P < 0,01$), and should be treated with caution.

There was no significant difference between irrigation treatments in the ripening rate for any of the harvest dates, even though fruit water potential showed significant differences in April and May. There was also a tendency for fruit water potential to be generally more negative with increasing water stress, but particularly so in the 80 kPa soil moisture tension treatment (section 2.2.2).

If all the results are plotted as days taken to ripen against the mean fruit water potential for the data group, the curve in Fig. 27 is obtained. There was little influence of fruit water potential on ripening for fruit taking longer than 6 days to ripen at room temperature. For fruit ripening faster than this, however, the estimate of fruit water potential did seem to markedly affect the ripening rate, with a more negative water potential at harvest causing more rapid ripening. However, fruits which ripened in two days appear an exception. A considerable amount of the variation for other points in Fig. 27 was due to the same phenomenon.

3.2.2.1.2 Harvest 1983

Results for 1983 are shown in Table 7 for fruit stored for 30 days at 5,5°C before ripening at 22°C, and Table 8 for unstored fruit. The same trend was found in relation to time during the season, whereby fruit water potential became increasingly negative as the season progressed, accompanied

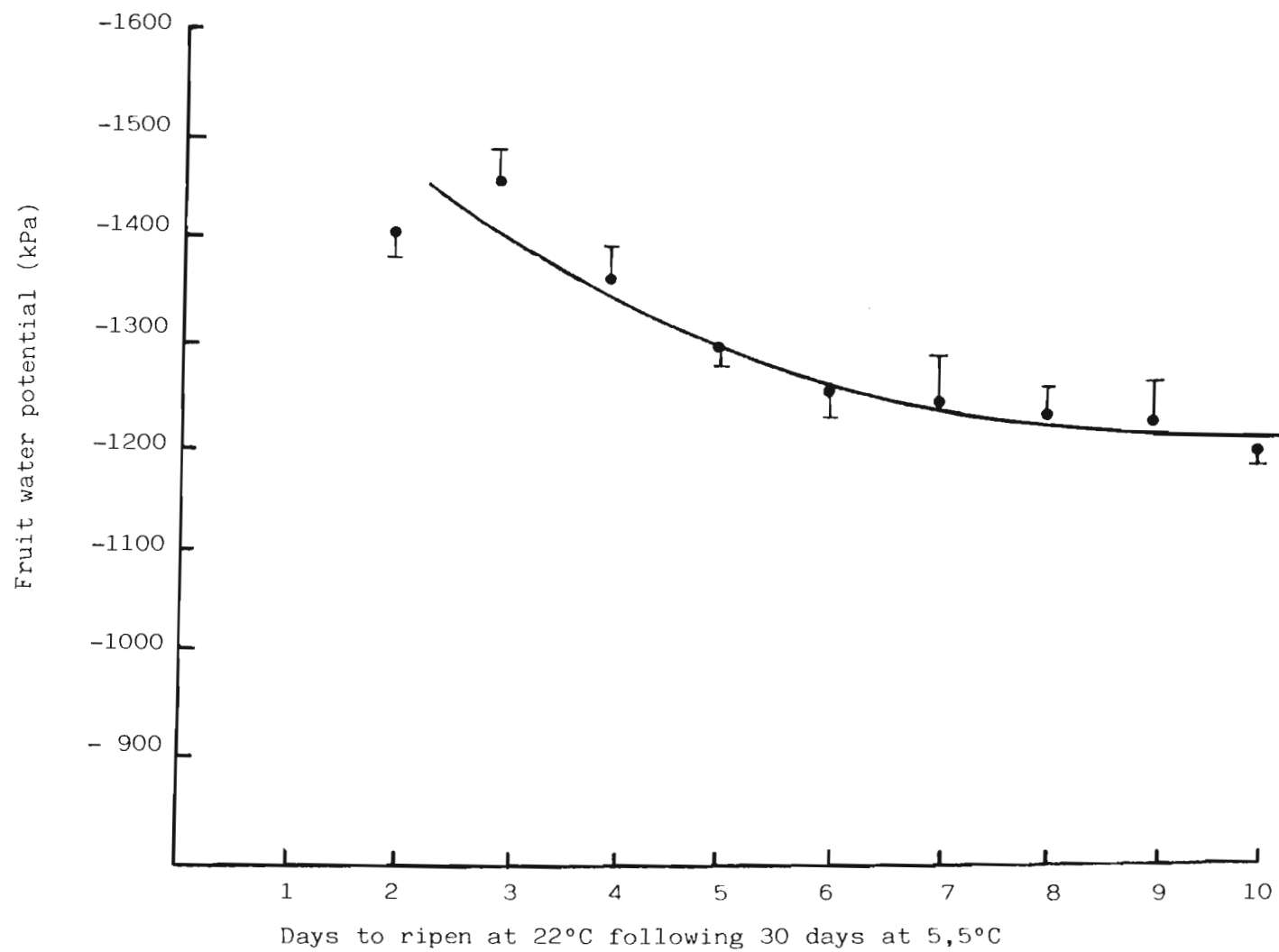


FIG. 27 Influence of 'Fuerte' fruit water potential at harvest on days to ripen after storage during 1982. Bars indicate S.E. of means

by decreasing times to ripen. Zauberman, Schiffman-Nadel & Yanko (1973) indicated that ripening can be slowed down but not stopped by low temperature storage, so the faster ripening times for stored as opposed to unstored fruit can be expected. Nevertheless, early in the season (April) stored fruit showed a marked ($P < 0,01$) increase in ripening rate for the 35 kPa and 80 kPa treatments as opposed to the 55 kPa treatment. Unstored fruit showed a similar trend, except that the time taken to soften was longer than in stored fruit. The same difference in treatments, in terms of days, was not evident. Significant differences did exist during the first two picking dates.

Table 7 Effect of irrigation regime on 'Fuerte' fruit softening after 30 days storage at 5,5°C during the 1983 picking season. Significance levels are indicated by * for $P = 0,05$ and ** for $P = 0,01$. S.E of means are shown in brackets

Picking date	Days to fruit softening for irrigation regime				LSD		CV%
	35 kPa	55 kPa	80 kPa		$P = 0,05$	$P = 0,01$	
04/07	4,1 (+0,1)	6,2 (+0,3)	4,3 (+0,5)	*	1,1	1,5	23,6
06/07	4,4 (+0,2)	4,0 (+0,2)	3,5 (+0,2)	N.S.			
07/05	4,9 (+0,1)	4,8 (+0,1)	4,9 (+0,2)	N.S.			
LSD $P = 0,05$	*	**	N.S.				
$P = 0,01$	0,4	0,7					
CV%	0,6	1,1					
	10,5	15,7					

Table 8 Effect of irrigation regime on 'Fuerte' fruit softening without storage during the 1983 picking season. Significance levels are indicated by * for $P=0,05$ and ** for $P=0,01$. S.E. of means are shown in brackets

Picking date	Days to fruit softening for irrigation regime				LSD		CV%
	35 kPa	55 kPa	80 kPa		$P=0,05$	$P=0,01$	
04/13	8,9 (+0,2)	9,5 (+0,2)	10,0 (+0,1)	*	0,4	0,5	4,3
06/08	8,6 (+0,2)	8,6 (+0,2)	7,9 (+0,3)	*	0,7	1,0	9,1
07/12	8,1 (+0,3)	8,2 (+0,2)	8,4 (+0,3)	N.S.			
	*	**	**				
LSD $P=0,05$	0,6	0,5	0,7				
$P=0,01$	0,8	0,8	0,9				
CV%	7,5	6,8	8,5				

The later in the season fruit was picked, the less difference was found in softening rates for fruits from the various irrigation treatments. This could imply that the fruit had become over-mature.

3.2.2.1.3 Harvest 1984

In 1984, fruit was picked in May, and allowed to ripen at 22°C without storage. Results are shown in Table 9. The dryland fruit ripened most rapidly. The only significant difference ($P<0,05$) occurred between fruit from trees irrigated at 80 kPa and dryland, and although possible trends are indicated, the lack of clear differences makes interpretation difficult.

Table 9 Effect of irrigation regime on 'Fuerte' fruit softening without storage during May, 1984. Significance at P=0,05 is indicated by *. S.E. of means are shown in brackets

Days to fruit softening for irrigation regime							
35 kPa	55 kPa	80 kPa	Dryland		LSD		CV%
					P=0,05	P=0,01	
8,6 (+0,3)	9,7 (+0,3)	10,4 (+0,8)	8,2 (+0,3)	*	2,1	2,8	16,6

The fruit water potential at the time of picking did not differ significantly between irrigation treatments in 1984 (section 2.2.2). This may imply that ripening physiology can be influenced by past irrigation and or rainfall history, and not only the stress situation immediately prior to picking.

Overall, a number of factors seem to play a role in avocado ripening, and the relative influence of short (immediately before picking) and long-term stress are not so clear from the results presented. The work of Adato & Gazit (1974), which implied that water stress plays a direct role in accelerated ripening, may reflect only the short-term effect, and further research is required.

3.2.2.2 Effect of irrigation regime on ethylene evolution

During 1983, the memory board of the integrator became faulty on day 8 after picking. Most fruits were approximately 50% soft by this stage, but because the entire pattern of ethylene evolution could not be determined, meaningful results cannot be presented. Results are therefore those of the 1983/84 season.

Most authors seem to evaluate the pattern of ethylene

production during ripening in terms of days after picking. While this will indicate how rapidly ethylene is produced, it is not very useful in evaluating the manner in which ethylene may be involved in the actual ripening process. This is due to the large variations in softening found in fruits between, as well as within treatments. Due to the rapid changes which occur in ethylene production over a short space of time, together with differences in individual fruit ripening rates, very large standard errors occur when ethylene evolution is plotted against time at 1 day intervals. It was therefore decided to compare ethylene evolution with fruit softness, as indicated by the firmometer. Even using this method, variation between fruits (as also indicated by Saltveit & McFeeters (1980) in cucumbers) made it difficult to plot values for individual fruits. The data for each treatment were therefore grouped according to softness. The histograms in Fig. 28a to d indicate the frequency of softness values obtained. Results were grouped, and the mean softness with corresponding rate of ethylene evolution plotted as in Fig. 29.

The various irrigation regimes resulted in different patterns of ethylene evolution. Fruit from the 55 kPa soil moisture tension and dryland treatments appeared to follow the most normal trend when compared with results in Leopold & Kriedemann (1975), and Eaks (1980). The first ethylene recorded was approximately $15,3 \mu\text{l kg}^{-1} \text{h}^{-1}$ when fruit was just over 30% soft. This was approximately the lower limit of accurate ethylene detection in the system used, and should represent an internal tissue ethylene concentration of $30 \mu\text{l kg}^{-1} \text{h}^{-1}$ according to the formula of Burg & Burg (1962b), whereby an ethylene evolution of $1 \mu\text{l kg}^{-1} \text{h}^{-1}$ was equivalent to $2 \mu\text{l kg}^{-1} \text{h}^{-1}$ internal ethylene. This contrasts with the basal tissue concentration of $0,03 \mu\text{l kg}^{-1} \text{h}^{-1}$ as found by Kosiyachinda & Young (1975) for 'Fuerte'. Burg & Burg (1962a), however, quoted a basal figure of $2 \mu\text{l kg}^{-1} \text{h}^{-1}$.

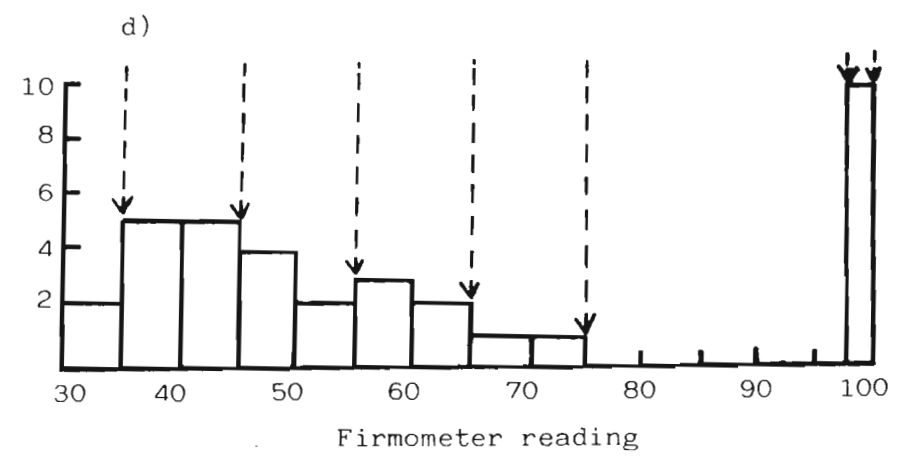
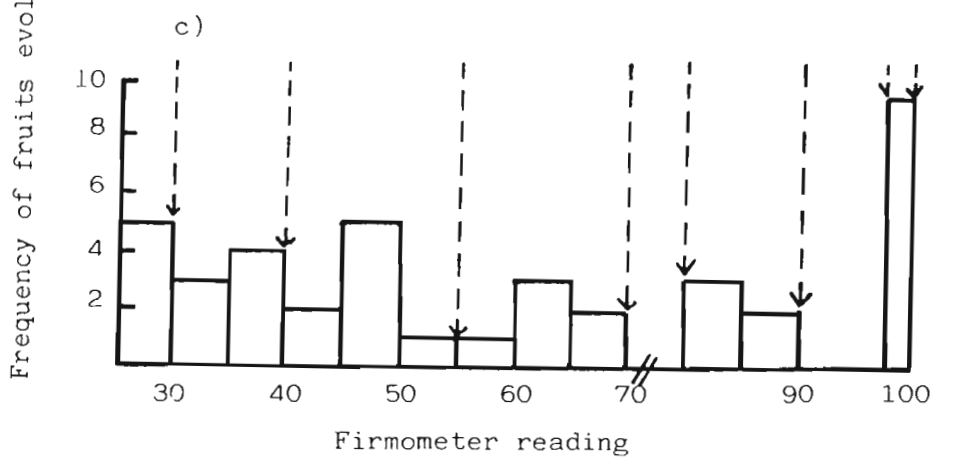
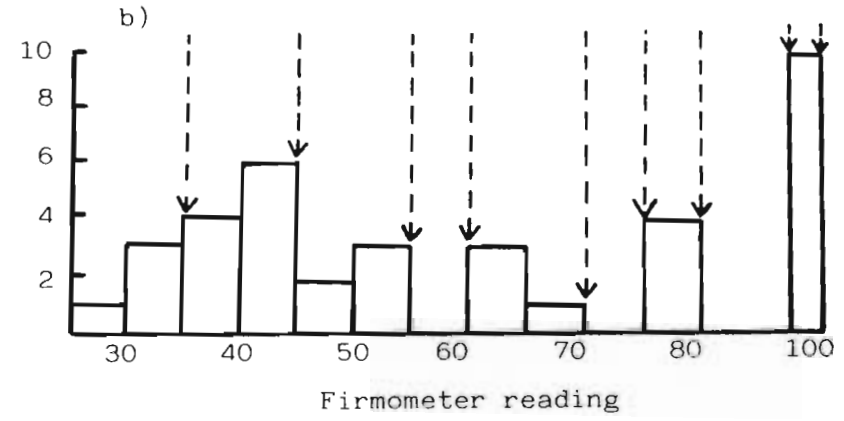
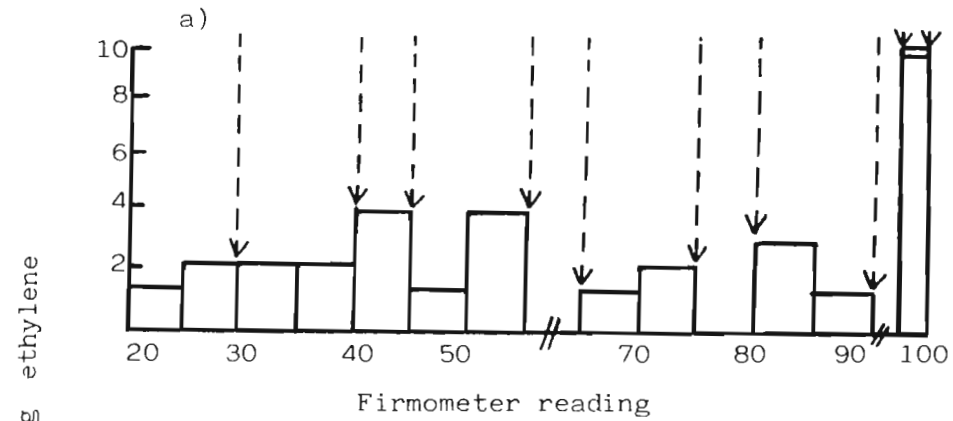


FIG. 28 Frequency distribution of 'Fuerte' fruits which showed ethylene evolution within a particular softness range for four irrigation regimes. a) Indicates the 35 kPa regime, b) 55 kPa c) 80 kPa and d) dryland. Dotted lines indicate boundaries for data grouping for use in Fig. 29

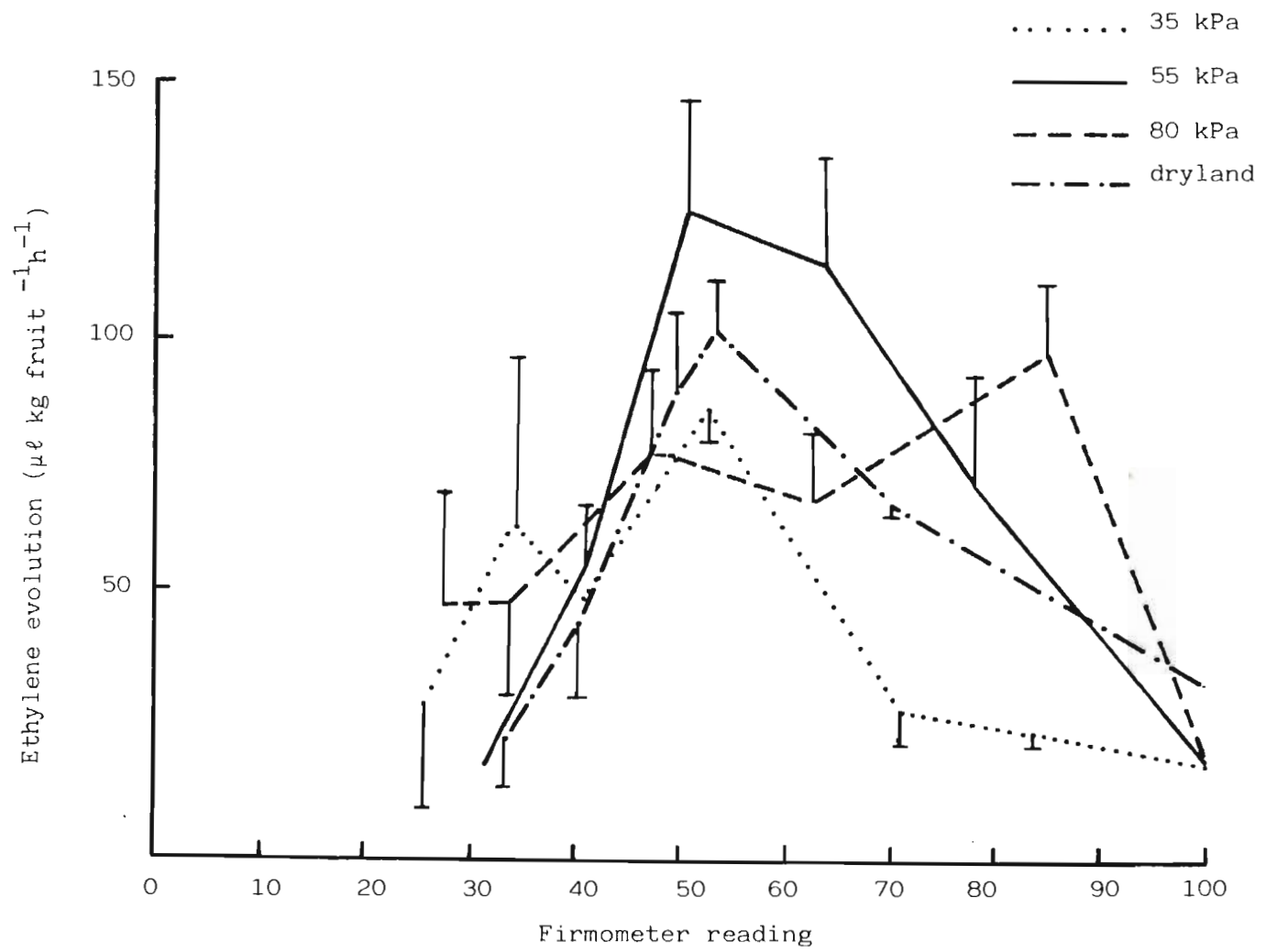


FIG. 29 Ethylene evolution during 'Fuerte' fruit softening for four irrigation regimes. Bars indicate S.E. of means

This indicates that the ethylene rise had already begun. Nevertheless, the sensitivity seems equally as good as that of Eaks (1980). Thereafter, a rapid and continuous increase in ethylene evolution with softening occurred, with a maximum value of approximately $125 \mu\text{l kg}^{-1} \text{h}^{-1}$ at a softness close to 50%. A fairly steady decline then occurred, until at 100% soft, slightly more than $15 \mu\text{l kg}^{-1} \text{h}^{-1}$ ethylene was produced. The maximum value of ethylene production compared reasonably with the approximately $150 \mu\text{l kg}^{-1} \text{h}^{-1}$ found by Eaks (1980), approximately $100 \mu\text{l kg}^{-1} \text{h}^{-1}$ by Awad & Young (1979), and $110 \mu\text{l kg}^{-1} \text{h}^{-1}$ by Zauberman & Fuchs (1981). This curve therefore followed normal characteristics.

Fruit from the treatment irrigated at 80 kPa soil moisture tension appeared to produce ethylene earlier, and more rapidly than those from the 55 kPa treatment, as the first ethylene was recorded in slightly harder fruit (approximately 27% soft) and at a higher level ($47 \mu\text{l kg}^{-1} \text{h}^{-1}$ as opposed to $15 \mu\text{l kg}^{-1} \text{h}^{-1}$ respectively). Thereafter, little change occurred until approximately 35% soft, after which a slow increase to 47% soft ($78 \mu\text{l kg}^{-1} \text{h}^{-1}$) took place. As softening continued, an approximately constant ethylene evolution occurred, followed by a second increase, peaking at approximately 84% soft. A rapid decline to the 100% soft stage followed. This curve may indicate a malfunction in the ethylene-forming system of these fruits. Both the lower primary peak level and the apparent slow increase to a later, major ethylene peak could be important to ripening.

As these results apply to only one season, caution is needed in their interpretation. If Tucker & Laties (1984) are correct in their theory of gene expression alteration by ethylene causing cellulase production, then normal softening could be affected by the ethylene evolution pattern. This could be an explanation for the apparent anomaly of slower ripening under more stressful conditions often reported by

the industry. The reasons for the slower appearance of ethylene, and as the effect of long-term stress on the various factors affecting ethylene formation, are unknown. The fact that long and not only short-term stress can play a role in ripening physiology has been demonstrated, as no difference in fruit water potential at picking was evident.

Fruit from frequent irrigation (35 kPa soil moisture tension) showed a somewhat different pattern to the 55 kPa treatment. Kapuya & Hall (1984) showed that ethylene levels even in a hydrophyte can change with waterlogging. Whether this treatment did become waterlogged (the 450 mm tensiometer readings seldom reached 35 kPa), or whether some additional stress due to P. cinnamomi occurred is unknown. Ethylene evolution began increasing when fruit was fairly hard, and somewhat earlier than other treatments. A slightly slower increase to a lower peak than the 55 kPa treatment was reached at less than 35% softness, although this should be cautiously interpreted considering the large SE of data at this point. The major peak occurred at close to 50% softness, (Fig. 29), although the mean value of ethylene evolution was only $88 \mu\text{l kg}^{-1} \text{h}^{-1}$ as opposed to the $125 \mu\text{l kg}^{-1} \text{h}^{-1}$ for the 55 kPa soil moisture treatment. Thereafter a rapid decline in ethylene production took place with softening, reaching $27 \mu\text{l kg}^{-1} \text{h}^{-1}$ at 70% softness, slowly declining to $14 \mu\text{l kg}^{-1} \text{h}^{-1}$ at 100% softness. Although this curve is clearly different to that of the 55 kPa soil moisture treatment, the highest peak ethylene production was reached at close to 50% softness, and probably ripening should not have differed markedly from the 55 kPa treatment. Ripening was slightly (but not significantly) more rapid for the 35 kPa treatment.

The dryland treatment showed an apparently normal ethylene curve, having only a single peak at near 50% softness. The rate of ripening was nevertheless the most rapid of all

treatments. Other, unknown factors can also be influenced by long-term irrigation practices, and can in turn influence ripening. However, as previously mentioned, the lack of 1983 crop in this treatment may have altered tree physiology to the extent that 1984 results could be misleading. The effect of stress acclimation is also unknown, but will be of considerable future interest. Only repeated work in the future will clarify the situation.

3.3 FRUIT CALCIUM RELATIONS

3.3.1 MATERIALS AND METHODS

During the 1982/1983 and 1983/1984 fruit growth seasons, 'Fuerte' fruit were analysed for mineral constituents, with particular emphasis on calcium content. Fruits were picked from as soon after set as possible until mid-way through the normal harvest period. Although fruit set in the avocado can occur over an extended period, a particular set period was identified, and thereafter all fruit picked corresponded to this set. A number of fruits were marked at the start of the season, and used as a basis for selection of data fruit from the same set period, by the criterion of fruit size.

In 1982/83, sixty fruits were picked for the first two dates from each irrigation regime with the exception of the dryland trees which, as previously mentioned, had a very poor crop. Fruits were then randomly divided into six groups of 10 fruits. Such a combined sampling was used due to the small mass of fruits at these stages. Half the fruits were picked from the northern and half from the southern sides of five randomly selected data trees. From the third picking date onwards, six single randomly selected fruits from the northern and southern sides were analysed. Fruits were picked 14 times during the season, this being related to the capacity of the analytical laboratory. From the results of the first season, the number of fruits analysed was increased but the sampling dates reduced to nine for the second season. In this case 36 fruits, 18 each from the northern and southern sides of the five data trees were analysed. Each analysis consisted of a composite sample of three fruits, again with the exception of the first two sampling dates, where 10 fruits comprised each of the six composite samples from both north and south sides of the trees.

Immediately after picking, fruits were weighed (for plotting fruit growth curves), peeled, the seed removed and the fruit flesh divided into proximal and distal sections as described for the PPO analysis (section 3.1.1). Thereafter, fruits were chopped into small pieces to facilitate drying at 70°C to constant mass. The dried samples were finely ground before analysis.

Analysis was done by the Soil Science and Chemistry department of the CSFRI, Nelspruit. The method used for calcium analysis was as follows: To a 0,5 g fruit sample was added 5 ml conc. HNO_3 and 2 ml H_2O_2 . The mixture was left overnight for digestion of organic material. Thereafter, 60% HClO_4 was added, and once the solution had clarified, it was made up to 25 ml with distilled water. Estimation of calcium was done by atomic absorption spectroscopy. Known standards were included after every 10 samples as a check. Results were calculated in $\text{m eq } 100 \text{ g dry mass}^{-1}$.

3.3.2 RESULTS AND DISCUSSION

While both the proximal and distal portions of fruits were initially analysed, it was found that the pattern of calcium change in these portions through the season was similar. As fruit physiological problems are usually first visible and most severe in the distal portion of avocado fruits, only these data are presented. The fruit calcium concentrations through the season, as well as fruit growth data are presented for two seasons.

3.3.2.1 The 1982/1983 season

The calcium concentration results are depicted graphically in Fig. 30. The general pattern was for calcium concentration in fruits from all irrigation regimes to increase rapidly very early in fruit growth (first 7 weeks), decreasing rapidly during the following 9 to 11 weeks, and then remaining at a relatively low and stable value until final fruit picking. This pattern is in accordance with the findings of Quinlan (1969) and Tromp (1978), who found that most of the calcium uptake in apple fruits occurred within the first six weeks.

The calcium concentration curve of Quinlan (1969) for apples is almost identical to Fig. 30. Wilkinson (1968) explained this trend on the basis of rapid uptake during the cell division stage of fruit growth, followed by slower uptake or even loss, during cell expansion. The avocado fruit shows some cell division throughout its growth, although maximum cell division occurs early in fruit development (Barmore, 1977), thereafter slowing down with cell expansion becoming predominant. No clear differences between treatments emerged, although fruit from the 55 kPa treatment had a higher calcium concentration than the other regimes, between 7 and 16 weeks after fruit set ($P < 0,01$ at 13 weeks). Surprisingly, considering the supposed effect of water stress (Bangerth,

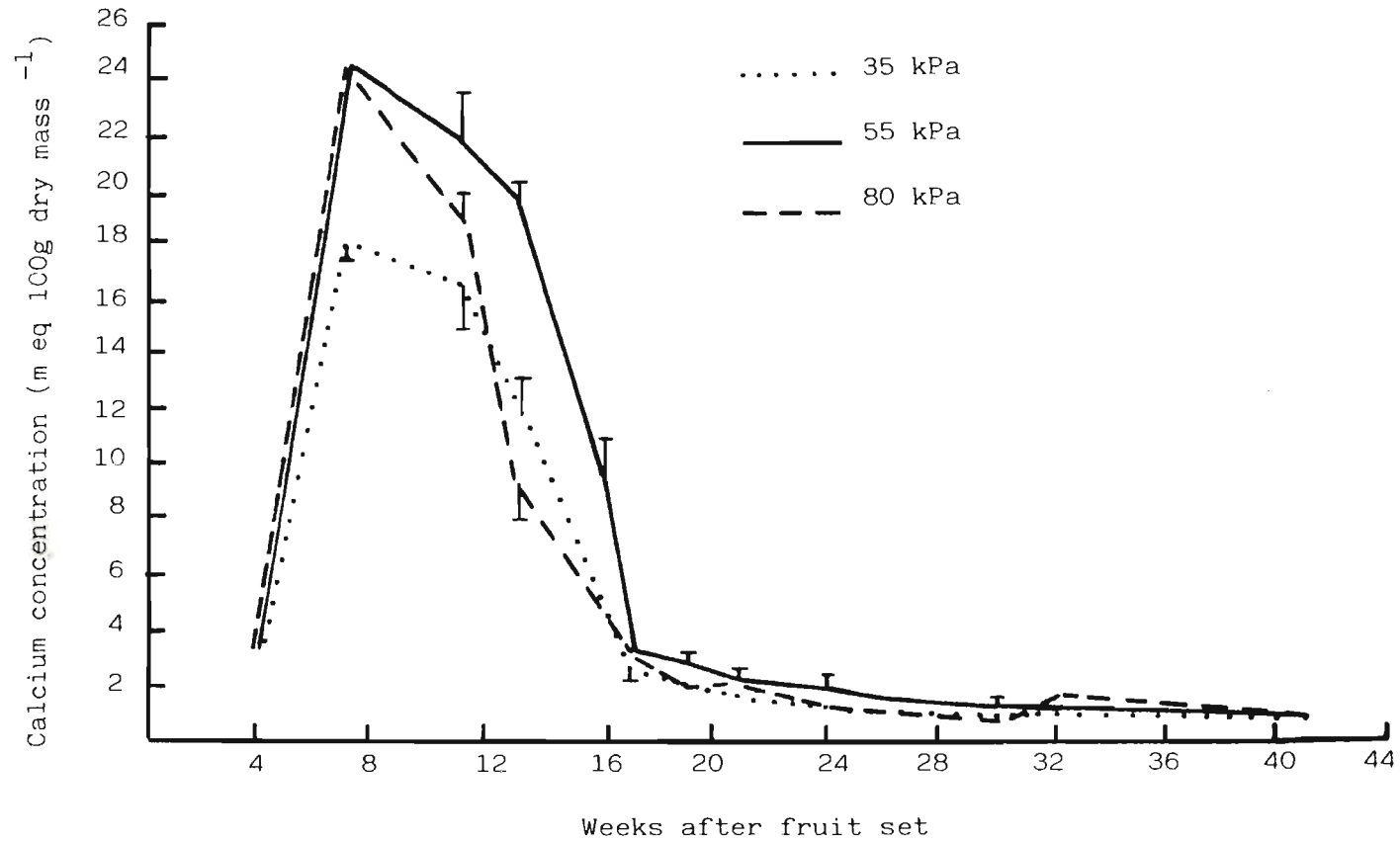


FIG. 30 Fruit calcium concentration changes in the distal portion of 'Fuerte' fruit during the 1982/1983 season. Bars indicate S.E. of means

1979) the treatment with the least supplementary irrigation had a high calcium concentration at 7 weeks following fruit set, having the same concentration as the 55 kPa regime at this stage. The very frequent schedule (35 kPa), however, showed a tendency to a lower concentration at this stage, although it was not statistically significant.

Examination of the fruit growth curves in Fig. 31 may explain at least some of these trends. The growth curves are sigmoidal in shape, in agreement with the work of Robertson (1971) and Barmore (1977). The initial rapid calcium uptake could be explained by the link between actively dividing cells and calcium accumulation (Bangerth, 1979). The fruit growth curves indicate that cell expansion was probably not occurring as rapidly in the 80 kPa treatment fruit as the others. As the fruits increased rapidly in size, uptake presumably did not keep pace with the rapidly expanding and dividing cells, resulting in calcium dilution. The initial advantage of the 80 kPa treatment was lost, because although the fruit expanded more slowly, uptake was presumably limiting after the first 7 weeks following fruit set. A very rapid decline in fruit calcium concentration then ensued. At 13 weeks after fruit set the 55 kPa treatment fruit had a significantly higher ($P < 0.01$) calcium concentration than the 35 kPa treatment, with the 80 kPa treatment even lower than the 35 kPa treatment. Whether this is physiologically significant is not known. However, cell division and rapid cell expansion occurs during this period, and the steeper the declining curve the more this infers an imbalance in uptake relative to growth. A deficiency at a critical period could therefore be encountered. The period of most rapid calcium change occurred during the time of normal fruit drop (the November drop) when large physiological changes occur and the competing spring growth flush develops. The role of calcium as a second messenger and not only the total concentration, may thus play an important role in fruit physiology at this

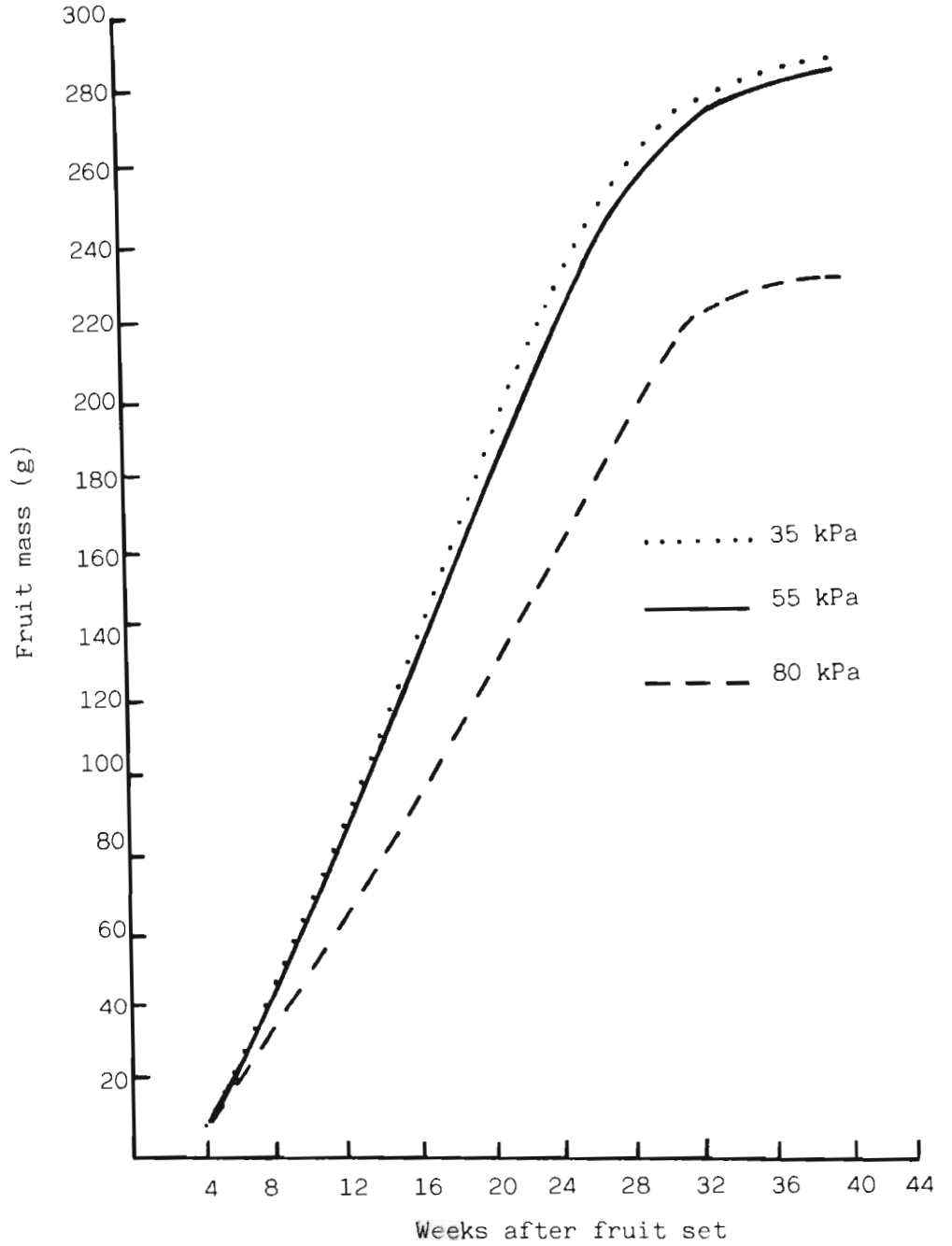


FIG. 31 Trends in 'Fuerte' fruit growth during the 1982/1983 season

time.

The calcium concentrations of all treatments were almost identical to each other from approximately week 16 onwards. This infers, if calcium per se affects fruit physiology, that critical influences of calcium occur early in the life of the fruit.

3.3.2.2 The 1983/1984 season

The pattern of calcium accumulation is shown in Fig. 32. The pattern differed slightly from the previous season, in that the initial increase in calcium concentration was not determined. Further, absolute levels were lower than the previous season. During the period 2 to 7 weeks after set when fruit of the previous year showed a marked increase in calcium concentration, all treatments were showing a rapid decline, particularly fruits from the 35 kPa treatment, which had shown a higher calcium concentration at 2 and 4 weeks than other treatments. By 8 weeks treatment differences had narrowed, although the more frequently irrigated regimes still had a higher calcium concentration ($P < 0,05$). Either maximal calcium concentrations were reached extremely soon after fruit set, or sampling was not frequent enough to detect rapid changes in concentration.

The higher calcium concentrations (Fig. 32) during the initial period of fruit growth (Fig. 33) were similar to the previous year, which strengthens the likelihood that critical changes in calcium concentration occur early during fruit development.

Further work will be necessary to confirm the degree to which these trends vary from season to season, and the actual role of calcium in avocado fruit physiology.

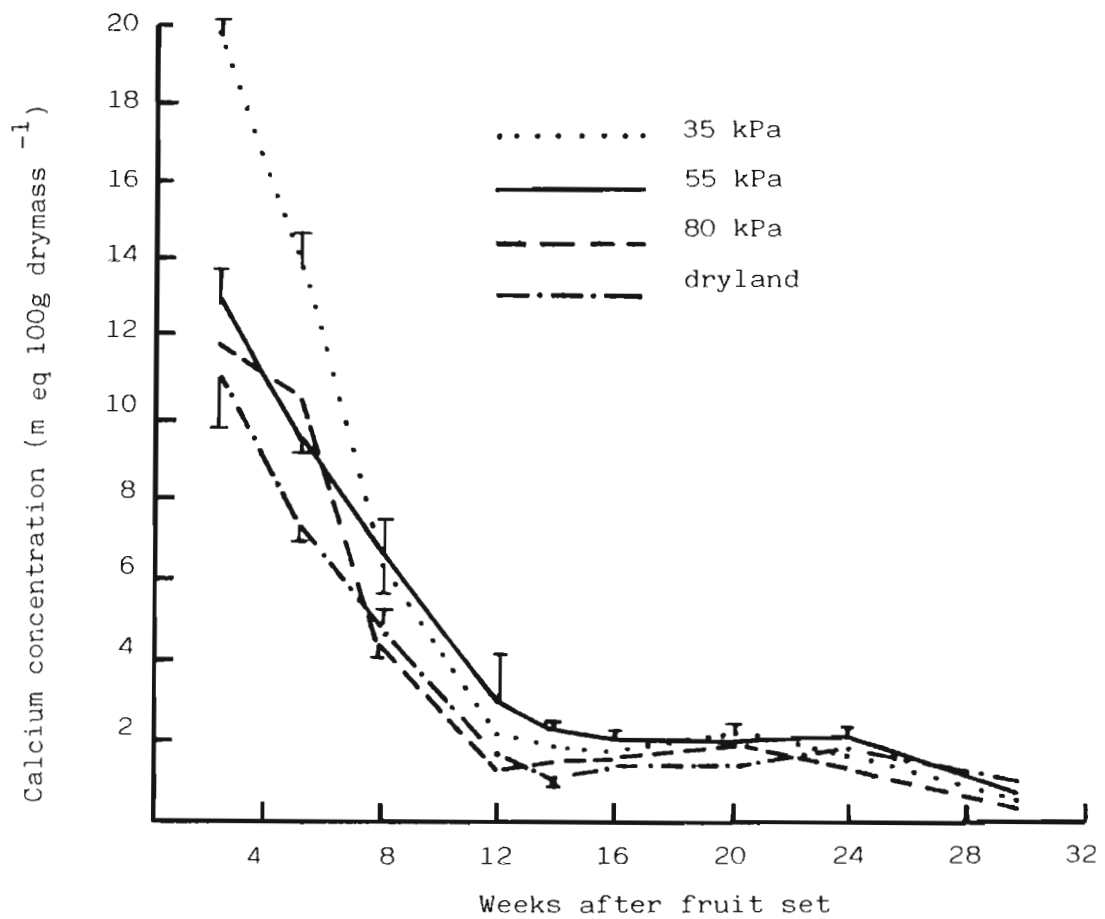


FIG. 32 Fruit calcium concentration changes in the distal portion of 'Fuerte' fruit during the 1983/1984 season. Bars indicate S.E. of means

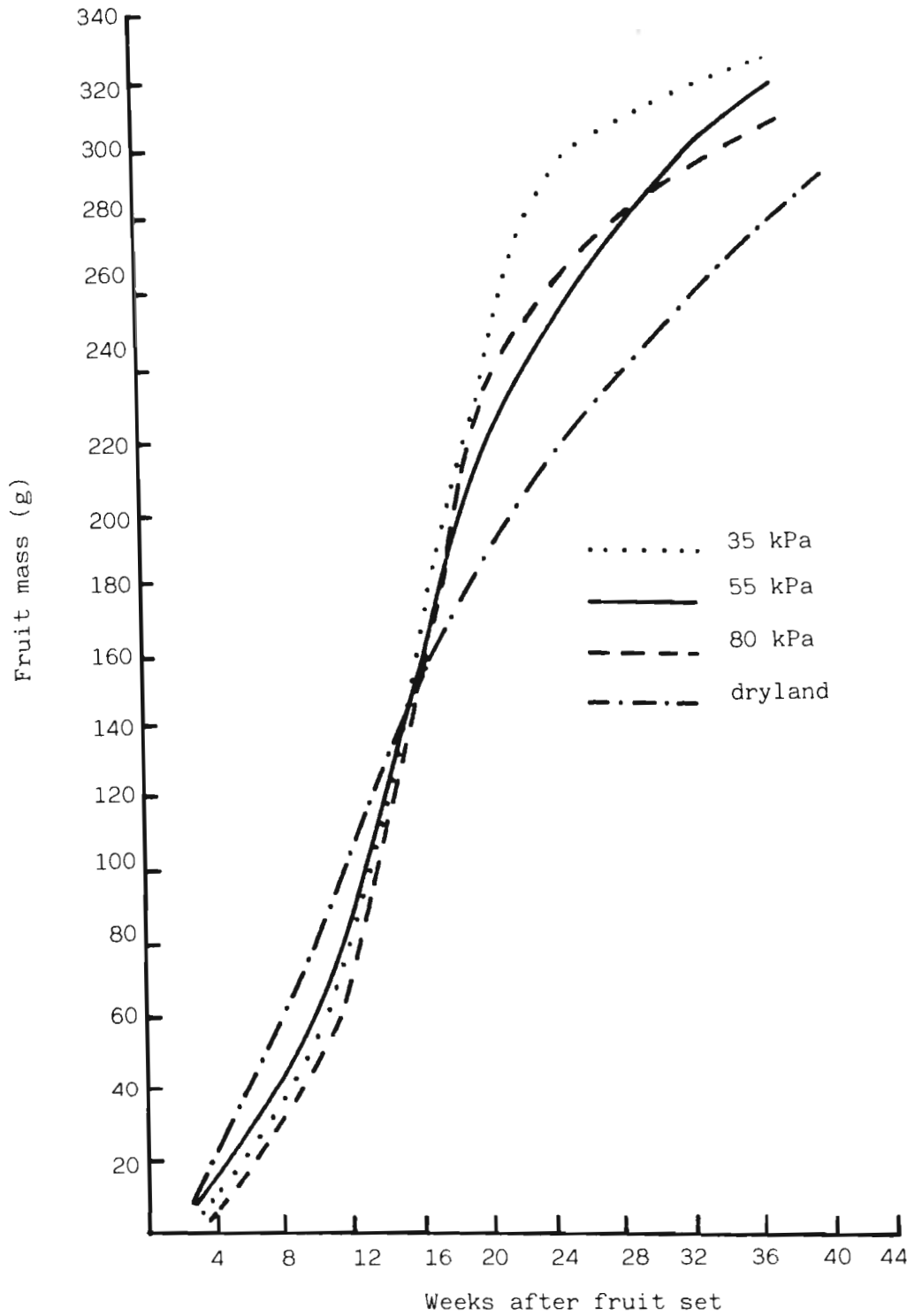


FIG. 33 Trends in 'Fuerte' fruit growth during the 1983/1984 season as influenced by irrigation regime

3.4 ABSCISIC ACID

3.4.1 MATERIALS AND METHODS

The same 'Fuerte' fruits (thus with the same moisture stress histories) analysed for PPO activity in 1983 were also used for abscisic acid analysis. Three classes of April-harvested fruit, viz. hard fruit, fruit indicating 50% softness on the firmometer (Swarts, 1981), and soft fruit, were analysed. For late-harvested fruit, only soft fruit were tested. In each case, distal pulp tissue from five fruit was combined for every sample, as it was not possible to assay as many samples as for PPO activity. Two replicates of each sample combination were assayed.

3.4.1.1 Extraction and assay of ABA

Various extraction and assay techniques for ABA are available. If large numbers of samples are to be assayed, a bioassay may seem appropriate. Bakken & Boe (1982) reported on two highly efficient bioassays which do not need additional growth regulators in a medium to ensure a response, and are rapid and easy to perform. However, as with all bioassays, the interactive effect of growth regulators not being assayed but present in the biological sample may be problematical. Considerable purification of the sample would therefore be necessary. The possible lack of accuracy and precision in bioassays has worried other authors (Letham 1967; 1978; Dekhuijzen & Gevers, 1975; Horgan, Palni, Scott and McGraw, 1981). According to Brenner (1981), limited sensitivity and selectivity of bioassays makes them questionable for plant growth substance analysis.

Of the physicochemical techniques, high performance liquid chromatography with UV detection has been successfully used (Ciha, Brenner & Brun, 1977) but the high oil content of

avocado fruit necessitates excessive pre-purification. Gas chromatography with an electron capture detector has been very successful for a methylated derivative of ABA (Seeley & Powell, 1977), but such facilities were unavailable. Brenner (1981) also notes the extreme care required in pre-purification.

Radioimmunoassay can overcome many of these problems, and is suitable for ABA extracts of limited purity (Weiler, 1982). Several ABA assays are available, but that of Cutting, Lishman, Hofman & Wolstenholme (1985) was chosen, since the work could be conducted in their laboratory. The possibility also existed for assay of free (active) as well as bound (inactive) ABA using the same antisera, unlike Weiler's (1980) technique. The procedures outlined below are those of Cutting et al. (1985).

3.4.1.1.1 Extraction of plant material

Fruit samples of 5 g each were homogenised in 50 ml 80% methanol, and extracted at 5°C for 48 h in the dark. After centrifuging at 1 000 x g for 10 min, the supernatant was evaporated to dryness under vacuum at 30°C. The residue was dissolved in 2,5 ml methanol. A pre-purification to remove the large quantities of oil present, plus pigments, was undertaken.

The plant extract was placed in 10 ml 0,5 M potassium phosphate buffer pH8, and reduced under vacuum to the aqueous phase. Diethyl ether (20 ml) was added, and after separation, the bottom phase collected, and acidified to pH 3 with phosphoric acid. 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 dissolved in 2,5 ml diethyl ether. In order to separate the free ABA from the extract, thin layer chromatography (TLC)

using silica on aluminium plates was conducted. Extract volumes of 20 μ l were chromatographed against authentic free ABA, using toluene : ethyl acetate : acetic acid (50:30:4) as developer. The R_f zone corresponding to free (+)ABA was eluted in 2,5 ml methanol.

3.4.1.1.2 Radioimmunoassay

All samples were duplicated in the RIA step. A 250 μ l aliquot of sample was used, and the RIA was conducted in 10 x 75 mm rimless soda glass test tubes. In addition to the unknown plant extract, standard concentrations of (+)ABA were assayed in order to construct a standard curve (Fig. 34). This was repeated for each group of samples assayed. A typical RIA run format consisted of total assay, non-specific binding determination (NSB), methanol (to determine the effect of methanol on the assay), a blank or 0 standard, standard (+) ABA concentrations and the unknown plant extracts.

The methanol in the plant extracts was evaporated in air at 37°C (water bath temperature) after which 100 μ l BSA in phosphate buffered saline (PBS) of pH 7 was added to all tubes (for coating) except those for NSB determination, where 100 μ l of 0,1 % gelatine in PBS pH 7 was used. All tubes were vortexed and allowed to stand for 15 min. Thereafter, suitably diluted anti- ABA (100 μ l) was added to the bottom of the tubes, except those for total assay and NSB. The anti-ABA used was that of Cutting *et al.* (1985), and the characteristics as determined by them accepted. Thereafter, 100 μ l tritiated (+)ABA was added to all tubes and vortexed. After incubating for 15 min at 37°C, tubes were again vortexed before stabilization of the reaction mixture in an ice bath for 2 hr. Separation of the bound (for activity counting) and free ABA was achieved with 800 μ l of dextran-coated charcoal (125 mg charcoal and 250 mg dextran in 50 ml distilled water) at 4°C (Herbert 1968). In the case of total

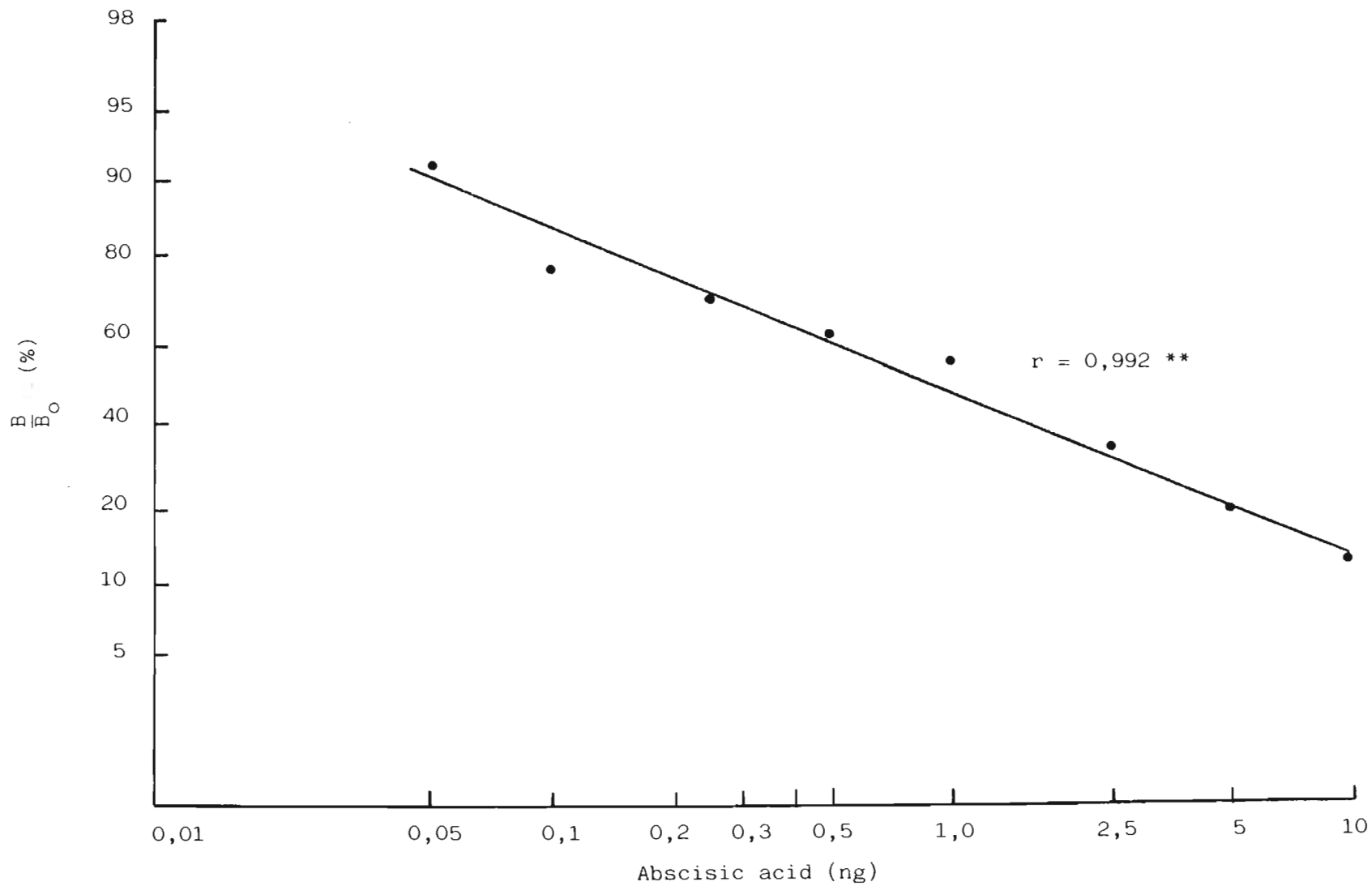


FIG. 34 A typical absciscic acid concentration standard curve in the log logit format. B is the proportion of tritiated ABA bound and B_0 is the zero standard.

assay, 800 µl distilled water was used instead. Tubes were vortexed, and after 10 min centrifuged at 2 000 x g for 10 min at 4°C, to pellet (and thus separate) the absorbed free fraction. The supernatant containing the anti-ABA bound fraction was decanted into standard scintillation vials containing 12,5 ml scintillation cocktail, and shaken until a single phase was obtained. The scintillant consisted of 12 g 2,5-diphenyloxazol and 0,3 g popop ((1,4-di-(2-(5-phenyloxazolyl) benzene) dissolved in 3 l toluene, with 1,5 l "Triton X-100" added. After 10 min scintillation vials were placed in a "Beckman LS 8100" liquid scintillation counter for activity determination.

3.4.1.2 Calculation of results

A linear calibration curve was obtained by the logit transformation (Chard, 1978). The curve was obtained by plotting logit B, where B = proportion of tritiated (+)ABA bound, expressed as a percentage of the 0 standard, against log known ABA. For the unknown plant samples dilution was taken into account, and results expressed as ng g fresh mass⁻¹.

A known standard was run through the entire extraction and assay procedure, for determination of recovery rate. Results were adjusted accordingly.

3.4.2 RESULTS AND DISCUSSION

The recovery of free abscisic acid through the assay was found to be 80%, and all results were adjusted accordingly. As was the case with PPO, results from the north and south sides of the trees have been combined, and are depicted graphically in Fig. 35. The most stressed (80 kPa soil moisture tension) hard fruit, samples were lost during the assay and could not be replaced. Nevertheless, some important trends for the April harvested fruit were evident.

Overall, there was a slight non significant increase in free ABA concentration as softening increased to the 50% level (which approximately corresponds to the ethylene peak) followed by a significant ($P < 0,01$) decline. This is in accordance with the findings of Adato, Gazit & Blumenfeld (1976), although the actual levels recorded were considerably lower.

It is particularly noticeable that fruit from the 55 kPa soil moisture tension irrigation regime showed the smallest change in ABA concentration during ripening, with no significant difference between firmometer readings of 20 and 50, thereafter showing a moderate non significant decline. This treatment also showed a tendency to a lower ABA level, although not significantly, immediately after picking than did the 35 kPa soil moisture tension treatment. At 50% soft, the 55 kPa treatment had significantly ($P < 0,05$) lower ABA than the 35 kPa treatment and considerably ($P < 0,01$) lower than the 80 kPa fruit.

The water potentials of the fruits analysed for ABA did not differ significantly. Thus factors other than water relations at the time of picking may result in higher ABA levels appearing during fruit softening. As differences were irrigation treatment related, a water stress at a period

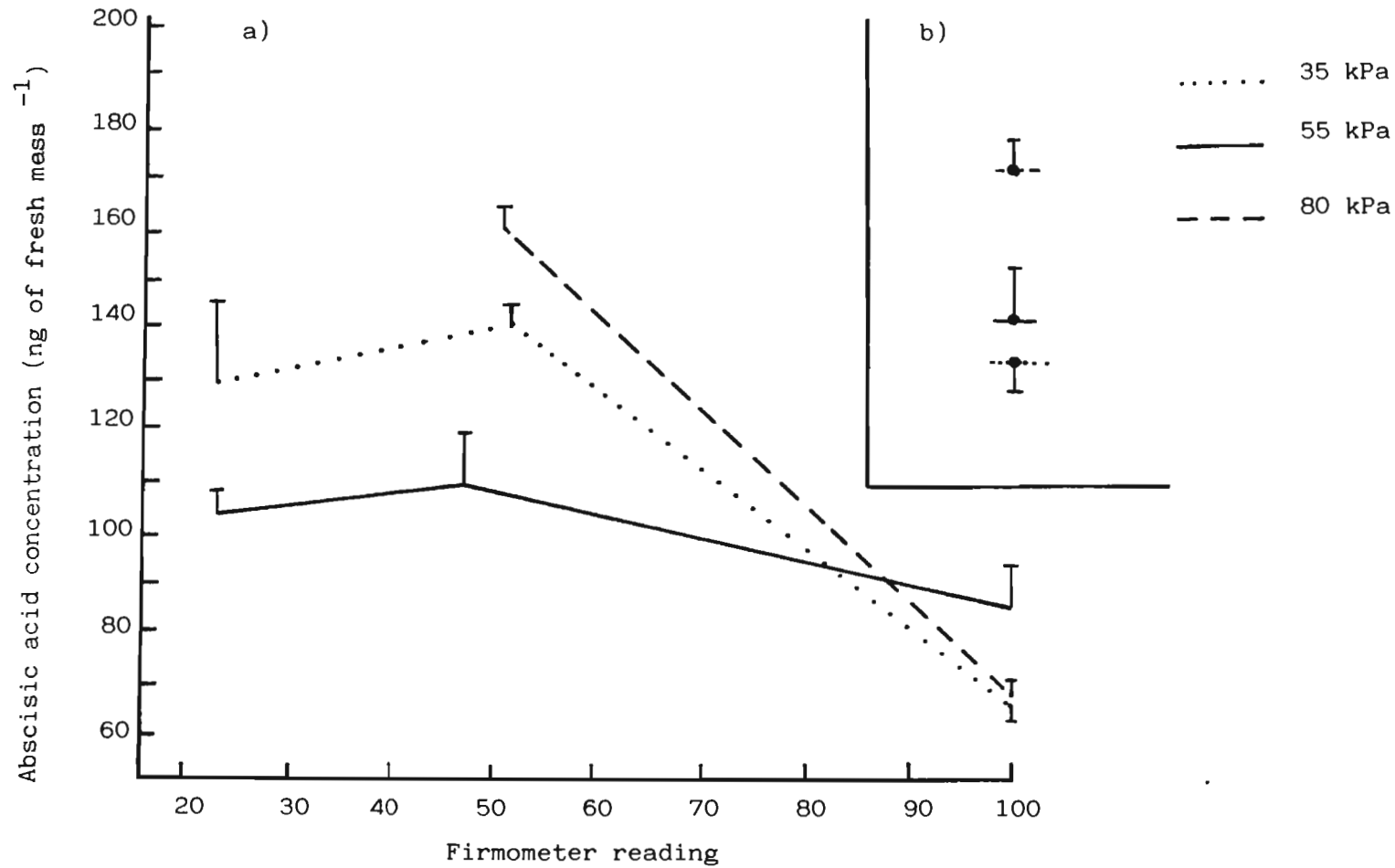


FIG. 35 Free abscisic acid concentration in distal 'Fuerte' fruit flesh as influenced by irrigation and softening, a) depicts changes for April harvested fruit and b) concentration in fruits harvested in July, and at a firmometer reading of 100. Bars indicate S.E. of means. Scale of b) is the same as for a)

earlier in the season is likely to be important. The period of likely maximum stress would have been before the summer rains, i.e. the first two months of fruit development, or the period of early cell division (Robertson, 1969), which did indeed correspond with dry conditions (Fig. 2). Fruit from over-watered trees may incur "stress" at any time.

The level at which ABA influences ethylene stimulation for normal ripening is unknown. However, no indication was found of a common level of ABA being reached during the pre-climacteric phase for any of the treatments. The levels of ABA reached at 50% softness at approximately the ethylene climacteric peak (see section on fruit ripening) differed considerably, although they must have been in excess of the level required for ethylene stimulation. Lieberman, Baker & Sloger (1977) concluded that ABA accelerates ageing during ripening. This could mean more rapid cell degeneration and therefore quality problems.

The soluble PPO activity showed a very similar trend in activity level when compared with ABA levels. There was in fact a linear correlation of 0,875 which was significant ($P < 0,01$). Recent work in apples has indicated a link between browning and ABA. Wills & Scott (1974) found that injection of ABA into apples caused 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 highly correlated with the degree of browning occurring later. Wills, Franklin & Scott (1978) found the level of free ABA was reduced by calcium. Calcium has been linked to membrane stability and physiological disorders by many workers.

The free ABA concentration for soft fruit harvested late in the season was extremely high (Fig. 35) when compared with early season results, as was the PPO activity.

3.5 GENERAL DISCUSSION

Water relations at the time of picking do appear to have some effect on post-harvest avocado fruit physiology. However, trends are either not entirely consistent, or large differences in physiology (such as PPO activity and ABA formation) occur in relation to the long-term irrigation treatments, without there being large (especially in normally accepted physiological terms) differences in short-term fruit water potential. Thus, poor irrigation management near the time of picking may, as demonstrated by Adato & Gazit (1974), cause more rapid fruit ripening, but the effect on other quality factors is unknown. The data presented, particularly that pertaining to PPO activity, ethylene formation and ABA, indicate deep-seated physiological differences, which are likely to have occurred early in fruit development. Fairly regular rainfall occurred from December 1983 and December 1984 until the start of normal harvest, in each case implying that this should not have been a major period of stress. The most likely stress period is therefore during the first two months after fruit set. Both the rainfall pattern (Fig. 2) and the seasonal variations in leaf water potential (Fig. 5) support this contention.

While the avocado fruit is unusual in exhibiting cell division throughout its development (Schroeder, 1960), maximum cell division is greatest in young fruits (Blumenfeld & Gazit, 1974). Thus, a substantial proportion of the final cell number could be produced during the period of possible stress and any ultrastructural damage at this stage could be lasting. This would explain the irrigation regime-related physiological results obtained.

Poor irrigation practices before and soon after fruit set could result in poor quality fruit at harvest, even if water relations improved later in the growing season. This is

particularly important in relation to fruit browning. The role of spring vegetative growth and competition with fruit for calcium has not been investigated, but deserves future attention. On the other hand, there is also the indication, confirmed by the work of Adato & Gazit (1974), that water stress near the time of picking can affect post-harvest physiology, particularly the rate of ripening. Such a situation could decrease the quality of potentially good fruit or further reduce the quality of an already damaged fruit. Water stress is therefore undesirable at any time during the fruit development period. However, if one considers normal rainfall in the avocado-producing areas of South Africa, then the period from November until harvest in early April (for 'Fuerte' in relatively warm areas) would not be particularly critical. Sufficient supplementary irrigation from fruit set (July/August) until onset of regular rainfall (normally in November) is, however, necessary if potentially good quality export fruit is to be obtained.

A history of water stress seems to adversely affect all the physiological attributes studied. There may be a direct link between water relations and physiological abnormalities outlined in the Results sections, while stress may also affect some common factor. As previously noted, membrane structure and stability is likely to be the common factor. The long-lasting effect of stress on fruit physiology, as well as evidence that a substantial release of bound PPO occurs during "suffocation" of fruit from the 80 kPa regime but not from 55 kPa fruit supports this possibility. Morre (1975) noted, however, that knowledge of the processes and factors affecting membrane formation in the case of different organelles is poorly understood. To discuss the manner in which water stress may directly or indirectly affect these processes is therefore difficult, and beyond the scope of this work. A similar problem exists in the case of ethylene formation, where little more than a broad outline

of the system is known, without knowledge of all limiting or potentially (depending on cell environment) limiting situations.

There is still considerable disagreement as to whether increases in PPO activity are due to activation by change in conformation, or release of bound PPO from microsome or other membranes. The work presented suggests that both occur, depending on cellular conditions. This is demonstrated by the effect of "suffocation" on stressed fruit, which showed an increase in total PPO activity, thereby indicating enzyme activation. A disproportionate increase in soluble activity as compared with total, showed release also occurred. Low temperature storage caused an increase in total PPO activity (section 3.1.2.1.1.2.2), activation therefore being indicated. Late in the season (July picking date) soluble and total PPO activities were similar, pointing to a release. A change in cytoplasmic pH could, for instance, result in conformational change (Kahn, 1977b). Poor container ventilation and resultant decrease in oxygen content could result in anaerobic respiration with the formation of organic acids (Conn & Stumpf, 1966), thereby changing cell pH. On the other hand, membrane collapse or alteration during the ripening process would be likely to result in the release of bound PPO. Further biochemical work will be necessary to clarify this aspect.

In this regard, Nel, Small & Botha (1984) found that particularly under anaerobic conditions, at pH 6, lipoxygenase (shown to be important in apple storage disorders by Feys, Naesens, Tobbyack & Maes (1980)), increased in activity. As these enzymes are probably membrane bound (Feys, De Mot, Naesens & Tobbyack, 1982) and according to Feys *et al.* (1980) involved in oxidation reactions of structural membrane lipids, this could explain the mechanism of membrane breakdown if it occurs. These authors consider that this

process is involved in onset of senescence, so that ABA could also be linked. Results of this study do not go far enough to permit conclusions to be drawn, but these aspects are avenues for future research. The extent to which these processes occur, and the role of long and short-term water stress will require considerably more basic biochemical research.

There is ample evidence in the literature to implicate calcium in fruit storage disorders (Bangerth, 1979; Millaway & Wiersholme, 1979). This study presents evidence that total calcium levels may only at some stages have been physiologically different as a result of irrigation regime, and that both dry as well as fairly wet (Shear, 1980) soil conditions may decrease uptake. If calcium was implicated in the expression of the PPO results, then this was most likely to have been 7 to 13 weeks after fruit set during the 1982/1983 season.

Any factors which could increase the calcium content in young fruits may be beneficial. However, the importance of calcium transport in and out of the cells, and availability at the correct positions at the correct time cannot be over-emphasised. Little seems to be known concerning these processes, but as Roux & Slocum (1982) noted, changes in intra- and intercellular calcium do occur in response to various stimuli, which in turn result in plant growth changes. These authors support the idea of calcium acting as a second messenger system. This is an important concept, as even though sufficient total calcium may be present, other factors such as water or other environmental stress may prevent it from playing its normal role. A fruit quality prediction system based only on total fruit calcium concentrations would be unreliable, and should be treated with circumspection. Study of the positional as well as concentration changes during critical stages of fruit development could improve knowledge of the role of calcium in

avocado fruit physiology.

It must again be stressed that the events leading to good fruit quality mostly occur early during fruit development, rather than immediately prior to harvest. It is obvious that the creation of favourable total fruit calcium levels by, for example, calcium sprays, is not going to prevent later physiological disorders unless other factors limiting normal cellular activity, such as water or temperature stress, are simultaneously eliminated.

An important link between water stress, physiological (as evidenced by high PPO activities) and ripening disorders, is ABA. Scriven & Wills (1984) established a link between ABA and increased fruit browning in apples. A further link, that of stress history, has now been added. Further, if ABA is involved in the initiation of ethylene biosynthesis as suggested by Bruinsma (1981), an earlier start to ripening as in the 80 kPa fruit (Fig. 29) which had a stress history, can be explained. High ABA levels due to stress, together with the known senescence promotion of ABA could effect the more rapid ripening as well as physiological disorders encountered late in the picking season. ABA infiltration studies will be required to confirm the existence of such a link.

From results of the rate of ripening after picking, 'Fuerte' should probably not be harvested later than approximately mid-May for sea export in the warm lowveld region of the Eastern Transvaal. These results correlate well with observations of fruit ripening in the Nelspruit region (Durand, 1981). Low orchard temperatures during winter are likely to increase ABA levels (Wang *et al.*, 1972), as presumably will low temperature storage. Additional work on the biosynthesis and physiological function of ABA in avocado fruits needs to be undertaken.

CONCLUSIONS

There is evidence that fruit water stress, as a result of tree water stress, can have a marked effect on fruit physiology, with adverse implications for the exporter. An extremely important aspect, however, is that the greatest physiological damage caused by water stress seemed to occur very early in the development of the fruit. This suggests ultrastructural damage, the most likely probably being membrane structure. Membrane effects could explain many of the fruit physiological changes. From this point of view it may be useful to examine ultrastructure during early development. Although an attempt was made, only limited success was achieved, and could thus not be included in the work reported on. Nevertheless, indications were, based on the work of Platt-Aloia, Thomson & Young (1980) that structural differences did exist between the treatments. The writer believes that this is a promising field for a detailed investigation. A study of the biochemistry of PPO as affected by pre- and post harvest stress is required, to clarify the mechanisms involved in the activation of latent PPO.

Calcium and calcium-binding proteins play a role in the development of physiological disorders of fruits (Bangerth, 1979; Millaway & Wiersholm, 1979; Roux & Slocum, 1982). From the evidence presented, it is likely that the same applied to the results being discussed. However, the second messenger status of calcium implies that one must rather look to other factors for solving physiological problems. A study of inter and intra cellular calcium concentrations and calcium binding proteins under varying stress conditions during fruit development is called for. While it cannot be construed that water relations are the only factors involved, a definite link seems to have been established. The interactions between tree, fruit and environment, particularly during the critical period outlined, is of

importance. Firstly, there is environmental water demand, and secondly, the ability of the tree to take up sufficient water to meet this demand. Sterne et al. (1978) portrayed this by means of a model where:

$$\psi_l = \psi_{\text{soil}} - (\text{flux} \times r_{\text{soil to leaf}})$$

where:

ψ = water potential

flux = absolute humidity between leaf and air / r
leaf to air

r = resistance component

This model was originally designed to describe the effect of P. cinnamomi on tree water relations, but may provide a useful basis for describing water relations under varying soil water and atmospheric demand conditions.

Of some importance is the question of canopy area. The higher the leaf area, the larger the evaporative surface. If this is not matched by root area, insufficient water uptake will occur. A study of root-shoot relationships may be worthwhile, and could perhaps have made interpretation of these data easier.

Promotion of vegetative growth will create a larger canopy, with all the effects previously discussed. Most important in this regard, is nitrogen fertilization. As Embleton, Jones & Garber (1959) noted, high nitrogen applications will result in excessive new foliage growth. If moisture is non-limiting, vegetative growth will be considerable. If the spring flush is allowed to develop in this way, canopy water demand will increase, which may or may not be met by uptake. There will also, as discussed in regard to calcium accumulation, be increased competition for calcium and other nutrients. Ludders (1980) found this to be so in apples, and recommended that nitrogen be applied in small, but frequent applications

throughout the growing season. In avocados, early stages of fruit development are critical for fruit quality determination, and the spring flush develops at about the same time. Reduction or control of the vigour of this flush may be beneficial. The practice of nitrogen application in late winter should thus be questioned. This was not a problem as far as the experimental trees were concerned, as no nitrogen applications were given in winter.

The possible role of leaf-fruit ratios in the development of physiological disorders should be examined. A study of the water flow dynamics within the trees of different irrigation regimes could be extremely useful. A study of the mechanisms (almost certainly plant growth substances) affecting the development of the spring flush and the relationship with water relations may be valuable, as would the effect of fruit-leaf competition, particularly in respect of calcium. Calcium tracer studies need to be undertaken. As indicated in Table 4, yields varied for the various irrigation regimes. The effect of differential yields on the development of stress in tree and fruit is, however, unknown, but likely to be substantial. Changes in photosynthetic activity, particularly where acclimation to water stress has occurred, require investigation.

The study under discussion involved seedling rootstocks. The South African avocado industry, due to the pressure of P. cinnamomi, is switching to clonal rootstocks, notably 'Duke 7'. While the significance of water relations effects on fruit quality has been established, and is unlikely to differ with 'Duke 7' rootstocks, the point at which environment becomes limiting may differ. Clonal rootstocks, by virtue of the method of propagation, have a shallow root system. The volume of soil available for water absorption is therefore more limited than a seedling rootstock, and this aspect will have to be examined in future research. Field

observations in fact indicate that avocado trees on clonal rootstocks require more efficient and careful management.

A question often asked by growers, particularly those who have insufficient water, is whether differential irrigation regimes should be used during the year. The period prior to and during early fruit growth is the most important time to ensure adequate tree water, while water stress at other times may be less harmful, although some adverse consequences are likely to result. Should irrigation become necessary due to insufficient rainfall, then 55 kPa soil moisture tension is a suitable level at which water should be applied on high clay soils. This figure is higher than that used under Californian conditions, where sandy soils are often planted to avocados. Further research on varying soil types is needed to refine this recommendation.

Some growers are of the opinion that a dry period in autumn and winter is necessary to ensure good flowering in spring. Observations of flowering and final total tree fruit mass discount this theory. Buttrose & Alexander (1978) indicated that low winter temperatures, which are likely to be encountered by avocados grown in the recommended cool, escarpment areas of South Africa, promote adequate flowering. Recent work by Sedgley, Scholefield & Alexander (1985) further confirmed the interaction between temperature and flowering. An anatomical study to investigate the effect of winter temperature and water stress on reproductive bud development under South African conditions would clarify the matter further.

An aspect which has long been problematical to the avocado export industry is the prediction of fruit quality. Historically, certain years have shown greater physiological problems than others. If a system could be devised to predict the occurrence of physiological disorders well before the

start of a picking season, arrangements could be made to export such fruit by air. This would eliminate the need for long periods of low temperature storage, which increases the likelihood of browning in the fruit. Measurements of parameters such as PPO, total or individual phenolics, abscisic acid or other growth regulators, or ripening patterns are not very useful, as these factors are important at the time of picking, but are unlikely to be very reliable a number of months before picking. Calcium analysis also has limitations, in that major changes of importance take place during a relatively short period, implying that very comprehensive sampling will be required. Even then, as previously discussed, calcium concentrations alone may not be the only criterion of importance.

Taking into account the trends found in calcium uptake, PPO activity, abscisic acid and ripening, plant stress during the period from fruit set to about the end of December is the most critical in terms of the influence on later fruit quality. Water stress is also not usually a problem during late summer. If "stress" during the early fruit development period could be determined, it may be possible to predict fruit quality. A prerequisite of such a model is that it be simple, using only parameters easily obtainable on an industry basis. It would be unrealistic to expect a very high degree of accuracy from such a model, but if a prediction of fruit quality relative to that of a known year could be obtained, considerable benefits to the industry would accrue.

As water stress is linked to fruit quality, it is proposed that an index of stress be computed, and compared with an index linked to a known fruit quality. The work reported could assist in determining the basis of such a stress index. The relationship between transpiration and evaporative demand in the form of the saturation deficit, for non-acclimated trees and non-limiting soil moisture conditions, is known.

The point of stress is at a value of approximately 28 to 30 hPa. Idso, Reginato, Clawson & Anderson (1984) examined leaf-air temperature differentials (which could be a measure of stress) and the relation to vapour pressure deficits. These authors and others cited by them, found stable relationships. The relationship between transpiration and SD is therefore likely to be stable, although future work could result in refinements, taking into account the individual effects of temperature and humidity. Ideally, transpiration (via the SD relationship) could be determined throughout each day, and an integrated value over the critical period calculated as was done to predict citrus greening by Green & Catling (1971). Unfortunately, values of temperature and relative humidity are not normally available on such a basis. Maximum and minimum values respectively are, however, and a maximum daily SD could be calculated. It is suggested that any day on which the SD rises above 30 hPa may be construed to as a stress day. A very low rate of transpiration would adversely affect calcium transport if due either to stress stomatal closure, or lack of diffusion gradient or closure due to low radiant flux densities. An additional factor should thus be added, to cater for cool, rainy days. A possible solution to these problems of modelling until further information is available, may be to use the maximum SD for non-stressed days, and integrate over the number of non-stressed days. The total stress index from September through December could therefore be calculated as follows:

1. Determine, by means of maximum SD values, all stress days.
2. For the remaining non-stressed days, integrate SD values over the time desired. The higher the value, the higher the transpiration rate and ultimately, higher fruit quality.

The model assumes non-limiting soil moisture, and no acclimation. Further work will be necessary to determine the effect of soil moisture on the SD/ transpiration curve. It must be stressed that this model is of a preliminary nature, but that it may nevertheless assist the industry. The addition of a radiant flux component, as well as better knowledge of the individual effects of temperature and humidity, could improve the accuracy. A component for tree vigour has not been included, as this aspect did not form part of the study, but may be advisable.

The work on PPO showed a considerable difference between fruit picked early and late in the season. The rate of ripening also increased as the season progressed. Further research is needed to determine whether a final picking date for export is necessary, to ensure high quality on the export market, and if so, what that date should be for various production areas.

In conclusion, it can be said that this work has, for the first time in avocados, established a definite link between fruit and tree physiology (particularly physiological disorders) and water relations. Further, the optimal irrigation regime for local (Burgershall Research Station) conditions has been confirmed. The work also forms the basis for identifying directions for future physiological research which would be of benefit not only to the knowledge of avocados, but also other subtropical tree crops.

SUMMARY

Fruit physiological disorders cause considerable losses to the South African avocado exporter. Ripening abnormalities and particularly internal flesh discoloration, are the most severe problems. An extensive survey of the likely causes revealed that the on-tree fruit condition played a considerable role. The variable irrigation practices and poor rainfall distribution and reliability in the majority of South African avocado growing areas may influence tree and fruit water stress. Most previous water relations work involving fruit studies has investigated the short-term effects immediately pre- or post harvest, but not longer seasonal effects, or the tree - fruit interactions. The aim of this study was therefore to investigate the seasonal effects of various irrigation regimes on tree water relations, and correlate these with post-harvest fruit physiological differences.

'Fuerte' trees on 'Duke' seedling rootstocks were used in the study, and the following supplementary irrigation regimes imposed, based on soil moisture tension at 300 mm depth in the root zone:

- 1) Irrigation at 35 kPa (frequent irrigation)
- 2) Irrigation at 55 kPa (moderate irrigation)
- 3) Irrigation at 80 kPa (infrequent irrigation)
- 4) Dryland (no supplementary irrigation)

The possibility of tree acclimation to varying water stress levels during the period of study was evaluated by means of measuring the osmotic potential and osmotic potential at zero turgor of single leaves at intervals throughout the study period, using the pressure-volume technique. It was found that signs of acclimation only occurred in the dryland trees. Such acclimation was dependant on prolonged water stress, and as such even the infrequent (80 kPa) irrigation treatment

did not develop any marked acclimation. There was nevertheless an indication that least stress occurred in the 55 kPa regime. Acclimation decreased considerably within approximately two months of regular summer rainfall.

A study of leaf water potential over three seasons showed considerable changes between summer and winter, particularly in the dryland and 80 kPa treatments, indicating greater stress in these two treatments as opposed to the 55 kPa and 35 kPa irrigation regimes. The period of maximum stress during fruit development occurred within approximately the first three months after fruit set.

Diurnal tree water relations during a summer, two warm winter days and a spring day were evaluated. Stomatal resistance, leaf temperature and transpiration were estimated with a steady state porometer, while the environmental parameters of air temperature and relative humidity were used to calculate atmospheric water demand, in the form of the saturation deficit. Leaf water potential was estimated by the pressure chamber method. Leaf water potentials were most negative in the dryland treatment, even pre-dawn. On the summer day, transpiration increased while stomatal resistance decreased during the morning, but reversed in the early afternoon, implying a temporary stress. In all cases except the dryland treatment, temporary stress occurred at a saturation deficit of approximately 28 hPa. Continuing acclimation in dryland trees appeared to decrease the response of stomata and transpiration to environmental demand. Temporary stress also occurred later, at a saturation deficit of approximately 35 hPa. The same parameters of stress were found during the spring day. The influence of low relative humidity and the effect of clouds on stomatal action was also noted.

The non-destructive estimation of fruit water potential required development of a psychrometric technique, which is

described and compared with the more traditional pressure chamber method. The fruit water potential at picking showed a more negative trend as the harvest season progressed for all the treatments, but this process was more rapid as the tree water stress increased.

The rate and intensity of fruit browning is known to be a function of polyphenol oxidase (PPO) activity. The activity of this enzyme in fruit was therefore studied in relation to the past irrigation and stress history of the trees. Fruit was picked at both the start (April) and end (July) of the normal export season in 1983, and during April 1985. Both stored and non-stored fruit (to investigate the interaction of stress and low temperature storage) were analysed, as were soft (eating ripe) and, in the case of the unstored treatment, hard fruit. Both soluble PPO activity (to indicate the immediate browning capacity) and total PPO activity, consisting of bound, latent and soluble forms (to indicate potential browning) were analysed.

The first picking date in 1983 showed a clear trend in the case of soft fruit. PPO activity was lowest for the moderately irrigated (55 kPa) treatment, which had also shown the least indication of tree water stress through the previous season. Highest PPO activity occurred in the 80 kPa treatment, with the 35 kPa treatment being intermediate. Low temperature storage increased PPO activity. The trends were also more pronounced for total PPO activity as opposed to soluble. The second picking date showed the same trends, but PPO activity was significantly higher. Fruit picked in 1985 showed the same trend as the 1983 fruit, but treatment differences were non-significant. Higher rainfall and lower water stress than during the 1983 season, occurred. A lack of container ventilation after storage caused an increase in soluble PPO activity, particularly in the 80 kPa treatment, indicating an interaction between the effects of past stress

history and container ventilation.

Fruit softening did not always show a good relationship with fruit water potential at the time of picking, implying that other factors occurring earlier in fruit development are also of importance. Ethylene evolution during ripening confirmed this. Fruit from the 80 kPa treatment particularly, but to some extent also the 35 kPa regime, indicated an earlier start to ethylene evolution with softening, as compared with the optimal 55 kPa treatment. Further, particularly the 80 kPa treatment, showed a difference in the ethylene-forming system, with the major climacteric peak occurring later during softening. Further work to confirm these trends is suggested.

Fruit calcium concentrations showed some differences related to irrigation regime, but mainly showed a pattern with time after fruit set. The period of approximately 7 to 16 weeks after fruit set appeared particularly important, and as this coincides with early fruit development, may relate to the influence of calcium on structural development, particularly membranes. This could explain subsequent post-harvest results.

Free abscisic acid was assayed using the radioimmunoassay technique. Levels of ABA were considerably higher at the 50% fruit softness stage in both the 35 kPa and 80 kPa (particularly the latter) treatments as compared with the 55 kPa treatment, for fruit picked in April. A possible link between abscisic acid and fruit browning (as shown by polyphenol oxidase activity) was discussed.

Tree water relations as influenced by both soil and climate, particularly during the first three months of fruit growth, have been shown to affect subsequent fruit quality. A preliminary quality prediction model, based on transpiration

and water movement through the tree during this period is proposed.

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