HORTICULTURAL AND PHYSIOLOGICAL ASPECTS OF VIGOUR CONTROL IN APRICOT (*Prunus armeniaca* L.) UNDER ORCHARD AND CONTROLLED ENVIRONMENT CONDITIONS

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ABSTRACT

In the absence of dwarfing rootstocks for apricot, techniques which reduce vegetative growth are important in the orchard management system. Studies were conducted in the orchard and in controlled environment (CE) rooms in order to explore the horticultural and physiological responses of apricot (*Prunus armeniaca* L.) to some vigour control techniques.

In the orchard in the humid climate of Palmerston North, New Zealand, five-year-old vigorous 'Sundrop' apricot trees on 'Golden Queen' peach seedlings trained on Tatura trellis at 1000 or 2000 tree ha^{-1} were used. The objectives were: a). to evaluate the trees' responses to 0.5 and 1.5 g. tree^{-1} soil applied Paclobutrazol (PBZ), dormant root-pruning and regulated deficit irrigation (RDI); b). to identify osmotic adjustment in fruits and leaves in response to internal water stress. Two-year-old 'Trevatt' apricot in an aeroponic system in CE rooms were used with the objectives: a). to examine the effects of root cytokinin and endogenous ABA on shoot growth and whole plant physiology; b). to study the mechanism of adaptation to high water stress.

In the orchard all treatments reduced vegetative growth. PBZ was more effective than the other treatments, and the lower rate (0.5 g. tree^{-1}) when applied annually gave more uniform growth reduction. The root-pruning and RDI had less effect, particularly in the second season of study. The deep soil, together with low temperature and evaporation, high rainfall and humid conditions during winter and early spring were limiting factors for RDI. The inhibitory effect of root-pruning was more persistent on wider spaced trees. In close planted trees root length density (RLD) declined with increasing depth, but roots were observed to 1.6 m explored soil depth. Root-pruning increased RLD, but no treatments effect was observed on root weight density (RWD) in the explored soil volume.

PBZ increased dry matter partitioning into crop in both seasons on close spaced trees, and fruit growth and final fruit size were increased without any detrimental effect on fruit quality. In the second year PBZ advanced flowering by 2-4 days, and increased fruit set, final fruit number, crop density and yield efficiency. In general RDI had no negative effects on flowering, fruiting, yield and final fruit size. In the second year it generally enhanced flowering, fruit set and fruit number. Root-pruning did not affect other flowering and yield parameters, but reduced fruit size in the first season. There was some evidence of advanced fruit maturity and increased total soluble solids by all applied treatments. Generally fruiting characteristics were improved, and vegetative growth reduced, more by PBZ than by root-pruning and RDI.

PBZ treated trees had the same water status as controls. Their net CO_2 assimilation rate (A) and stomatal conductance (g_s) were improved, and from later stage I and during stages II and III of fruit growth fruit carbohydrates were increased. RDI and root-pruning increased net CO_2 assimilation rate (A) and stomatal conductance (g_s) on some occasions. Root-pruned trees developed an increased internal water deficit in the leaves and fruits especially at the time of highest water demand during fruit stage III. There was evidence on occasions in RDI and root-pruning of osmotic adjustment in leaves and fruits maintaining turgor (Ψ_p).

An aeroponic system with intermittent misting gave good control of plant water stress. When water stress was developed gradually plants were able to maintain their turgor at high internal water deficit (-2.2 and -3.0 MPa of Ψ_{xylem} and Ψ_l respectively). Osmotic adjustment occurred in both partially and fully expanded leaves of all treatments, BAP combined with water stress showed bigger osmotic adjustment. Water stress reduced vegetative growth, and increased root:shoot ratio. Shoot tip ABA increased as water stress increased. BAP reduced the growth inhibition and rise in shoot ABA of water stressed plants, maintained net CO_2 assimilation rate (A) and stomatal conductance (g_s), and increased root:shoot ratio.

In the Name of ALLAH the Most Merciful the Most Beneficent

I dedicate this thesis to:

My parents Haj Mohammad Hassan and Haj Fatemeh Arzani for their patience and moral support; my wife Fatemeh Arzani for her patience, support and encouragement, and my children Ali, Mohsen and Mina Arzani.

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GLOSSARY OF ABBREVIATIONS

A	net CO_2 assimilation rate (μmol m^{-2} s^{-1})
ABA	abscisic acid
a.i.	active ingredient
BAP	6-benzylaminopurine
BAP+WS	6-benzylaminopurine with water stress induced by intermittent misting
BSA	bovine serum albumin
CE	controlled environment
CD	crop density (fruit number cm^{-2} TCSA)
CHO	carbohydrate
cv.	cultivar
Cultar®	Paclobutrazol formulation
dafb	days after full bloom
dae	days after establishment
dats	days after treatment started
DW	Dry weight
DWI	stage I of fruit growth based on dry weight
DWII	stage II of fruit growth based on dry weight
DWIII	stage III of fruit growth based on dry weight
EC	electrical conductivity (mS)
ELISA	enzyme linked immunosorbent assay
E_{ps}	evaporation over the planting square
E_{pan}	pan evaporation
ET_{crop}	crop water requirement (mm day^{-1})
ET	evapotranspiration
FC	field capacity
FGR	fruit growth rate (mm, g. or cm^3 day^{-1})
FT_2	fruit size at time 2 (mm, cm^3 or g.)
FT_1	fruit size at time 1 (mm, cm^3 or g.)
FW	initial fresh weight (g),
GA_3	gibberellic acid

GLM	General Linear Model
g_s	stomatal conductance (m mol m^{-2} s^{-1})
H-PBZ	high rate of Paclobutrazol (1.5 g $tree^{-1}$)
HPLC	high performance liquid chromatography
IAA	indole-3-acetic acid
ICI	Imperial Chemical Industries Ltd
J	water flux density (g $m^{-2}s^{-1}$)
L^*	lightness (refers to colour)
ℓ	total root length (cm)(refers to root study)
LAR	leaf area ratio (ratio of total leaf area to whole plant dry weight, m^2 g^{-1})
L-PBZ	low rate of Paclobutrazol (0.5 g $tree^{-1}$)
MAb	Monoclonal antibody
MPa	mega pascal (1Mpa = 10 bar)
NF	total number of fruits at the time of fruit harvest
NFT	nutrient film technique
$n\ mol_{cho}$	moles of solute (refers to Van't Hoff's equation)
NZ	New Zealand
PBZ	Paclobutrazol
P.E.	pan evaporation
PEG	polyethylene glycol
P-index	partitioning index (kg yield cm^{-2} TCSA $year^{-1}$)
PNP	*p*-nitrophenyl phosphate
PP333	paclobutrazol
PPFD	photosynthetic photon flux density (µmol m^{-2} s^{-1})
PVP	Insoluble Polyvinylpyrrolidone
R	the universal gas constant (refers to Van't Hoff's equation)
r	distance from tree (refers to root study)
r	resistance to diffusion of water between Ψ_{w1} and Ψ_{w2}
RCBD	randomized complete block design
RDI	regulated deficit irrigation

Rdwt	dry weight of fine or woody roots (g)
RH	Relative humidity (%)
RLD	root-length density (cm of roots cm^{-3} of soil)
RM	repeated measurement analysis
RPM	revolutions $minute^{-1}$
RWD	root-weight density (g of roots cm^{-3} of soil)
RWC	relative water content (%)
Savant	automatic Speedvac concentrator
TSS	total soluble solids
T	fruit temperature in °K (refers to Van't Hoff's equation)
TBS	buffer containing Tris, $MgCl_2$ and NaCl
TBST	washing buffer TBS containing 0.05% (v/v) Tween 20, and 0.1% (w/v) BSA
T_2	time 2 (day)
T_1	time 1 (day)
TCSA	trunk cross sectional area (cm^2)
TCSA GR	growth rate of trunk cross sectional area (cm^2 $year^{-1}$)
$TCSA_{t2}$ & $_{t1}$	TCSA recorded at time 2 (t_2) and time 1 (t_1)
TY	total harvested fruit $tree^{-1}$ (g.)
TW	turgid weight (g)
V	volume of solvent in litres (refers to Van't Hoff's equation)
V	soil sample volume (cm^3)(refers to root study)
V_o	osmotic volume at full turgor
VPD	vapour pressure deficit (mb)
WS	water stress induced by intermittent misting
WSb	water stress induced with intermittent misting (see 7.2.5.2)
YE	yield efficiency (g. of fruit cm^{-2} TCSA)
z	depth to midpoint of sample (refers to root study)
Ψ_{cho}	osmotic pressure (MPa) of each individual recorded carbohydrate (refers to Van't Hoff's equation)
Ψ_f	fruit water potential

Ψ_g	gravitational potential
$\Psi_{glucose}$	osmotic pressure of glucose (the partial contribution of glucose in osmotic adjustment)
Ψ_l	leaf water potential
Ψ_m	matric potential
Ψ_p	turgor or pressure potential
Ψ_s	osmotic potential
Ψ_{So}	osmotic potential at full turgor
Ψ_w	water potential
$\Delta\Psi_w$	water potential difference between two points (Ψ_{w1} - Ψ_{w2})
Ψ_{xylem}	xylem water potential
θ	angle relative to row direction (refers to root study)

CHAPTER ONE

GENERAL INTRODUCTION AND OBJECTIVES

New Zealand is one of the finest fruit growing regions in the world (Childers, 1983). The fruit industry in this country is mainly export dependent and the whole fruit industry sector tries to increase the profitability of fruit production. According to Marshal (1991), in 1991 New Zealand exported $860 million worth of fresh fruit, and earned a further $82 million for exports of processed fruit. New Zealand has a target of $2 billion for horticultural exports by the year 2000 (Monigatti, 1991), which is attainable and might be achieved by the fruit industry alone. Davies (1991) noted that the overall goal for the New Zealand fruit industry in the future is making horticulture a leading export earner and to project this country as the world's leading horticultural exporter and marketer.

The New Zealand fruit industry comprises pipfruit, kiwifruit, summerfruit, citrus and exotics. During the 1980s the fruit industry was one of New Zealand's fastest growing export industries (Monigatti, 1991). The export value of apricots increased by 440 percent over two years to $2.8 million in 1991 (Anon, 1991) and in the 1992-1993 season 2,421 tonnes of fresh apricots were exported worth $3.14 million plus $0.16 million of processed apricots (Anon, 1993). The production of high quality fruits with low production cost to keep exports competitive are key objectives for the fruit industry of New Zealand.

Correct decisions at the time of orchard establishment, as in choosing good varieties for planting on the right rootstocks and in suitable environmental conditions, together with consideration of other orchard management factors help in producing good quality fruits with lower production costs and higher returns. O'Rourke (1994) suggested that the choice of rootstock, combined with the decision on number and spacing of trees ha^{-1}, have a major effect on future costs and returns. For example, a larger number of trees ha^{-1} on vigorous rootstocks can create serious management, yield and quality, problems as the trees mature.

The latest apricot rootstock trial reported in New Zealand (Knowles *et al.*, 1994) concluded that 'Golden Queen' peach seedlings are still one of the most suitable rootstocks for 'Sundrop' apricot. They found that 'Marianna 9.52' plum tended to give some reduction of tree size while maintaining good fruit quality. However, fruit size of 'Sundrop' on this rootstock was smaller than on 'Golden Queen'. O'Rourke (1994) pointed out that orchard returns are determined by the percentage of desirable grades and sizes of fruits included in the output, and that within the fresh fruit category larger sizes may sell for three to four times the price of smaller fruits.

The precocity of bearing, advancement of maturity, and pruning requirements are other factors affecting production costs and orchard management. The management of tree canopies and of tree vigour are important in the determination of yield, quality and production costs. Factors such as cultivar, soil type, tree spacing and environmental conditions influence tree management decisions aimed at maintaining the correct balance between vegetative and reproductive growth.

Several options are available for reducing tree size of apple and pear, but size controlling rootstocks for other species are limited (Martin, 1989), and a range of size-controlling rootstocks are not available for stone fruits (Geisler and Ferree, 1984a). It has been suggested that limited establishment of high density plantings of apricot in the past might be due to the lack of genetically dwarfing rootstocks (van den Ende and Kenez, 1985). Whilst the choice of dwarfing rootstocks for the stonefruit grower is very limited, any management system which tends to reduce vegetative growth might give an opportunity for reducing production cost and improving productivity and fruit quality.

The application of some vegetative growth control techniques, such as paclobutrazol (PBZ), root-pruning and regulated deficit irrigation (RDI), in managing fruit tree vigour has been reported in the literature. The responses of fruit trees to these techniques of reducing vegetative growth, the time and type of applications and some physiological effects on fruit trees will be discussed.

This thesis includes study on mature 'Sundrop' apricot trees in orchard conditions, followed by study of young 'Trevatt' apricot trees under controlled environment conditions and soilless culture in an aeroponic system. The field study was carried out over two growing seasons followed by a root study at the beginning of the third season (1992-1993). The orchard study examined whether PBZ, root-pruning and RDI have potential for controlling vigour without having undesirable effects on fruit quality and yield of mature 'Sundrop' apricot trees grown on Tatura trellis at two different tree spacings in the environmental conditions of Palmerston North, New Zealand. This variety of apricot has attractive fruit with good potential for export, but the tree grows tall, upright and vigorously (Glucina *et al.*, 1990). The other objectives of the orchard study were:

I. To examine the effects of these treatments on the internal water relations of apricot.

II. To determine whether the mechanism of response to root pruning appeared similar to the mechanism of response to water stress.

III. To examine the mechanism of stress adaptation in apricot and determine whether osmotic adjustment occurred in leaves and fruits.

The second experiment was carried out in controlled environment conditions (chapter 7). It used two-year-old budded 'Trevatt' apricot trees for a period of

12 weeks in two adjacent controlled environment rooms at The National Climate Laboratory, Hort+Research, Palmerston North, New Zealand with the following aims:

I. To examine whether root cytokinin may have an effect on shoot growth and whole plant physiology.

II. To determine concentrations of abscisic acid (ABA) in shoot tips to examine whether ABA played a role in controlling shoot growth under water stress conditions.

III. To further examine the mechanism of stress adaptation in apricot under higher stress conditions than in the orchard study, and determine the possible existence of osmotic adjustment in fully and partially expanded leaves.

The overall discussion of both the orchard and the controlled environment experiment will evaluate the potential of a range of growth control strategies for apricot and discuss the mechanism of tree response to drought and root-pruning and indicate possible directions for future research related to this study.

CHAPTER TWO

REVIEW OF LITERATURE

2.1 GENERAL OVERVIEW OF APRICOT

2.1.1 Classification

The apricot *Prunus armeniaca* L. is a member of the Rosaceae family, subfamily Prunoideae, genus Prunus L., sub-genus Prunophora. The apricot is diploid with 8 pairs of chromosomes (Meblenbacher *et al.,* 1991) but occasional tetraploids have been reported (Bailey and Hough, 1975). Apricot is a deciduous, perennial woody species.

2.1.2 History and origin

According to the literature the cultivation of apricot goes back a long time. For instance, apricot culture was practised more than 3000 years ago in China (Crossa-Raynaud and Audergon, 1987; Rezeghi, 1982) and slowly spread through central Asia, Iran, Armenia and Syria (Crossa-Raynaud and Audergon, 1987; Rom, 1991). Apricot was brought to Italy about 100 B.C., to England in the 13th. Century, and to North America by 1720 (Westwood, 1993).

2.1.3 Geographic / Climatic requirements and production regions

The apricot is grown in many parts of the world, and is a popular fruit considered by many people to be one of the most delightful fruits (Bailey and Hough, 1975; Meblenbacher *et al.,* 1991). Ecological adaptation is found in regions with a continental climate with about 500 mm rainfall with sufficient warm weather during the growing season. Only in suitable climatic conditions has apricot become pomologically important (Bailey and Hough, 1975). Apricots are grown both in central Asia with a warm summer and long and cold winter as well as in Mediterranean climates with mild, short, dry winter and hot, dry summer (Meblenbacher *et al.,* 1991). Western and central Mediterranean countries produce more than one third of the world's apricot production (Guerriero *et al.,* 1988). The main production regions for apricot in recent years were Turkey, USSR, Italy, Spain, Greece, France, United States, Morocco and Pakistan (Westwood, 1993).

In Australia and New Zealand apricot culture is not a big industry. According to the FAO production yearbook (1979-1983), as cited by Gathercole (1988), the production of apricot in Australia was about 30,000 tonnes per annum which was 2% of the 1.5 million tonnes worldwide apricot production. The major apricot production region in New Zealand is Central Otago (85%) and just 12% of the total planted area is in the more northerly area of Hawke's Bay (Glucina *et al.*, 1990).

2.1.4 Rootstocks, Growing Habit and Training systems

No successful genetically dwarfing rootstock for apricot has been found and this may be one of the reasons why there has been little development in high-density planting of this fruit in the past (van den Ende and Kenez, 1985). However, Quarta *et al.* (1985) reported the existence of five promising seedlings with dwarf growing habit in Italy which provided opportunity to use them as parents in a breeding programme. Sansavini and Giannerini (1991) reported that the Russian hybrid (*P. cerasifera X P. spinosa*) PI 304921 reduced vigour by at least 20%.

Currently, in different soil types and different climatic regions with various ecological conditions, different types of rootstocks have been used by apricot growers. Hartmann *et al.* (1990) suggested that apricot, peach and in some cases myrobalan plum seedlings are commercially suitable as rootstocks for apricot. They stated that apricot trees on apricot roots live longer and produce heavier crops than trees on either peach or plum roots, if grown on well-drained loam soils. In unirrigated orchards or where drought conditions prevail, apricots on peach seedling roots make better growth than those on apricot roots.

While the choice of cultivar and rootstock may have an influence on canopy density, tree management may also contribute significantly to optimising tree size and light penetration. Differences in fruit tree canopy form due to training and pruning methods can substantially modify light transmission to the various regions of the tree canopy. Meblenbacher *et al.* (1991) stated that there is a wide range of tree size among apricot species from small to large trees, and while trees of Central Asian cultivars are large European cultivars generally have smaller trees. Crossa-Raynaud and Audergon (1987) reported large, vigorous and long lived apricot seedlings under low rainfall and saline conditions in North Africa (Plate 2.1). Also, cultivars vary in growth habit from very upright to drooping or weeping. In some cultivars, fruits are borne on short spurs, however in others, flower buds are produced primarily on one-year-old shoots (Meblenbacher *et al.,* 1991). Jackson (1969) and Jackson and Sweet (1972) previously reported that flowers of apricot may be initiated either on the current year's wood (young apricot) or on wood (spur) at least two years old (mature apricot). According to Westwood (1993) flower buds in apricot develop in late summer on both current season's shoots and on short older spurs. The solitary flowers are produced from lateral buds.

Sansavini and Giannerini (1991) reported that training system becomes more critical when planting density is increased. Systems like palmette, free spindle, spindle and Tatura trellis are suitable for densities over 600-800 trees per hectare. The traditional planting system with wide spacing (6m x 6m and 6m x 5m) was largely abandoned in favour of high density plantings in recently established Italian orchards.

Plate 2.1 Huge apricot seedlings cv. 'Mech Mech' in a Tunisian oasis in North Africa. (After Crossa-Raynaud and Audergon, 1987).

In Australia the Tatura trellis (V shape) system was developed and used for high density apricot plantings, which allowed a smaller tree size despite the lack of dwarfing rootstocks. This allowed both higher light penetration within the canopy and higher land use efficiency due to smaller distances between trees (van den Ende and Kenez, 1985).

2.1.5 Some recent studies on apricot

Most researchers agree that apricot cultivars are adapted to specific ecological regions, so for most cultivars commercial cultivation is restricted to those areas in which they perform well. Apricot is mostly grown in temperate regions, but the development of new cultivars may provide the basis for the extension of profitable apricot production to new areas of the world such as very mild temperate or selected subtropical regions (Meblenbacher *et al.,* 1991).

Paunovic (1988) reported that more than 3500 articles have been published regarding apricot, of which 70% were produced after 1950. Early work dealt more with plant protection, while more recent studies have been concerned with rootstocks, breeding and selection, marketing, processing and some new technologies. Nevertheless, there is still a lack of knowledge in key areas of apricot culture such as the control of vigour in the absence of a satisfactory dwarfing rootstock. A recent report from a rootstock trial in New Zealand (Knowles *et al.,* 1994) showed that 'Golden Queen', 'P. zailisky' and 'Pixy' were the most promising rootstocks which produced intermediate sized trees of 'Sundrop' apricot.

In New Zealand apricot is a popular summer fresh fruit as well as being dried and processed (Glucina *et al.,* 1990). The cultivar 'Sundrop', introduced to this country in 1970, was selected by Summerland Research Station, British Columbia in Canada. This is a mid season variety with attractive fruit shape and colour, medium in size averaging 65 g. After evaluation of some factors such as fruit characteristics and resistance to diseases among 71 cultivars they suggested that 'Sundrop' might have outstanding success in many New Zealand districts. Noiton *et al.* (1993) and Noiton (1994) reviewed the recent breeding programme in New Zealand to introduce new cultivars suitable for each specific climatic region. 'CluthaGold', 'CluthaStar' and 'CluthaLate' were released earlier and 'CluthaEarly', 'CluthaGem' and 'CluthaSan' were released in 1992.

Regardless of the attractiveness of 'Sundrop' fruit, this cultivar grows tall, upright and vigorously. The present study mainly focused on investigating some techniques of vegetative growth control such as mild water stress, root-pruning and the use of paclobutrazol (Cultar®) on mature Sundrop trees grown on Tatura trellis. The study also examined the effects of these techniques on physiological factors which are involved in vegetative and fruit growth in this variety under humid environmental conditions.

2.2 MANAGING THE GROWTH OF FRUIT TREES

Techniques for the management of plant growth have been practised for centuries, including pruning and grafting as methods of plant improvement and growth control. For example, bonsai, which is one technique of sophisticated tree size control, has been long practised by the Chinese and Japanese. They have used this method as an art for centuries and bonsai specimens may be passed from generation to generation. In this type of size control the techniques involved are bending, twisting, root restriction, and precise control of soil composition and water and nutrient supplies. In this system growth is restricted rather than being removed by pruning (Barden *et al.*, 1987).

In terms of fruit production orchardists are concerned with supplying good quality fruit to the market with low production cost. The management of the tree canopies and vigour is important in the determination of yield, quality and production costs. Factors such as cultivars, soil type, tree spacing and environmental conditions influence tree management decisions aimed at maintaining the correct balance between vegetative and reproductive growth. Physiologists, horticultural scientists and growers seek to understand the interacting complex of factors influencing tree performance, and to optimise orchard productivity.

It is obvious that vegetative growth is necessary for maintaining vigour and developing new fruit bearing wood surface in fruit trees (Forshey and Elfving, 1989; Martin, 1989). Therefore, the ultimate objective of tree management practices is the manipulation of the vegetative growth and fruiting relationship (Forshey and Elfving, 1989). Many factors are involved in achieving this, including species, appropriate rootstock and cultural practices such as pruning, irrigation and plant spacing. All these are important for controlling tree vigour in the specific production region. So, management of environmental factors and cultural practices needs integration with cropping. For example both excessive and improper pruning favour vegetative growth at the expense of fruiting. On the other hand, overcropping which often leads some species to biennial bearing complicates management of vegetative growth. Thinning practice becomes important in overcropping to reduce the sensitivity to biennial bearing as well as to increase fruit quality in the current season (Forshey and Elfving, 1989).

Controlling vigour of fruit trees gives great economic advantage in the fruit production industry at the present time. Later in this review some evidence of this advantage is discussed, for example Chalmers *et al.,* (1981, 1985) demonstrated that using regulated deficit irrigation (RDI) to control vigour of peach trees resulted in reducing production costs (eg. pruning and irrigation) and increasing yield efficiency. Forshey and Elfving (1989) stated that in commercial orchard management increase in production efficiency has the highest priority. On the other hand producing unproductive wood adds to the production cost and at the same time reduces the saleable product. However in the final analysis, the ratio between input and output determines the success or failure of the orchard, however this may be affected more by economic than by pomological considerations.

Physical, chemical and environmental methods of growth control, as well as genetic control by the breeding of more efficient cultivars and rootstocks, is an advancing science. Elfving (1988) suggested two categories of factors for managing vegetative growth in fruit trees. First, physiological factors (those related to inherent factors, including genetic potential for scion and rootstock growth) and second, horticultural factors (such as shoot and root-pruning, training system, water control and hormonal practices). Martin (1989) pointed out that orchard management practices such as crop load adjustment, pest management, and fertilizer application particularly nitrogen, will affect the outcome of any growth control measures.

Barden *et al.* (1987) summarized growth control techniques in four categories as:

i. Genetic control, such as the use of dwarfing rootstocks, or selecting low vigour cultivars
ii. Physical control, including pruning and training, root-pruning, disbudding
iii. Chemical control, for instance, chemical pinching or the use of growth retardants
iv. Environmental control, eg. the control and manipulation of light, temperature, water, CO_2 and nutrients, although some of these are not applicable to orchard fruit trees.

From a practical point of view, understanding the physiological responses of fruit trees to each vigour control technique is essential and a prerequisite for using a technique at the correct time on a specific plant in a specific geographical region. Careful research will give this opportunity to the orchardist for increasing yield efficiency and perhaps reducing production cost, allowing more profit in return.

This review will discuss the response of some fruit trees to root-pruning, paclobutrazol and water stress which are examples of physical, chemical and environmental manipulation of vigour control.

2.2.1 ROOT-PRUNING

Ferree and Geisler (1984) reported that the practical use of root-pruning had generally been discontinued in current orchard management. They stated that relatively few experimental studies had reported on the effect of root-pruning under field conditions. Recently this technique has been developed as a successful commercial vigour control technique for established orchard trees (Ferree, 1989; Geisler and Ferree, 1984a; Schupp and Ferree, 1988b, 1989).

Root-pruning is a very effective technique to control vigour of both young and fully grown fruit trees (Geisler and Ferree, 1984b; Schupp and Ferree, 1990). Mika (1986) reported that root-pruning is very seldom recommended or applied in practice, except at the time of transplanting trees from nursery to orchard.

The mechanisms for the influence of root-pruning on tree growth are complex. Randolph and Wiest (1981) suggested that there are three possible ways growth may be influenced by root-pruning:

i. limited water absorption which may induce water stress
ii. reduction in CO_2 assimilation and nutrient uptake
iii. the source of growth-regulating hormones may be reduced

Geisler and Ferree (1984a) suggested that root-pruning has potential as a size-controlling mechanism in woody plants. However, the interaction of factors such as degree and time of root-pruning, soil moisture and crop load did not allow a predictive response based on current knowledge. They suggested additional investigation would be essential of many aspects in the response of plants to root-pruning, to utilize this technique on a commercial scale. In their review of the influences of root-pruning on plant growth, they reported no work on the effect of root-pruning on apricot plants in commercial orchards.

2.2.1.1 Effects of root-pruning on growth of fruit trees

2.2.1.1.1 Response of roots to pruning

Young (1982) and Young and Werner (1982) pointed out that root-pruning alone or combined with shoot pruning of young apple trees caused a rapid growth of new roots regardless of shoot response to pruning. Young apple trees subjected to removal of half the root system in early summer responded with a 30% reduction in shoot growth and showed an increase in relative growth rate (RGR) of remaining roots (Maggs, 1965). Ferree (1993) found that root-pruning of apple caused a reduction in the total number of roots, and tended to increase the number of smaller roots in the 0-30 cm soil depth.

Richards and Rowe (1977a) reported that root restriction of hydroponically grown peach seedlings reduced root growth and also limited shoot growth. They demonstrated that exogenous application of the synthetic cytokinin benzylaminopurine largely overcame the effects of root restriction on shoot growth. They suggested that roots exert control over shoot growth by synthesis and/or translocation of growth regulator(s), possibly cytokinins. Short and Torrey (1972) found a high concentration of cytokinin in root tips which inhibited lateral root formation and removal of root tips reduced the cytokinin pool in the roots and thus released lateral root formation close to the cut. The assimilate distribution changed in favour of roots thus suppressing shoot growth during the regeneration of the root system, until reestablishment of the root:shoot balance (Geisler and Ferree, 1984b).

There is a difference between the response of cropping and noncropping fruit trees to root-pruning. It has been suggested fruits and seeds are stronger sink for current assimilate during the growing season (Kramer and Kozlowski, 1979). Schupp and Ferree (1989) pointed out that root-pruning is more effective in reducing vegetative growth on cropping apple trees than on young noncropping trees. They concluded that inhibition of root regeneration occurred due to interaction between reproductive and vegetative growth. Non-cropping trees regenerated the cut roots quickly, and under favourable environmental conditions multiple root-pruning would be necessary during the growing season to achieve substantial growth reduction in non-cropping trees. Root regeneration on 'Golden Delicious' apple trees subjected to root-pruning plus deblossoming was abundant, but low in cropping trees (Schupp *et al.,* 1992). Geisler and Ferree (1984a) pointed out that root-pruning and root restriction both inhibit the normal development of root system.

The distance between trees has an effect on root distribution. For instance in wide spaced apple trees the root system was composed mainly of horizontal roots with relatively few vertical sinkers. At closer spacing, the horizontal portion was reduced and the root system was composed mainly of vertical sinkers (Atkinson *et al.*, 1976; Atkinson, 1976, 1978). In addition, in closer planting systems the root density increased and soil exploitation was more uniform. Individual tree canopy volume and surface area as well as length and weight of roots decreased with increasing planting density. The shoot weight per tree was reduced in the same proportion as the root weight per tree. Thus, tree root:shoot ratio was not affected by changes in plant spacing (Atkinson *et al.*, 1976; Atkinson, 1978, 1980; Boswell *et al.*, 1975a,b; Kaufmann *et al.*, 1972).

2.2.1.1.2 Response of shoots to root-pruning

Root-pruning of apple trees caused a reduction in shoot growth (Schupp and Ferree 1988b, 1989) and also suppressed the relative growth rate of new shoots (Young, 1982). Root-pruning limited the shoot growth of peach, apple, pear and grape (Poni *et al.*, 1992) and 'Colt' cherry rootstock (Asamoah and Atkinson 1985). Arnold and Young (1991) reported reduction of shoot extension on apple seedlings following root-pruning. However, Alexander and Maggs (1971) reported that root-pruning of two-year-old Sweet orange seedlings had no significant effect on mean shoot length. The reduction of shoot elongation appeared within a month of root-pruning at a distance of 0.4 m from the trunks of 'Bellaire' peach trees (Santos *et al.* 1991). Geisler and Ferree (1984b) reported that the greatest reduction in shoot growth occurred during the second and third week following root-pruning of young apple trees.

Geisler and Ferree (1984b) pointed out that less leaves developed following root-pruning of young 'Golden Delicious' apple trees and they were smaller, but specific leaf weights increased, while previously expanded leaves were not affected. Generally, as root-pruning severity increased, the rate of leaf growth decreased. Root-pruning resulted in decreased leaf number, total leaf area, and dry weights of all leaves, shoots and roots of young apple trees, relative to controls (Ferree 1989). Schupp and Ferree (1990) applied root-pruning at different times to one year old container-planted apple trees, and found that root-pruning regardless of timing reduced shoot extension for 4 weeks and reduced shoot diameter and total dry weight at tree harvest, 107 days after bud break. McArtney and Belton (1992) found light penetration into the tree canopy improved and the need for summer pruning decreased following root-pruning of apple.

Two possible effects of root-pruning are reduction in water uptake and a decrease in root synthesis of cytokinin (as discussed in 2.2.1 and 2.2.1.1.1). Possibly, the immediate effect of root-pruning is a shortage of water uptake which can not match the water loss by transpiration. Geisler and Ferree (1984b) found the immediate response after root-pruning of young apple trees during spring was reduction of photosynthesis, stomatal closure and wilting due to partial removal of the root system and reduction in water uptake. However, the general recovery of wilted leaves during the first 72 hours after root-pruning indicated that trees adjusted to reduced water uptake and also this adjustment allowed the recovery of photosynthesis. Schupp and Ferree (1988a) found the reduction of shoot extension and of size of newly expanded leaves were apparent on root-pruned apple trees 21 days after treatment, and that the immediate injection of cytokinin into the scion stem just above the graft union after root-pruning was ineffective in overcoming these responses to root-pruning.

2.2.1.1.3 Root:shoot ratio and functional equilibrium

The ratio between the weights of the underground part of the plant and the aerial part (root:shoot ratio) is often measured by ecologists and physiologists.

The change in this ratio observed under various conditions has often followed a predictable pattern. The interaction between root and shoot is definitely related to metabolic synthesis in and translocation from shoots, and to water and mineral uptake by the roots (Vaadia and Itai, 1969). Cytokinin may play a role as a chemical signal or messenger between roots and shoots (Chibnall, 1954 as cited by Vaadia and Itai, 1969). It has been reported that root tips are a site of production of cytokinins that are translocated in the xylem sap and can influence shoot growth (Skene, 1975). There is competition between roots and shoots for carbohydrates and the organs which are closer to the source are more successful in obtaining their requirements. However, the growth of one organ is dependent on the translocation of essential substances produced by another organ and thus on physiological activity (Brouwer and De Wit, 1969). This type of interdependence can be called a functional equilibrium. Disturbing this equilibrium by partial excision of the supply organ (for example root), reduced growth rate of the dependent organ (shoot). Plants try to reestablish their root:shoot ratio, and rapid restoration of the originally existing ratio occurred after root excision as a consequence of the reduced growth rate of the dependent organ and an increased growth rate of the supplying organ (Brouwer and De Wit, 1969). Geisler and Ferree (1984a) discussed the possible response of plants to root-pruning. They suggested that after the reduction of the root:shoot ratio immediately after root-pruning, the supply of water, mineral nutrients, and hormones from the roots to shoots declines. This could reduce shoot growth. A greater proportion of assimilate is directed to root growth and the plant starts to recover its root:shoot ratio by increased root growth. At the same time a reduction in the growth rate of shoots can be observed. Later, more growth-promoting substances such as cytokinin are produced by the numerous active root tips and translocated to the shoots. Once regained, the root:shoot ratio characteristic for the plant is maintained with shoot and root growth continuing in balance.

Research with a wide range of plants has shown their root:shoot ratio remained constant in a stable environment, except that it progressively decreased with plant age and size. Root-pruning initially reduces the root:shoot ratio and the reaction of plants is to restore this inner balance by increasing root growth through directing more assimilate to the root system. As growth is redistributed in favour of the roots relative shoot growth is reduced (Kramer and Kozlowski, 1979). Time is needed to allow plants to restore the balance between roots and shoots. For example, Richards and Rowe (1977 a,b) reported that root-pruning of hydroponically grown peach seedlings caused an immediate and rapid response of increased root growth and depression of shoot extension and leaf emergence. A redistribution of growth was observed after 25 days as a 20% increase in root dry weight and a 23% reduction of the increase of the above ground dry weight. Ferree and Geisler (1984) found total shoot dry-weight increment decreased with increasing root-pruning severity, while dry-weight accumulation in roots was almost unchanged due to stimulation of regrowth.

2.2.1.1.4 The effect on trunk cross sectional area (TCSA)

Trunk circumferences of orange and apple trees were smaller in high density plantings and were greater at wider spacing (Boswell *et al.*, 1975ab; Schneider *et al.*, 1978). Root-pruning for four years at full bloom of 15-years-old apple trees (planted at a spacing of 3.7 m x 6.7 m) caused a reduction of trunk cross-sectional area increase, and also reduced the time needed for pruning while it improved light penetration through the canopy. On 4-years-old 'Melrose'/M.7A apple trees root-pruning reduced the increment in TCSA (Schupp and Ferree, 1989). Schupp and Ferree (1988b) suggested that root-pruning at 60 cm from the trunk to a depth of 25 or 50 cm had a greater effect than root-pruning at 80 cm distance, which may have been due to their cutting a more substantial portion of the root system. Pruning depth had no influence in the conditions of their experiment. Atkinson (1980) reported that 70% of apple root weight occurred in the top 30 cm of the soil profile across many different orchard sites.

2.2.1.2 Effects of root-pruning on physiological processes

2.2.1.2.1 Accumulation of mineral nutrients

Root efficiency in nutrient uptake depends on the amount of surface in contact with the soil and the permeability of the root surface. Kramer and Kozlowski (1979) claimed it reasonable to assume that nutrient uptake may decline immediately after root-pruning, because the root volume absorbing nutrients is reduced. Often plants regenerate new roots after root-pruning so increasing the absorbing surface. Thus the uptake of nutrients will be the same or even improved when the root system is regenerated.

However, Schupp and Ferree (1987a, 1989) found that the mineral nutrient levels in leaves were not influenced by root-pruning of apple trees. They showed that dormant root-pruning increased the Ca level in the fruit flesh (Schupp and Ferree, 1987a).

2.2.1.2.2 Water relations

It has been demonstrated that removing a part of the root system caused reduction in water absorption and created a degree of water stress in the plant. If water absorption lags behind transpiration, a water deficit develops, stomata close and transpiration is reduced (Kramer and Kozlowski, 1979). Early-season root-pruning of peach decreased stomatal conductance, and there was no effect on diurnal xylem water potential. The closure of stomata appeared to moderate diurnal water deficits at levels similar to control (Santos *et al.* 1991). Work on young 'Colt' cherry rootstocks by Asamoah and Atkinson (1985) showed that root-pruning reduced total water use during the 10 days after treatment. However, Richards and Rowe (1977a) reported that root removal on peach seedlings induced water stress but, water uptake recovered after 10 days.

Leaves wilted for 72 hours following root-pruning of young 'Golden Delicious' apple trees (Geisler and Ferree, 1984b). The response of plants to root-pruning was related to the severity of root removal. With 59% root removal, photosynthesis was reduced by 47% and xylem water potential was reduced significantly for 6 hours with recovery apparent after a day. Photosynthesis and transpiration started to recover after 10 days, but even after 28 days trees subjected to severe root removal had lower rates than on the control.

Schupp *et al.* (1992) reported that after root-pruning of 'Golden Delicious' apple, leaves had lower water potential, transpiration and stomatal conductance than the controls.

2.2.1.2.3 Photosynthesis and assimilate partitioning

Poni *et al.*(1992) reported that root-pruning reduced photosynthesis and stomatal conductance two days after treatment on potted apple, grape, peach and pear trees. When they continued root-pruning a general decline in transpiration occurred. Photosynthesis was highly correlated in essentially all cases with stomatal conductance and transpiration. Ferree (1989) reported similar findings on young apple trees, and found that while carbohydrate levels in the leaves were unaffected by root-pruning, the levels in the roots were increased.

Root-pruning in combination with a lack of adequate soil moisture has a synergistic effect in reducing net photosynthesis. The work on 'Jonathan' apple trees by Ferree (1992) showed that trees subjected to root-pruning under severe drought conditions had reduced net photosynthesis and transpiration, while root-pruned trees given supplemental water (57 l.week^{-1}) showed increased transpiration and also greater fruit size at the time of harvest. Geisler and Ferree (1984b) reported that net reductions in photosynthesis and transpiration appeared to account for the overall reduction in tree dry-weight accumulation after root-pruning.

2.2.1.2.4 Flower initiation and fruit development

Root-pruning may influence flower bud initiation and development, this being more related to the time of root-pruning. Work on apple trees showed that root-pruning before June drop (northern hemisphere) caused an increase in root growth and resulted in a fourfold increase in blossom clusters compared to control trees. Later root-pruning resulted in less root regeneration especially on those trees with higher fruit load. In these trees decreased cytokinin production by active root tips resulted in a detrimental effect on spur flower initiation (Hoad and Abbott, 1983 cited by Geisler and Ferree, 1984a).

Ferree (1992) found when 'Jonathan' apple trees were subjected to annual root-pruning for 6 years either at dormant, full bloom or mid-June (northern hemisphere) stage the tendency for biennial bearing was reduced during this period with no relationship to time of pruning, generally root-pruning reduced preharvest fruit drop. Root-pruning reduced cumulative yield at bloom (14%) or mid-June (20%).

2.2.1.3 Practical use of root-pruning techniques

It is important to know the depth and distribution of the root system in the particular soil type to allow more precise root-pruning. Kramer (1983) reported that development of root systems involves complex interactions between roots and shoots and between roots and their environment. Geisler and Ferree (1984a) reported that tree spacing has effects on the form of the root system.

Rogers and Head (1969) reported that the root system of mature apple trees under orchard conditions consisted of horizontal main roots 25-50 cm below the soil surface and spreading from 1.5 to 3 times as far as the branches. They concluded that root-pruning is a dwarfing technique for apple trees, but did not recommend it due to the fact that main roots were scattered irregularly around the trees. Using suitable dwarfing rootstocks for controlling vigour was recommended, rather than root-pruning (Rogers and Head, 1969). However, Olien *et al.* (1993) pointed out that root-pruning has been an effective, nonchemical means to reduce vigour of apple trees.

2.2.1.3.1 Time and type of root-pruning

The optimum distance from the tree trunk for root-pruning and the amount of roots to be removed are important. Schumacher (1975) as cited by Geisler and Ferree (1984a) cautioned that if too few roots were cut, excessive root development was induced and shoot growth increased. However, the tree might die following severe root-pruning, especially in dry years. Correct root-pruning reduced vigorous shoot growth and increased flower bud initiation.

Santos *et al.* (1991) reported that the time of root-pruning and the distance from the tree trunk modified effects on vegetative growth of 'Bellaire' peach. They found that cumulative shoot elongation was reduced by 39% following root-pruning 0.4 m from the trunk in early season before flowering, while later pruning had less effect. Reduced shoot elongation was evident within a month of root-pruning at 0.4 m after all treatment dates, but at 0.8 m this varied with the date of pruning. Schupp and Ferree (1989) found root-pruning at 60 cm distance reduced shoot growth more than at 80 cm. In addition, canopy light penetration and spur quality increased. They reported that root-pruning during dormancy or at full bloom was very effective in controlling vegetative growth.

Olien *et al.* (1993) suggested that the optimum distance for root-pruning was 60-90 cm from the tree trunk of 8-year-old peach trees. Shoot growth was more responsive in the first year, but in the second year yield was reduced by the previous season's root-pruning.

Root-pruning during the dormant (late August in New Zealand) stage reduced mean shoot length, and the total number and length of shoots removed per tree during summer pruning of 'Braeburn' apple (McArtney and Belton, 1992). The time of root-pruning was important in achieving this result and dormant pruning was more effective than at petal fall.

2.2.1.3.2 The effect of root-pruning on fruit quality and cropping

McArtney and Belton (1992) showed the effect of root-pruning on apple fruit size was related to the tree's crop density. When the number of fruits was high, root-pruning had a negative effect on fruit size, otherwise no evidence of decreasing fruit size was found. However, Schupp and Ferree (1987a, 1988b, 1989) reported that root-pruning of apple trees at either the dormant or June drop stage (northern hemisphere) reduced average fruit size and pre-harvest drop, but increased yield efficiency (yield per cm^2 TCSA), fruit colour, and soluble solids. Trees 3-years-old were more sensitive to the reduction of fruit size than 4-years-old trees. Root-pruning while dormant increased the level of fruit soluble solids. Elfving *et al.* (1991), following a comparison between the effects of Daminozide, root-pruning, trunk scoring and trunk ringing of 'McIntosh' apple, found that fruit size was not affected by root-pruning at full bloom, while soluble solids concentration was increased.

Schupp *et al.* (1992) reported that root-pruning of 'Golden Delicious' apple trees had no effects on return bloom, fruit set or yield, but reduced fruit size.

Ferree (1992) found that root-pruning of 'Jonathan' apple reduced cumulative yield and cumulative yield efficiency, reduced fruit size and gave a decrease in large fruit and an increase in small fruit in 3 of the 6 years of the study.

2.2.2 PACLOBUTRAZOL

The early discoveries of plant hormones by physiologists were rapidly followed by studies of practical application by horticulturists, and the possibility of using chemical plant growth regulators to improve fruit production has been of interest to scientists for many years. Triazoles were relatively recently developed as one of a number of highly active growth retardants which have the potential for chemical growth regulation in horticulture (Davis *et al.*, 1988).

Imperial Chemical Industries (ICI) developed PP333, or paclobutrazol (PBZ), marketed with the trade name Cultar® (Davis *et al.*, 1988; Williams *et al.*, 1986), Bonzai, Clipper and Parlay (Davis *et al.*, 1988). PBZ [(2*RS,3RS*)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1*H*-1,2,4-traizol-1-yl)pentan-3-ol] (Anon, 1982) has low solubility in water (30 ppm) and its half life in soil varies considerably, usually being between 3 and 12 months (Lever, 1986). Appendix 3.1 shows some physical and chemical properties of PBZ (Anon, 1982).

Graebe (1987) suggested that gibberellins and auxins are the groups of phytohormones that promote longitudinal shoot and leaf growth, mainly by affecting cell elongation. Rademacher (1988) reported that several triazole plant growth retardants including PBZ reduced cell elongation and the rate of cell division, which caused reduction in shoot length of plants, without being phytotoxic or causing malformations. Davis *et al.* (1988) and Lever (1986) stated that the growth-retarding properties of PBZ are largely attributed to interference with gibberellin biosynthesis by inhibition of the oxidation of kaurene to kaurenoic acid in the biosynthetic pathway, and it is often referred to as an "antigibberellin".

PBZ has been shown to reduce shoot growth, increase flower bud formation and alter the acropetal movement of assimilates by changing the source-sink strength pattern (Hodairi and Canham, 1990 a,b; Lever *et al.*, 1982; Quinlan and Richardson, 1984; Williams, 1984), Hodairi and Canham (1990 a) reported an increase in movement of assimilate to roots. PBZ uptake by plants is passively through roots, stem tissue and foliage (Anon, 1982). The movement of soil-applied PBZ within the plant is acropetal in the xylem to leaves and buds and PBZ shows no phloem mobility (Anon, 1982; Lever, 1986; Reed *et al.,* 1989). The movement of PBZ in soil is low and is dependent upon soil water movement and the absorption coefficient of the particular soil type (Lever 1986). Root uptake can be an efficient way to maintain a supply of chemical to the growing apex of a young plant (Shearing and Jones 1986). In general, stone fruit respond to PBZ more rapidly than pome fruit. Toxicological studies with PBZ showed there is no hazard to users or consumers when used as directed. PBZ has a low level of residue in fruit after application (Lever 1986).

2.2.2.1 Effects of PBZ on growth of fruit trees

2.2.2.1.1 Response of roots to PBZ

The effect of triazoles on plant roots have not been investigated in as much detail as effects on shoots. However, in general PBZ has caused reduction in shoot growth and increased root:shoot ratio (Davis *et al.*, 1988; Grossmann, 1992 and Hodairi and Canham 1990a). The reduction of shoot growth was associated with redistribution of assimilate leading to increased root dry weight (Hodairi and Canham, 1990a).

The work by Rieger and Scalabrelli (1990) showed that PBZ supplied in nutrient solution culture to 'Nemaguard' peach rootstock increased root:shoot ratio, but decreased root length and hydraulic conductivity with increasing PBZ concentration. Root-shoot ratio increased following either foliar spray or soil drench application to spur- or non-spur-type 'Delicious' apple trees (Lehman *et al.* 1990b).

Miller (1982) found that PBZ at 0.5 mg ml^{-1} applied to apple by pressure injection method reduced shoot terminal extension by 80%, but root weight was not affected. Williamson *et al.* (1986) reported that 37 mg PBZ applied to each container-grown peach seedling reduced vegetative growth, leaf size and shoot dry weight, but increased root tip diameter and reduced unsuberized root length. Microscopic study of root tip cross-sections and longitudinal sections showed that growth and development of the root cortex and stele was modified by PBZ. Although, the stele diameter was increased, this may have been related to increased cell size and radial increase rather than longitudinal elongation of the inner-most cortex parenchyma cells.

2.2.2.1.2 Response of shoots to PBZ

The activity of growth retardants is usually measured as an effect on shoot growth. Growth retardants including PBZ reduce internode elongation and thus plant height without affecting the number of internodes and leaves, although leaf area expansion may be inhibited (Grossmann, 1992; Steffens and Wang, 1986).

Reduction in shoot growth has been reported by both foliar sprays and soil application of PBZ. Soil-applied PBZ decreased shoot growth of mature 'Starkrimson Delicious' apple (Lehman *et al.*, 1990a) and 'Bing' sweet cherry (Jacyna *et al.*, 1989). A similar result was reported by Curry *et al.* (1989) with early season trunk application of PBZ on 'Red-Gold' nectarine and 'Perfection' apricot. Blanco (1986) showed that spray or soil applied PBZ to nectarine and peach inhibited shoot growth, however soil application had a greater effect than foliar application, particularly when used early in the growing season. Soil or spray application of PBZ reduced vegetative growth of peach cv. 'San Pedro' for 2-3 years depending on dose (Loreti *et al.*, 1989). Gaash (1986) observed the effect of a foliar spray of PBZ on vegetative growth of plum, apple and apricot 2-3 weeks after treatment and it lasted 2-3 months. However, the effect of soil applied PBZ was observed 4-6 weeks later and lasted the entire season. Cobianchi (1989) reported a reduction of vegetative growth of nursery cherry trees over 2 years by a single foliar PBZ application with 116 mg/tree in May (northern hemisphere). Tukey (1986) found that annual sprays of PBZ at 1000 ppm to mature apple trees on M.9 or M.26 reduced vegetative growth favourably and enhanced production. The reduction of vegetative growth continued with annual spraying even when the concentration decreased to 500 ppm. PBZ also promoted spur development, and reduced spur leaf size and terminal shoot elongation. Buban (1986) found that spray application of PBZ to 'Gloster' apple/M.9 rootstock reduced shoot growth until late summer. At about the end of July (northern hemisphere) shoots from treated trees were only 60% of control, but this difference was reduced by the end of season due to a moderate secondary growth flush. Miliou and Sfakiotakis (1986) reported a reduction of shoot growth with 3-4 foliar spring spray applications of PBZ to ten-years-old apple trees. However, this inhibition mostly occurred early in the season of shoot growth (May-July, northern hemisphere).

Costa *et al.* (1986) found soil applied PBZ reduced shoot growth of 'Independence' nectarine. Shoot growth of untreated trees had two peaks, the first of moderate intensity occurred in late May and there was a second stronger peak, in late-June or early-July (northern hemisphere). Treated trees showed the strongest reduction of shoot growth at the times of these two growth peaks. Ogata *et al.* (1989) found soil applied PBZ reduced peach and cherry tree growth in the second year after treatment, but in the third year the trees tended to grow more strongly than untreated trees. Facteau and Chestnut (1991) found that a soil application of PBZ at 0.30 $g \cdot cm^{-1}$ trunk diameter to five-years-old sweet cherry decreased terminal shoot extension during the season of application and for at least 3 years following.

2.2.2.1.3 The effect on trunk cross sectional area (TCSA)

Blanco (1987) reported a reduction of growth of shoots and trunk cross sectional area of 'Redhaven' peach after treatment with PBZ. Coston (1986) found that 1.6 g in 1 litre per tree applied either to a soil area of 4 m^2 under a peach tree canopy or applied to one point under the tree canopy caused a reduction in lateral shoot extension. In the following year tree height and shoot length were decreased and the trees showed a weeping growth habit.

Marini (1987) found that different rates of PBZ soil applied to five-year-old 'Redhaven' peach reduced trunk cross sectional area in the year of application, but had no influence in the year after treatment. However, Webster (1990a) reported that soil or foliar applied PBZ to plum reduced trunk girth increment, and this like mean extension growth was reduced both in the year of treatment and the subsequent year.

Casper and Taylor (1989) reported that PBZ applied to container-grown 'Loring'/Lovell peach seedling reduced post-treatment trunk cross sectional area increase by 26%, while GA_3 increased TCSA growth by 45%. Martin *et al.* (1987) showed that soil-injected PBZ reduced TCSA increase of 'Flavorcrest' peach trees and reduced shoot growth.

2.2.2.2 Effects of PBZ on physiological processes

2.2.2.2.1 Accumulation of mineral nutrients

Rieger and Scalabrelli (1990) found that leaf nutrient content of 'Nemaguard' peach treated with PBZ was altered, with levels of N, P, K, Fe, Mo decreasing while Ca, Mg, B and Mn increased. Coston (1986) found that PBZ applied to peach trees reduced leaf size, but increased specific leaf weight and chlorophyll and nitrogen contents in the leaf.

2.2.2.2.2 Water relations

Steffens *et al.* (1983) as cited by Steffens and Wang (1986) stated that under non-water stress conditions apple seedlings to which PBZ had been applied took up less water than control plants. It was later found that the rate of water loss was much slower from leaves on PBZ-treated trees compared with control plants (Wang and Steffens 1985). Detached control leaves lost 15% of their initial fresh weight within one hour, but it took two hours for the PBZ-treated leaves to reach the same weight loss.

Biasi *et al.* (1989) reported that on peach seedlings under greenhouse conditions shoot growth was reduced more by PBZ than by reduced soil water content. PBZ reduced root fresh and dry weight and total water consumption. PBZ combined with low soil water content reduced stomatal conductance, although at 100% field capacity PBZ increased stomatal conductance.

2.2.2.2.3 Photosynthesis and assimilate partitioning

Elfving and Proctor (1986) found photosynthesis was generally stimulated in apple trees treated with PBZ. The direct morphological evidence for the antigibberellin effect of PBZ is reduction in vegetative growth and the secondary effect is alteration in sink strength within the plant, allowing greater partitioning of assimilates to reproductive growth (Lever, 1986). Costa *et al.* (1986) suggested that the control of vegetative growth in the first phase of fruit growth is important, reducing competition between vegetative and fruit growth, allowing a greater amount of assimilates to be diverted to fruits as a result of the reduction in vegetative growth. Steffens and Wang (1984, 1986) reported that 0.1 mM PBZ sprayed on apple seedling leaves reduced the increase in leaf area and leaf weight on new shoots 21 days after treatment, but weight, diameter and length of fibrous roots increased. Also, carbohydrate concentration in the uppermost leaves, stem and especially in the fibrous roots increased while total plant carbohydrate was not markedly changed.

Casper and Taylor (1989) reported that PBZ applied as a foliar spray to container-grown peach seedlings reduced vegetative growth, but total plant dry weight and root:shoot ratio were not affected. However, GA_3 applied two weeks after PBZ application increased vegetative growth and plant dry weight by 38% and decreased the root:shoot ratio relative to PBZ treatment.

2.2.2.2.4 Flower initiation and fruit development

Both soil and foliar application of PBZ to sweet cherry, sour cherry and peach reduced shoot growth in the first season and enhanced flowering and fruiting in the year after treatment (Edgerton 1986). Bubán (1986) reported that spray application of PBZ to 'Gloster' apple caused a large return bloom which increased the crop in the subsequent year.

PBZ increased flower and fruit number in the next season may necessitate extra fruit thinning in those species where excess fruit number causes a problem with fruit size (Lever, 1986). Williams *et al.* (1986) reported that a high dosage of PBZ caused excessive fruit set on most tree fruit species and therefore resulted in smaller fruit and increased the potential for biennial bearing. However, Webster and Quinlan (1984) found that foliar application of PBZ did not increased flowering of plums in the subsequent year.

Miller and Swietlik (1986) reported an increase in fruit set on 4-year-old 'Golden Delicious' apple following soil applied PBZ. When Knight and Browning (1986) applied GA_3 to 'Conference' pear it led to the production of small fruit and inhibited flower initiation for the following year's crop due to the large number of fruit set in the year of application. PBZ applied together with GA_3 at flowering time resulted in a reduced fruit set and improved fruit size, while providing a reduction of vegetative growth. Webster (1990b) reported that 1.6 g soil applied PBZ to 9-year-old sweet cherry trees advanced flowering date by several days for 3 years after application and enhanced fruit set in the year of application. Jacyna *et al.* (1989) found that PBZ soil applied to 'Bing' sweet cherry advanced flowering by 6-7 days, but the length of flowering was not affected, and increased fruit size. Foliar PBZ sprays to plum trees reduced initial and final fruit set, yield and fruit size in the year of treatment, but did not in the year after treatment (Webster, 1990a). In contrast, soil applied PBZ had either a negligible or a slight beneficial effect on fruit set, yield and fruit size, but the increase in fruit size was noted for several years after soil application.

2.2.2.3 Practical use of PBZ

In a mature orchard once the available space has filled with productive vegetation, the question is how to achieve minimum vegetative growth to maximize yield (Martin, 1989). Effectively reducing the number and length of vegetative shoots by suitable growth retardants in a number of situations would be of considerable benefit to fruit growers. The reduction of vegetative growth of trees, such as apricot, for which no dwarfing rootstocks or compact scion cultivars are available, makes chemical growth regulation an attractive possibility. PBZ may allow planting of such fruits in high density orchards which would allow improved cropping efficiency. Quinlan and Richardson (1984) stated that reducing the vigour of fruit trees is a major problem facing the orchardist, and results suggested PBZ can give long-lasting control of tree growth.

A survey of Australian stone fruit growers by Hillier and Rudge (1989) showed PBZ had good potential for growth control and increasing yield an average of 24%. A majority of growers noted fruit colour was enhanced and fruit size, fruit number and yield increased. This was achieved by PBZ providing good vegetative vigour control and increasing fruit size and at the same time allowing trees to carry more fruit. Similar benefits were shown by Martin (1989) and over a three year experiment PBZ increased peach grower returns by an average of US$ 2018 ha^{-1} (Table 2.1).

Table 2.1 Economic evaluation of paclobutrazol in a 'Flavorcrest' peach orchard located in California (Yoshikawa *et al.*, 1987 as cited by Martin 1989).

	Dollars/ha	
	Control	*Paclobutrazol*
Expenses		
Paclobutrazol application	0	150
Paclobutrazol chemical	0	?
Winter pruning	1040	1040
Summer pruning	162	0
Fruit thinning	1262	2142
Harvest - $1/box	1625	2757
Fruit Packing -$2.45/box	3981	6755
TOTAL:	$8070	$12844
Income		
Packed boxes/ha (Control 1625; Pachlobutrazol 2757)		
Total gross at $6/box	$9750	$16542
Net return		
Total gross income	9570	16542
Total expenses	8070	12844
Net profit/ha	$1680	$ 3698

Williams (1984) pointed out that using growth retardants to control tree size might lead horticultural practice to eliminate the use of rootstocks to control tree size. It would be possible to propagate trees by micropropagation and transplant them to the orchard site on their own roots in a high density planting, then at a suitable time after adequate training a GA inhibiting agent such as PBZ could be used. However, Hoffmann (1992) in his survey of plant growth regulators (PGR) suggested that the action of PGR is highly specific as to plant species, variety, organ, the stage of development and strongly dependent on its rate of application. Unfavourable weather conditions might cause damage or unwanted side-effects. Stan and Burloi (1989) after 5-years study of the effects of PBZ on sweet cherry, peach and plum under Romanian conditions reported a reduction of vegetative growth and pruning weight for 2-3 years after treatment. They pointed out that more research was needed to evaluate the effects of PBZ application over an extended period of 2-3 years cycles in high density plantings, and to determine any other physiological and biochemical changes associated with long term GA biosynthesis inhibitor application. Also, Davis *et al.* (1988) reviewed the literature on the use of triazoles on fruit trees, and suggested that before using triazoles on a large scale on fruit trees, more information was needed regarding the long term effects of repeated applications on reproductive and vegetative growth. They pointed out that triazoles had been available for research only since 1980, and time had not been adequate to carry out long-term experiments to see the long-term effects of using this type of growth retardant. Williams *et al.* (1986) suggested on-site trials for determination of proper rates and type of PBZ application for commercial use in controlling tree size. They stated that as there were no dwarf rootstocks for most stone fruits, chemical control of vigour was a welcome possibility. However, they concluded that more research and many orchard trials on the various tree fruit species would be necessary to find all of the benefits and problems associated with using growth retardants for vigour control.

2.2.2.3.1 Type and time of PBZ application

Lever (1986) suggested that the optimum application method depended upon species, natural growth pattern and training system as well as cultural method. In general, either soil or foliar application had proven satisfactory on stone fruit while spray application to pome fruit performed better. However the spray regime needed to be integrated with optimal production practices (eg use of pesticide), and tree age and planting density considered to optimize tree productivity in the long term for commercial use. Ramina *et al.* (1986) reported that foliar application of PBZ had a stronger effect on peach trees, while soil application induced a more uniform effect. Foliar application of PBZ at 2000 ppm or soil application at 1-2 kg ha^{-1} to 'San Pedro' peach enhanced yield and fruit size (Loreti *et al.* 1989). Williams *et al.* (1986) suggested that a mid- or lower-trunk spray or paint, or soil application in a continuous narrow band on both sides of tree rows were satisfactory methods of application. They considered that about 1 g soil applied PBZ to sweet cherry, peach, nectarine or plum of 15 cm trunk diameter was sufficient to control vegetative growth over two seasons. They reported that pome fruit trees were less responsive than stone fruit and needed more chemical, and considered spray application unsuitable because PBZ is not translocated out of leaves into growing points. However, Quinlan and Richardson (1986) reported that PBZ may reduce growth by either soil or foliar application. They suggested under English environmental conditions foliar application to pome fruit trees gave a more consistent and rapid response than soil application. They recommended multiple low-rate sprays as being more effective for long-term vigour control than a single application with the same amount of chemical. Webster (1989) considered that soil application of PBZ was the more effective method for plum with rates of 0.4 to 3.2 g active ingredient per tree, depending upon rootstock, scion cultivar, soil type and tree size and age. Gaash (1986) reported that either spray or soil application of PBZ to apple, plum and apricot reduced vegetative growth under a Mediterranean climate, with dose response curves being related to species, clone, rootstock and tree size. For instance, big trees with longer shoots and greater trunk girth needed a larger amount of chemical.

Erez (1986) pointed out that timing of application has an important role in the control of vegetative growth by PBZ. Autumn or winter application was more effective than spring in peach orchards. However, the proper combination of time and dosage would enable achievement of the required effect. Bonomo *et al.* (1986) compared May and July (northern hemisphere) application of PBZ to young 'Golden Delicious' apple trees. They suggested that May application inhibited shoot growth more, while July application had less effect, but produced inhibition in the year after application.

2.2.2.3.2 The effect of PBZ on fruit quality and cropping

Elfving and Proctor (1986) reported that foliar or soil applied PBZ in spring to six-years-old 'Summerland McIntosh'/M.26 or 12-years-old 'Spartan'/M.26 apple trees had no effect on fruit or yield in the year of application. In the year following, mean fruit weight and yield of 'McIntosh' apple were unaffected while there were reduction in 'Spartan'. They concluded that the reduction in fruit size at higher PBZ dosage was possibly due to a direct long term effect on the fruit itself, perhaps on cell division. Erez (1986) found that 1 g PBZ per peach tree soil applied in autumn had a positive effect on fruit size, but had no effect on fruit number. It appeared a low dosage of PBZ was able to inhibit vegetative growth, but had only a minor effect on the fruit, while higher dosages showed a marked effect on the fruit. Accordingly, he suggested the effect on fruit size may be indirect and result from a relative strengthening of the fruit's sink power.

Lever (1986) reported that PBZ was able to have direct and indirect effects in improving fruit colour and size. The direct effect being a reduction in vegetative growth and increased fruit sink strength, the indirect effect by reducing vegetative growth, and so increasing light penetration to the fruits. Facteau and Chestnut (1991) found that application of PBZ to sweet cherry did not affect fruit size, soluble solids concentration or firmness. However, number of fruit/cm shoot growth was increased, but fruit number per flower bud decreased with increased PBZ concentration. Ogata *et al.* (1986) reported that application of PBZ to nectarine and peach trees had no effect on fruit quality aspects such as acidity and soluble solids, however flesh firmness was decreased.

Marini (1987) reported that fruit maturity, yield and number of fruits were not affected by PBZ application to peach trees in the year of application. Ramina *et al.* (1986) found that marketable yield of peach trees treated with PBZ increased and that fruit ripening was delayed. PBZ applied to nectarine trees did not affect yield in terms of total crop weight and fruit number, although mean fruit weight was larger (Blanco, 1986). However, Williams (1984) reported that PBZ advanced maturity and increased fruit yield of apple.

Webster and Andrews (1985) reported that a spray application containing 1 or 2 $g \cdot l^{-1}$ PBZ reduced fruit number and increased final fruit size of 'Victoria' plums when applied at full bloom. Soil-injected PBZ had no effect on 'Flavorcrest' peach yield in the year of application, whereas yield was increased in two subsequent years (Martin *et al.*, 1987). The cumulative yield increased from 30.1 to 41.8 $t \cdot ha^{-1}$ over the 3-year experiment. Also, fruit size was increased and fruit maturity advanced. No measurable fruit quality loss was observed, except in soluble solids, which were reduced in two of the three years.

Tukey (1986) found that spray application of PBZ to apple trees resulted in heavy fruit set and smaller fruit size. Miliou and Sfakiotakis (1986) reported that apple trees treated with PBZ produced smaller and flattened fruit with shorter pedicels, and a small increase in soluble solids and firmness. Privé *et* *al.* (1989) showed that the apple pedicel has an internal structure similar to a stem and is sensitive to growth retardants. Applied PBZ reduced the pedicel and fruit cell length and decreased fruit fresh and dry weight and fruit length and diameter in both 'McIntosh' and 'Spartan' apple trees.

2.2.3 WATER STRESS

Water is one of the most important environmental factors for plant growth, its importance has been recognized in the past and its role continues to be studied by researchers (Kramer, 1983). The amount of available water and temperature are two primary environmental parameters that frequently limit plant growth (Kanemasu and Asrar, 1985). Shortage of water, supplied by rainfall or irrigation, in addition to high temperature and evaporation may cause drought conditions for growing plants. Since agriculturists first observed the effect of water stress on the physical appearance, yield and quality of the crops that were being grown, they have tried to find out how plants react to water stress. Kramer (1983) suggested that water can affect plant growth by influencing physiological processes such that every plant process is affected by the water supply. The degree of this response varies and depends on environmental conditions as well as on species, size, shape and the other morphological and physiological character of the plants. Bradford and Hsiao (1982) stated that low plant water status was associated with various physiological responses, such as growth reduction and stomatal closure, which may reduce plant water consumption and overall plant productivity. Bennett (1990) reported that the water status of plant cells affected the physiological and metabolic activity of the cells and their expansion and growth.

The free energy per unit quantity of substance, specifically per gram molecular weight (i.e. the free energy mol^{-1}), is called the chemical potential. The chemical potential of water may be used as the basis for a property of water in plant-soil-air systems. The chemical potential of each system containing water is equal to the water potential of that system, and water potential of pure water is assumed to be zero (Salisbury and Ross, 1992). Begg and Turner (1976) and Jones *et al.* (1985) suggested the components of the water potential (Ψ_w) equation as:

$$\Psi_w = \Psi_p + \Psi_s + \Psi_g + \Psi_m$$

> where: Ψ_p is pressure potential, Ψ_s is osmotic potential, Ψ_g is gravitational potential and Ψ_m is matric potential.

The gravitational potential (Ψ_g) increases by only 0.01 MPa for each 1m height, so would be less than 0.05 MPa for most fruit trees and is usually ignored (Jones *et al.* 1985). According to Kramer (1983) Ψ_p and Ψ_s are the essential components of this equation and in the specific situation (for example for most fruit trees) we can assume the following equation:

$$\Psi_w = \Psi_p + \Psi_s$$

> where Ψ_w in plants is almost always negative, and becomes lower as water stress increases. The turgor pressure Ψ_p is produced by diffusion of water into protoplasts exerting a pressure outwards on the enclosing walls, is responsible for cell expansion and growth and usually is positive. Loss of turgor pressure results in wilting. Ψ_s is osmotic potential which is the component produced by solutes dissolved in the cell sap, mainly the vacuolar sap.

The differences in water potential in all parts of a system is equal to zero at equilibrium (i.e., the differences in water potential are 0; $\Delta\Psi_w = 0$). However, some factors, such as vapour density and pressure, temperature and solute, are able to have a direct effect on these differences (Salisbury and Ross 1992). The water potential in any system is decreased by those factors that reduce the relative vapour pressure, including addition of solutes, and increased by those factors that increase the relative vapour pressure such as elastic cell wall pressure on the cell contents and increase in temperature (Kramer, 1983).

The increase in $\Delta\Psi_w$ acts as a driving force, moving water molecules from one point to another within the system. The quantity of water molecules or solute particles crossing a unit cross-sectional area in time, is proportional to the magnitude of the driving force and inversely proportional to the resistance to diffusion. Fick's First Law equation shows this mechanism of movement:

$$J = \frac{\Psi_{w1} - \Psi_{w2}}{r} \quad or \quad J = \frac{\Delta\Psi_w}{r}$$

> where: J is the water flux density (g $m^{-2}s^{-1}$), $\Delta\Psi_w$ or (Ψ_{w1} - Ψ_{w2}) is the water potential difference between two points, which is the driving force for diffusion of water, and r is the resistance to diffusion of water between Ψ_{w1} and Ψ_{w2} (Salisbury and Ross, 1992).

As a consequence of water movement through soil and the plants, Begg and Turner (1976) pointed out that water evaporates from the mesophyll cells of the leaves causing a reduction in Ψ_w. Then water moves toward the point of lowest water potential along a gradient distributed throughout both the plant and the soil. They concluded that since the plant can extract water from the soil only when the water potential in the plant is lower than that in the soil, the water in the plant is seldom in equilibrium with the water in the soil. When the plant is becoming water stressed, the amount of plant water loss exceeds water uptake by the roots. Syvertsen (1985) constructed a summary of contributing factors which influence water movement into and out of the plant system at the soil-root and leaf-air interfaces (Table 2.2).

Water deficit or water stress refers to conditions in which plant water potential and turgor is decreased enough to interfere with normal functioning. The level of water stress at which this occurs depends on the type of plant and the stage of development (Kramer, 1983). Water stress in the plant results from

Table 2.2 Summary of contributing factors that influence water movement proposed by Syvertsen, 1985.

Soil to root (supply)	Leaf to air (demand)
Rain, irrigation	Tissue capacitance
Canopy interception	Plant water potential gradients
Soil water potential gradients	Stomatal conductance
Soil H_2O conductivity	Turgor, ABA
Root distribution, density	Radiation
Root conductivity	CO_2 concentration
Genetic (rootstock)	Temperature
Temperature, aeration	Humidity gradient
Water potential gradients	
Nutrition	

a decline in soil water potential over a period of time and is also influenced by evaporative conditions, which are affected by daily changes in net radiation and humidity (Morgan 1984). The progressive changes in soil and plant water potential with reducing supply of available soil water are presented schematically in Figure 2.1. The upper limiting curve shows the progressive decline in water potential of the soil bulk and the other curves show the water potential in the leaves and roots, assuming that transpiration proceeds for 12 hours and then ceases for 12 hours. This diagram shows how the depletion of available water in the soil affects leaf and root water potential. At the beginning of developing a water deficit leaf water potential is able to recover to the degree of soil water potential, but as the soil dries the recovery decreases. When movement of water toward the roots in drying soil becomes too slow wilting occurs during the day (day 3 and 4 in Figure 2.1). Permanent wilting occurs when leaves are not able to recover their turgor during the night (day 5 in Figure 2.1). In the development of water stress, the rate of transpiration, the rate of water movement through the soil to the roots, and the

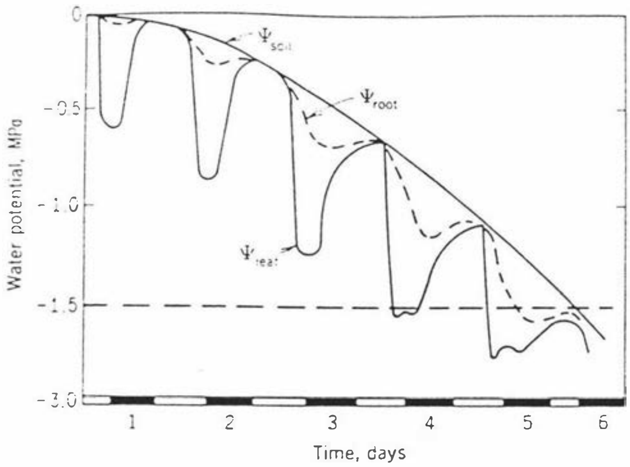


Figure 2.1. Schematic diagram of the development of plant water deficit (Slatyer, 1967). Dark areas on the X-axis represent nights.

relationship between soil and leaf water potential are three important factors. The degree of response to water deficit which causes wilting is a plant related characteristic (Kramer, 1983). It usually occurs when the soil water potential drops to -1.5 MPa (Kramer, 1983). However, positive turgor is necessary for cell expansion and plant growth, and maintaining positive turgor potentials at low tissue water potential can be an important mechanism for maintaining plant growth under water stress conditions (Hsiao *et al.*, 1976).

From early this century numerous papers and books on water stress have been published. The objective of this review is not to cover fundamentals and basic concepts, but to review the relevant literature relating to fruit trees and this study.

2.2.3.1 Effect of water stress on growth of fruit trees

2.2.3.1.1 The effect of water stress on roots

Some interactions between root and shoot growth and their importance in controlling vegetative growth are described in the root-pruning section (see

2.2.1.1.3). The importance of roots and their function in absorbing water and mineral nutrients are discussed in detail in some text books (eg. Kramer, 1983; Kramer and Kozlowski, 1979; and Salisbury and Ross, 1992).

Under water stress conditions the growth of the roots usually suffers less than that of the aerial parts (Kramer, 1983). However, these two parts are entirely inter-dependent, shoots supplying carbohydrates to the roots which supply water and mineral nutrients to the top of the plant. So it would be surprising if there were not a close coordination between growth and activity of the root and shoot systems (Richards, 1983). The top:root dry weight ratio is an index of growth commonly used to illustrate the coordination between roots and shoots, this being subject to change during the growth and phenological development of plants. This "functional equilibrium" was discussed in section 2.2.1.1.3. However, the physiology of shoot growth in drying soil can be modified as a function of soil drying, even when shoot water relations are not altered. This suggests plants have a sense of communication between roots and shoots by some means other than a reduction in the flux of water to the shoots (Davies and Zhang, 1991).

Root zone water management to decrease vegetative growth without affecting fruit growth by regulated deficit irrigation (RDI) was a new concept developed by Chalmers and co-workers in Australia. Chalmers and van den Ende (1975b) suggested this management could be an important technique to control vegetative growth and production of peach trees. Jerie *et al.* (1989a) suggested that maintaining a high density orchard without using dwarfing rootstocks required control of the wetted root zone water stress with RDI. There was good potential for achieving this in shallow well drained soils and/or in a dry climate, during periods of rapid shoot growth.

Sinclair and Ludlow (1985) described root water uptake and transpiration effects on stomatal behaviour and plant water balance as having three stages.

In the first stage soil water content is high, the root's rate of water uptake matches the transpiration rate, and stomata would be open. Stage II occurs when transpiration is high and root water uptake rate can not match the transpiration rate. In this stage root uptake dominates the water loss rate, and stomata adjust the water balance in the leaves (Cowan, 1965). Stage III begins as the soil dries further, root water uptake declines, and stomata lose the capability to adjust the balance between root water uptake rate and transpiration rate. In this situation the stomata are closed and transpiration rate is dependent on the vapour conductance of the epidermis. Gollan *et al.* (1986) showed that in a plant growing in drying soil, the decline in soil water status eventually induced stomatal closure and reduced stomatal conductance. They concluded that roots were sensitive to a reduction in soil water content and sent a signal to the leaves which induced stomatal closure.

Jones *et al.* (1983) found that midday xylem water potential was higher in plants subject to a moderate water stress than in irrigated plants over a period of several weeks. This status was due to reduced stomatal conductance and is apparently an example of over control of water potential. Davies and Zhang (1991) questioned why plants in unwatered soil showed low conductance when their shoot water potentials were high. They concluded that one explanation is that the stomata do not open because plants can sense the availability of water in the soil and regulate stomatal behaviour accordingly, whatever the water status of their shoots. They cited the suggestion of Jones (1980) and Cowan (1982) that such detection involves chemical message transfer from roots to shoots. Davies and Zhang (1991) stated that root signals not only influence stomatal behaviour (and therefore carbon gain) but also regulate leaf initiation, leaf expansion, and other developmental processes. Davies and Zhang (1991) claimed observations of higher leaf water potential in dry than moist soil argued against the general relevance of leaf water potential as a regulator of plant development. Leaf water potentials can vary from minute to minute (for example under cloudy conditions), therefore they suggested it was clear rapid changes in leaf water potential are not an appropriate control signal for plant development. However, Kramer (1988) has suggested that leaves dehydrate before roots because they lose water to the atmosphere, and therefore would be a more sensitive indicator of water stress. He concluded that it was possible to produce experimental conditions in which shoot physiology and stomatal behaviour were affected by root signals. This required roots to be stressed before shoots and in the field usually shoots are affected by water stress before roots. Thus, he assumed it unlikely that roots are the primary sensor of water stress in the field.

2.2.3.1.2 The effect of water stress on shoot growth

Plant growth is evidence of cell expansion which is believed to be the physiological process most sensitive to water stress (Hsiao 1973). However, both cell division and cell expansion are affected by water stress. In general, water stress reduces shoot growth more than root growth, because more severe water deficits develop in the transpiring shoots (Kramer 1983). In addition, water stress would be expected to markedly reduce leaf area development due to the sensitivity of cell expansion (Hsiao 1973).

Water stress stopped shoot growth of ‘Bing’ sweet cherry, and stress developed more rapidly on ‘Colt’ rootstock than on ‘Mahaleb’ (Southwick *et al.* 1993). A similar result was reported by Proebsting *et al.* (1989) in both 1- and 2-years-old peach trees, and caused a reduction of vigour of ‘Flordaprince’ peach trees (George and Nissen 1992b). Work by Higgs and Jones (1991) showed that when apple trees were subjected to water stress there was a 40-50% reduction in the number of shoots and up to 62% in the weight of shoots removed by summer pruning.

Water stress applied to peach trees in both stage I and II of fruit development reduced shoot growth, but shoot growth stopped on both control and stressed trees at the same time, in July (northern hemisphere)(Li *et al.* 1989). The work of Chalmers *et al.* (1984) showed that the vigour of intensive peach and pear orchards could be successfully managed using deficit irrigation. Water deficit was applied during the early part of the growing season to limit shoot growth. At the beginning of final rapid fruit growth full irrigation was resumed and, by harvest, the fruits were similar to, or larger than, those from continuously irrigated trees.

2.2.3.1.3 The effect of water stress on trunk cross sectional area (TCSA)

Water deficit in general reduces TCSA. Girona *et al.* (1993) reported an 8% reduction in trunk growth of peach trees subjected to RDI. Secondary growth of TCSA of peach trees was reduced by 60% when irrigated with 12.5% E_{ps} (evaporation over the planting square)(Chalmers *et al.*, 1985) and increased on trees received 100% E_{ps} (Mitchell and Chalmers 1982). The increase in growth of TCSA in pear trees was linear with increasing level of water replacement (Mitchell *et al.*, 1984). Huslig *et al.* (1993) found TCSA of peach trees subjected to water stress increased when irrigated during stage III of fruit growth, however there were no significant differences in TCSA among irrigated treatments.

Westwood and Roberts (1970) found a linear relationship between TCSA and above ground weight of lightly pruned apple trees. The work on 'Bartlett' pear by Mitchell *et al.*, (1989) showed the increase in TCSA was related to the annual growth of the tree, with a lower increase obtained on closer spaced trees.

2.2.3.2 Effects of water stress on physiological processes

Water in plants is linked to water in the soil via root epidermal cells, and to atmospheric moisture via stomata. The movement of water has been discussed briefly in previous sections, but is described in detail in the literature, for example Kramer (1983), Passioura (1988) and Salisbury and Ross (1992). When water in the soil is adequate, the movement of water in plants is controlled by transpiration which is regulated by both stomatal aperture and evaporative demand. When the plant is in drought conditions this movement through the plant system is regulated primarily by soil water supply and root conductivity (Syvertsen 1985).

Water stress is characterized by decrease in water content, turgor, and total plant water potential, resulting in wilting, partial or complete stomatal closure, and a decrease in cell enlargement and plant growth. Severe water stress conditions cause cessation of growth, decrease or cessation of photosynthesis, disturbance of many metabolic processes, and finally death of the plant (Kramer, 1983).

2.2.3.2.1 Accumulation of mineral nutrients

Some of the materials absorbed by the roots must be moved to the leaves to allow assimilation. Minerals that are absorbed into the roots typically move up the plant in the xylem to the leaves, and then solutions move through the phloem tissue from assimilating organs to utilizing organs (Salisbury and Ross 1992). When the soil dries, it affects water uptake by the plant and the transport of nutrients is markedly reduced (Nye and Tinker, 1977).

The reduction of leaf turgor potential that accompanies the loss of water from leaf tissue directly affects many important morphological and physiological processes, such as leaf enlargement, stomatal aperture, photosynthesis and N assimilation (Kramer 1983). The work on apricot in South Australia by Loveys *et al.*, (1987) showed that between December and March relative water content (RWC) declined and leaf osmotic potential fell by approximately 0.7 MPa, while the concentration of all ions increased. They found that potassium was the most abundant cation in apricot leaves and substantial amounts of calcium and magnesium were also present.

2.2.3.2.2 Water relations

The importance of water for plant growth and the effect of a water deficit on vegetative growth of organs was discussed in previous sections. Syvertsen (1985) observed that water is generally considered to be the most important factor limiting plant growth. There is no doubt that a plant water deficit can limit yield and quality of crops. Jones (1990) pointed out that a clear understanding of how water deficits affect crops and of the natural adaptive processes by which different species respond to limited water availability should enable us to optimize management procedures (i.e. how and when to irrigate). He summarized the complex series of environmental and physiological controls and some of the interactions between them such as:

a. The soil water potential and those factors controlling it such as rainfall and water use, soil depth and texture, root distribution and activity and crop type and spacing.
b. Evaporation rate and environmental factors affecting water loss (ie., radiation, humidity, temperature and wind speed) as well as the physiological factors, leaf area and stomatal conductance.
c. Hydraulic conductances of flow pathways within the plant.
d. Water potential and its relationship with the other measurements such as turgor pressure, osmotic potential and water content and the possible existence of osmotic adjustment.

Water potential is the chemical potential of water, and pressure potential and osmotic potential are the main components of water potential (Kramer, 1983; Salisbury and Ross, 1992). Turgor pressure plays an important role in cell enlargement (Kramer 1983), and the most likely mechanism through which water stress can affect metabolic processes is by changes in turgor pressure (Hsiao, 1973). Munns (1988) argued that while turgor must be above zero for growth to occur, turgor does not control cell expansion. Kramer (1983) pointed out that water stress caused a reduction in osmotic potential of plant tissue in some plants. This contributes to the maintenance of turgor and presumably to maintenance of normal metabolic activity. Part of the decrease in osmotic potential as plants are subjected to water stress is caused by concentration of cell solutes resulting from water loss. Osmotic adjustment refers to net accumulation of solute in the cell and must be distinguished from the passive increase in concentration caused by loss of water (Turner and Jones 1980). So, we are able to summarize the changes of Ψ_s during the period of water deficit as two types:

i. Passive changes in osmotic potential which are the result of water loss and then reduction in cell volume.
ii. Active changes in solute which result from the net accumulation of solutes by transport through the cell membrane or biochemical alteration of cell material. This is called osmotic adjustment.

In the literature the term osmoregulation is sometimes used synonymously for osmotic adjustment. The term osmoregulation will not be used in this thesis. Munns (1988) considered the term should be used with caution, as it was not universally used to refer to the same phenomenon. She reported that a large amount of literature has been published in recent years on changes in osmotic potential, but most studies looked at the simple measurement of osmotic potential rather than trying to test the hypothesis of causes and effect of osmotic adjustment. So, physiological knowledge of such view has not been improved. It is clear that osmotic adjustment itself cannot promote growth, and the accumulation of solute which accounts for the reduction in osmotic potential must be diverted from other essential processes such as protein and cell wall synthesis. However, osmotic adjustment is one of the elements in the physiological adjustment of plants to drought conditions.

Osmotic adjustment refers to a reduction in osmotic potential (Ψ_s) greater than can be explained by concentration of solute during dehydration (Kramer, 1983). This explanation of active osmotic adjustment agrees with that of Turner and Jones (1980) as referred to by Morgan (1984), and suggests changes in Ψ_s must be distinguished as to whether or not they are due to a reduction of cell volume by water loss, or due to net accumulation of solutes. In the case of passive osmotic adjustment, the osmotic potential is inversely related to the osmotic volume:

$$\Psi_s = \Psi_{s_o} \frac{V_o}{V}$$

where: Ψ_{So} and V_o are the osmotic potential and osmotic volume at full turgor.

Reduction in Ψ_s arises from the amount of solutes present at full turgor. When solute accumulates, the Ψ_s corresponding to a value of V less than V_o will be lower (more negative) than that predicted by this equation (Morgan, 1984) and the active osmotic adjustment may be calculated as the difference between the measured and predicted values (Morgan, 1980). In a recent study of apple trees under water stress conditions Wang and Stutte (1992) explained this issue of active and passive osmotic adjustment. They adjusted Ψ_s to 100% RWC using the following equation to eliminate the passive osmotic adjustment related to tissue dehydration:

$$\Psi_s \ at\ 100\%\ RWC = \frac{measured\ \Psi_s \times RWC}{100}$$

In a situation of lowered leaf water potential osmotic adjustment enables cell enlargement and continuing growth, keeping stomata open and photosynthesis operating (Kramer, 1983). Maintenance of turgor by osmotic adjustment could therefore be an important adaptational response in some species during drought periods (Kramer, 1983 and Morgan, 1984).

Osmotic adjustment occurs in such organs as roots and leaves and the capacity for adjustment is higher in expanding organs (Morgan, 1984). The work on apricot by Loveys *et al.* (1987) showed that, at low leaf water potential, osmotic adjustment allowed the maintenance of high stomatal conductance and photosynthesis. In apricot leaves, when water potential was low, turgor was maintained because the osmotic potential had fallen to a low value during development of the water stress. The drop in leaf osmotic potential was partly accounted for by a decrease in relative water content (RWC). There was a net accumulation of solute between December and March (southern hemisphere), shown by measuring osmotic potential at full turgor. Ruggiero *et al.* (1988) evaluated osmotic adjustment in young 'Cafona' apricot trees subjected to different water regimes. They found apricot showed a good ability to adjust leaf Ψ_s, with a noticeable long term adaptation to increasing water stress. During the season as Ψ_s decreased, the accumulation of soluble sugars increased.

Ranney *et al.* (1991) found that expanding and mature leaves and roots of 'Colt' cherry trees subjected to a period of increasing water stress maintained their turgor at water potentials of -2.08, -2.09 and -1.85 MPa. Lakso *et al.* (1984) found leaf osmotic potential of 'McIntosh' apple reduced with increasing leaf age. Osmotic potential of leaves at the growing shoot tip were higher than mature leaves by 1.0 to 2.0 MPa.

Osmotic adjustment in expanding cherry leaves resulted either from increased translocation of sorbitol to young leaves or from a decreased rate of sorbitol metabolism (Ranney *et al.*, 1991). Sorbitol is the primary photosynthetic product and translocated carbohydrate in many woody Rosaceous species. Sorbitol is synthesized in older leaves in apricot (Bieleski and Redgwell 1985) and in photosynthetic tissue it can approach 60-80% of total soluble carbohydrates (Bieleski 1982). Sorbitol was the solute chiefly responsible for osmotic adjustment in apricot leaves (Loveys *et al.*, 1987). Wang and Stutte (1992) found sorbitol constituted a major carbohydrate component in apple leaves and had an important role in osmotic adjustment during water stress. They offered the following possible explanations for the effect of water stress on sorbitol accumulation.

i. It may increase the partitioning of newly fixed carbon into sorbitol;

ii. It may induce the enzymatic pathways that break down starch and/or sucrose, which increase the substrate (glucose-6-p) for sorbitol synthesis;

iii. It may reduce the rate of sorbitol transport from the leaves compared with that of sucrose.

In relation to plant water status, undoubtedly stomata have an important role. Hall *et al.* (1993) suggested that stomata are important in controlling the balance between water loss and carbon gain, i.e. biomass production. Stomatal conductance is influenced by environmental conditions, such as water stress, which are able to affect photosynthetic efficiency (Hall *et al.*, 1993; Kramer, 1983). Wang and Stutte (1992) found that in apple trees as water stress developed photosynthesis declined, and stomatal resistance increased.

However, under water stress conditions a total water status characterized by decreases in water content, turgor and plant water potential may be influenced by some environmental factors like temperature. George and Nissen (1992a) found air temperature to be the most important environmental factor influencing plant water status of Atemoya (Custard apple) in both field and controlled environment conditions. Larsen *et al.* (1989) evaluated diurnal water relations of apple, apricot, grape, olive and peach under arid environmental conditions in Jordan. They reported that apricot, olive and grape had similar transpiration rates and lost less water per unit leaf area than peach or apple.

Apricot and peach reached maximum leaf conductance at midday, and apple and olive in the afternoon.

Stomatal conductance and daily leaf and stem water potential of peach trees decreased linearly with decreasing water content in the upper 60 cm of soil (Garnier and Berger, 1987). Predawn leaf water potential was insensitive until soil dried to a threshold of available soil water content. Jones (1990) reported there was some evidence that predawn leaf water potential was more sensitive than midday leaf water potential for measuring plant water status. Similarly Natali *et al.* (1985) reported that predawn xylem water potential was a useful indicator of available soil water content.

However, leaf water status varies with age (Steinberg *et al.*, 1989). Predawn xylem water potential of immature folded leaves was 0.24 MPa lower than mature leaves under both wet and dry conditions. Asian pear subjected to water stress had lower predawn xylem water potential than fully irrigated trees, and xylem water potential of water stressed trees followed a more rapid decline during the morning and a slower recovery during late afternoon (Caspari *et al.* 1993). Plant water use by stressed plants was lower than by irrigated plants.

RDI applied to apple trees caused a reduction in soil moisture and xylem water potential (Ebel *et al.*, 1993). Chalmers and Wilson (1978) found leaf water potential in peach was lower and limb shrinkage greater during DWIII than DWII of fruit growth. The hydraulic gradient increased from 0.1 MPa m^{-1} in DW II to 0.2 MPa m^{-1} in DW III. They suggested that total plant water use increased substantially during periods of high assimilate demand by the fruit during stage III of fruit growth. Peach trees varied in their response to water stress. Wilting, the most obvious symptom of water stress, did not happen in unfruited peach trees until quite late in an irrigation cycle even in periods of high temperature and evaporation. However, heavily cropped peach trees wilted much sooner, even on the day following irrigation if the environmental stress was high.

2.2.3.2.3 The effect of water stress on photosynthesis and assimilate partitioning

These processes are critical control points where environmental factors and physiological mechanisms interact to regulate exchange of energy and materials (water, carbon, oxygen and minerals) (Syvertsen, 1985). There are two major contact points between plants and their environment. One exists between shoot tissue and the aerial environment, where CO_2 and O_2 are exchanged in leaves and H_2O diffuses out of stomata. The other exists between root and soil, where water, mineral nutrients and oxygen enter the plant system.

The main environmental factors affecting photosynthesis of a leaf are water, light, the ambient CO_2 and O_2 concentrations, mineral nutrients and leaf temperature (Lawlor, 1987). However, water is often the most limiting factor for photosynthesis in natural or agricultural ecosystems (Salisbury and Ross, 1992). Farquhar and Sharkey (1982) reported that water stress causes the closure of stomata and uptake of CO_2 is therefore restricted.

Kanemasu and Asrar (1985) pointed out that transpiration and photosynthesis have a major role in crop yield models. Leaves strongly control transpiration which is an energy consuming process. However, photosynthesis is a quantum-requiring process which is dependent to a large extent upon the interception of visible light and the diffusive conductance of CO_2. The energy for transpiration and the quanta for photosynthesis are provided by solar radiation. Therefore, stomatal conductance is an important factor in photosynthesis, and stomatal control of leaf conductance is an important means by which plants limit water loss (McDermitt. 1990). Flore *et al.* (1985)

showed stomatal conductance (g_s) and photosynthesis were reduced in apple and cherry by mild water stress.

Partitioning of the carbohydrate produced by photosynthesis is also important. Despite studies on the influence of various cultural factors in growth and fruiting of fruit trees, little is known about how the tree allocates its resources or the priorities by which it allocates them (Chalmers and van den Ende, 1975b). Several experiments, particularly on fruit development, suggest that hormones play a role in determining sink strength. Chalmers and van den Ende (1977) demonstrated in peach that the kind and concentration of hormones can affect the mobilizing ability of a sink-hormone directed transport phenomenon. However, both growth and cropping depend on a ready supply of carbohydrates and nutrients. The available carbohydrate resources are divided between the several sinks, determining the pattern of growth (Oliveira and Priestley, 1988). Kramer and Kozlowski (1979) have suggested there is a ranking of sink strengths during the growing season: fruits and seeds > young leaves and stem tips > mature leaves > cambia > roots > reserves.

In the area of physiology, there have already been some studies on the relationship between production and utilization of carbohydrates in the partitioning of dry matter. Patrick (1988) demonstrated that partitioning of photoassimilates is the end result of a coordinated set of transport and metabolic processes governing the flow of assimilates along an array of source-path-sink systems. The activities of these processes are not static, but may change both during the day and during development of the plant. Wardlow (1990) reported that it is important therefore to recognize that carbon partitioning is sensitive to environmental changes that may differentially influence the relation between growing organs and between growth and photosynthesis.

Under water stress conditions the level of water-soluble carbohydrates in the leaves of some species is increased. This accumulation, or osmotic adjustment, in the leaves of plants under water stress also probably results from a reduction in growth and slowing in carbohydrate utilization prior to a reduction in photosynthesis (Munns, 1988).

However, the response to water stress is dependent on the stage of plant development. The work by Chalmers and van den Ende (1975b) on 4-15 years old peach trees showed that with increasing tree size and age the distribution of dry weight changed considerably. The ratio of vegetative growth made by the top of the tree to root growth changed from 1 in young trees to 4 in the oldest trees, but there was a strong allometric relation between the growth of both portions. They suggested root growth of a large tree required less than 1% of the total annual dry weight increment, but in a young tree this proportion was 20%. In larger and more mature trees a greater proportion of assimilate supply was distributed to fruits, while in the young and smaller trees a larger proportion went to the frame and root growth. Under the conditions of their experiment trees older than 9 years did not allocate increasing proportions of assimilate to the fruits.

Chalmers *et al.* (1975) found the increase in photosynthesis in DWI and DWIII was due to the increase in fruit growth rate which increased assimilate demand at these stages of fruit growth. They found that photosynthesis is closely related to changes in carbon requirements at different stages of fruit growth. Over two-thirds of the dry weight of a peach fruit was accumulated during the last 1/3 of the growing season, and the rate of assimilate use by the fruit increased in DWI and DWIII and decreased in DWII (Chalmers and van den Ende, 1975a).

Syvertsen (1985) suggested that an awareness of the interactions among plant nutrition, hormone balance, and drought stress, and their integration within the whole plant system, is important for understanding how water and carbon budgets affect horticultural plant productivity. Schulze (1986) similarly suggested that in understanding whole-plant performance under drought conditions, the interactions between external and internal factors are important. The production of plant biomass is not only a function of carbon metabolism, but also significantly determined by concurrent fluxes of water and nutrients and the process by which these resources are partitioned.

2.2.3.2.4 The effect of water stress on flower initiation and fruit development

The time of imposing water stress and the stage of flower bud development as well as of fruit growth are important factors in irrigation management. Water stress reduced flower bud formation of apricot trees, and in the following season flower number and yield of such stressed trees were decreased. However, the time of imposing water stress in the first season was important as the time of flower bud development was sensitive to drought conditions (Uriu, 1964). Discontinuing irrigation after harvest reduced flower bud formation, fruit set and yield of apricots in the desert areas of California. Other work on apricot trees showed fruit number per unit length of shoot was higher in irrigated than un-irrigated trees in the subsequent year (Marangoni *et al.*, 1988). Mitchell *et al.* (1984) demonstrated RDI increased flower density in the subsequent year on pear. The final rate of fruit growth was faster than control on trees subjected to deficit irrigation during stage one of fruit growth and then irrigated.

2.2.3.2.5 The effect of water stress on endogenous ABA

Abscisic acid (ABA), as a plant growth inhibitor (Weaver, 1972), interacts with growth promoters and thus has important effects on growth phenomena. The increase of ABA level in water stressed plants has been reported in the literature (eg. Davies *et al.*, 1986; Davies, 1987; Mansfield, 1987; Robinson and Barritt, 1990), and its physiological effects have been reviewed in the literature (eg. Milborrow, 1974; Walton, 1980; Zeevaart and Creelman, 1988).

Loveys and Düring (1984) reported an increase in ABA in leaves of grapevine subjected to drought conditions, the effect being doubled at midday compared with predawn. Loveys (1984) claimed ABA has a key role in control of stomata in field-grown vines (*Vitis vinifera*) by optimizing the relationship between CO_2 uptake and water loss. He concluded that water stress induced the synthesis of ABA in leaf mesophyll tissue, then movement of this ABA to the stomata caused their closure. Flore *et al.* (1985) suggested that the role of stomatal conductance in controlling water loss is obvious. They cited Dubbe *et al.* (1978) who found that water stress induced ABA build up in leaves, and reduction in stomatal aperture was related to increase of this hormone. Downton *et al.* (1988b) found endogenous levels of ABA increased in leaves of grapevine (*Vitis vinifera L.*) subjected to water stress. The leaves responded in a similar manner to that observed when exogenous ABA was applied. The concentration of ABA increased as leaf water potential decreased. Stomatal closure caused by the abscisic acid had the major effect of reducing photosynthesis (Downton *et al.,* 1988a).

However, Schulze (1986) concluded the ABA content of leaves was not closely related to stomatal regulation. Burschka *et al.* (1986) as cited by Schulze (1986) found the same ABA content in stressed and well irrigated *Arbutus* trees whether the stomata were open or closed, although there were big differences in leaf water potential. Injection of ABA into the transpiration stream caused rapid stomatal closure. Loveys *et al.* (1987) found ABA to be relatively unimportant in the control of stomatal conductance in apricot.

Schulze (1986) considered three responses of stomata were of major importance in explaining the control of gas exchange under drought conditions:

i. a direct response to humidity
ii. a direct response to root functioning (probably related to the metabolism of cytokinin, which tended to open stomata and counteracted the effect of ABA in the leaves)
iii. a response to mesophyll internal conditions (the metabolism of ABA and the content of ABA in the bulk leaf were not related to its action on stomata).

Apparently, only a very small amount of the total ABA content needed to be released into the apoplast adjacent to the guard cells for a significant response of the stomata.

2.2.3.3 Practical use of water stress

Water is a limited resource in many areas of the world, and water management for irrigation in horticultural industries is particularly important in those production regions. Water stress can be used to control tree size, save water and reduce time and costs for pruning, increase early bearing and yield, reduce the problem of biennial bearing and poor fruit set, increase flowering, control fruit quality and reduce certain disorders (Chalmers *et al.,* 1985) when integrated into an appropriate orchard system. These benefits have been documented for a number of crops including peaches (Chalmers *et al.,* 1981 and 1985; Mitchell and Chalmers 1982), pears (Mitchell *et al.,* 1984, 1986 and 1989), and prune (Lampinen *et al.*, 1991).

In addition, water is one of the more important factors determining fruit size and total yield of fruit crops, so good irrigation management of fruit crops is essential for high returns (Jones *et al.,* 1985). Sometimes the high cost of irrigation, the limited availability of good quality water, and the demand for more efficient fruit production has encouraged the search for an improved understanding of plant water requirements. Chalmers *et al.* (1985) after applying RDI to peach trees concluded that regulated deficit irrigation was an excellent tool for a powerful new management system that would be attractive to orchardists in suitable areas. This might easily be achieved using 1/3 less irrigation water, with currently available computer controlled irrigation technology.

2.2.3.3.1 Time and type of imposed water stress

The regulated deficit irrigation strategy suggested by Chalmers and co-workers has attracted the attention of other horticulturists due to its ability to control excessive vegetative growth and for increasing water use efficiency without having a negative effect on yield. Reduced wetted root volume and plant water deficits have been studied as potential management tools for the control of vegetative growth of commercial fruit trees (Chalmers *et al.* 1981; Mitchell *et al.* 1984).

Chalmers *et al.* (1981) reported that root competition (tree density), summer pruning and RDI appeared to inhibit tree growth. RDI was most effective when it was combined with the root competition of a high density planting. Southwick *et al.* (1993) suggested that in a high density sweet cherry orchard a combination of early season water stress and summer pruning may be the best combination for growth control. Chalmers *et al.* (1981) pointed out that regulated deficit irrigation is based on the idea that vegetative growth of stone fruit trees can be restricted if RDI is applied during the period of slow fruit growth and rapid shoot and frame growth. At this stage shoot growth was reduced by RDI (Figure 2.2). Jerie *et al.* (1989b) reported that RDI has greatly facilitated the management of pear, cherry, peach and nashi orchards established on vigorous rootstocks under in density planting.

The relationship of shoot and fruit growth, and when growth occurs during the season, are critical factors in determining the time of applying water deficit within the RDI strategy. Chalmers *et al.* (1985) showed vegetative growth of deciduous fruit trees was greatest in the spring, at which time fruits grow

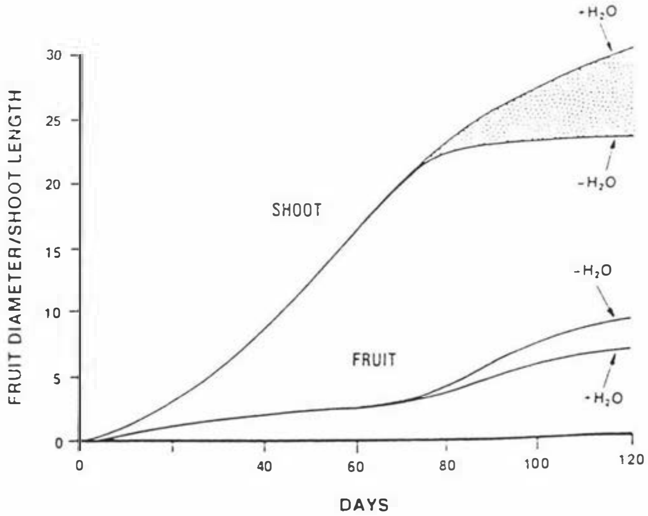


Figure 2.2 Regulated deficit irrigation (RDI) applied during pit hardening of young high-density peach trees (From Seeley 1990).

slowly (Figure 2.3). Vegetative growth of peach trees was reduced by 80 and by 70% when their daily water replacement was reduced to 1/8 and 1/4 respectively of the evaporation from a class A pan during the early stage of fruit growth (Chalmers *et al.*, 1984).

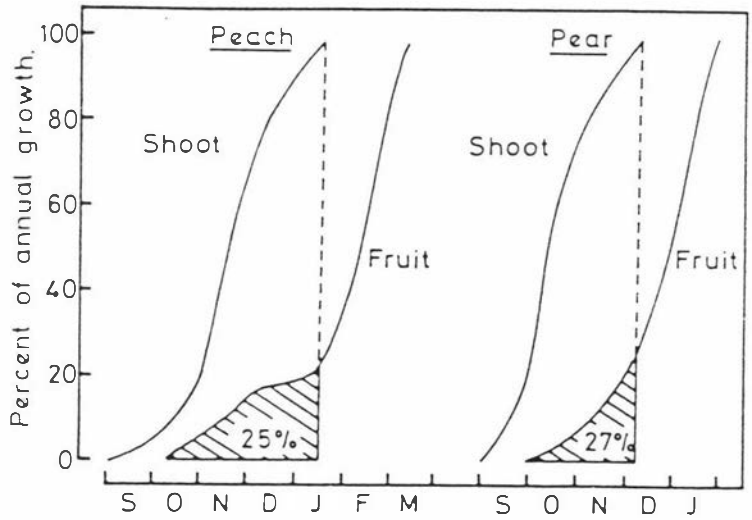


Figure 2.3 The period of accumulation of shoot and fruit growth of 'Golden Queen' peach and 'Bartlett' pear proposed by Chalmers *et al.*, (1984 and 1985) and Chalmers, (1989).

Mitchell and Chalmers (1982) irrigated at 100, 50, 25 and 12.5% replacement of E_{ps} (evaporation over the planting square) ultra-densely planted peach trees. Reduced irrigation during fruit's stage one, when vegetative growth was greatest, reduced vegetative growth, summer pruning and TCSA increase. They concluded that frame growth could be controlled without depressing yield by restricting irrigation in the first and second phase of fruit growth. The optimum level of E_{ps} replacement varied and depended on soil type, tree age and vigour, and environmental conditions.

Mitchell and Chalmers (1982) reported that replacement of 12.5% E_{ps} to mid January (southern hemisphere) followed by 100% replacement to harvest would replace 6 megaliters of water per hectare, compared with 9 megaliters for 100% replacement throughout the season. They concluded that at the lower levels of water replacement a large saving in irrigation water was obtained.

2.2.3.3.2 The effect of water stress on fruit quality and yield

Using water stress commercially to control vigour requires an understanding of changes in fruit growth, maturity and quality. In addition, successful fruit growing requires good management for maintaining sufficient vegetative and root growth to support maximum carbon fixation and assimilate partitioning for good fruit size, yield and quality (Proebsting *et al.* 1989).

Ebel *et al.* (1993) found RDI of apple caused a reduction in fruit size and titratable acidity while it increased soluble solids concentration. Fruit colour and firmness were not affected by the water deficit. Irving and Drost (1987) showed an increase of 0.8% in soluble solids and 37% reduction in shoot growth when water stress was applied to the first phase of 'Cox's Orange Pippin' apple. However it had no effect on fruit size. Water deficit during phase III increased peach fruits soluble solids (Li *et al.*, 1989). Chalmers *et al.* (1985)

demonstrated that peaches grown at lower leaf water potential had higher soluble solids, skin colour and firmness (after cool storage) and lower water content.

Mitchell *et al.* (1984) reported that final fruit size and yield were not reduced by RDI treatment on pear. Li *et al.* (1989) showed it was possible to reduce vigour of peach trees without affecting fruit size and quality if water stress was imposed at a particular stage of fruit development, normally stage I and II of fruit growth. Lötter *et al.* (1985) suggested that a decrease in total available soil water during the main phase of fruit development had a negative effect on fruit size. Li *et al.* (1989) found fruit size increased following imposed water deficit during the first phase of fruit growth of peach trees, while those trees subjected to water stress during either phase three or throughout the fruit growth period, produced smaller fruit. However, when trees were subjected to water stress at pit hardening (phase II) or during the first two phases there was no effect on fruit size. They also found that such water stress reduced pre-mature fruit drop.

CHAPTER THREE

ORCHARD EXPERIMENTS:

GENERAL MATERIALS AND METHODS

3.1 INTRODUCTION

The positive effects of controlling vegetative growth of fruit trees by root-pruning, PBZ and mild water stress have been reviewed in chapter 2. The former two treatments may be readily applied in most situations. However, the humid climate of Palmerston North demanded a special management technique to allow investigation of the use of water deficit to control tree vigour.

The method chosen was to isolate the root zone of the experimental trees from natural rainfall. However, three plots of the water stress treatment in the close planted experiment were flooded after heavy rain in late October 1990 in the first season. Subsequently, the required level of water stress did not develop in these plots, and they were eliminated from the analysis.

Silver leaf disease (*Chondrostereum purpureum*) is another limiting factor in apricot growing in this particular climate. In the 1991-1992 season some trees in both the close and wide spaced blocks showed moderate to severe symptoms and were eliminated from the analysis. In the close planted experiment 4 trees (from root-pruned and RDI) were affected by silver leaf, but infection in the wide planted experiment was so widespread that it was limited to a few measurements and observations on apparently healthy trees.

3.2 PLANT MATERIAL, CULTURAL AND TRAINING SYSTEMS

The material selected for this study was apricot (*Prunus armeniaca* L. cv.

'Sundrop') on 'Golden Queen' peach seedling rootstock. The two-years-old budded trees had been obtained from a commercial nursery (Pattullo's Nursery, Hastings) and planted in winter 1987 in North-South rows at spacings of 5 m × 1 m (close planted, 2000 tree ha^{-1}) and 5 m × 2 m (wide planted, 1000 tree ha^{-1}), with a 2.5 m wide herbicide strip and 2.5 m sod alleyways (clover and ryegrass).

The trees were growing with natural rainfall and trickle irrigation and normal orchard practices, and containment pruning was practised to maintain tree height and spread on the Tatura trellis training system (Plate 3.1). In making measurements the tree canopy was divided into three sections (bottom, middle and top positions), in which the middle position was at 120-255 cm canopy height. This technique gave a more homogeneous canopy micro climate within each section for sampling and assessment through the experiment.

3.3 EXPERIMENTAL SITE

The experiments were carried out in the Fruit Crops Unit orchard, Massey University, Palmerston North (altitude of 30 metres, longitude of 175° 37'E and latitude of 40° 23'S), in the North Island of New Zealand.

3.4 ENVIRONMENT

3.4.1 Climate

The climate of the area is typically humid temperate with no distinct summer dry season. Figure 3.1 shows the climatic data of the area of this study, based on monthly means of 30 years from 1964-1994 at the nearest weather station, AgResearch Grasslands, Crown Research Institute (CRI), Palmerston North (≈1 km away from experimental site).

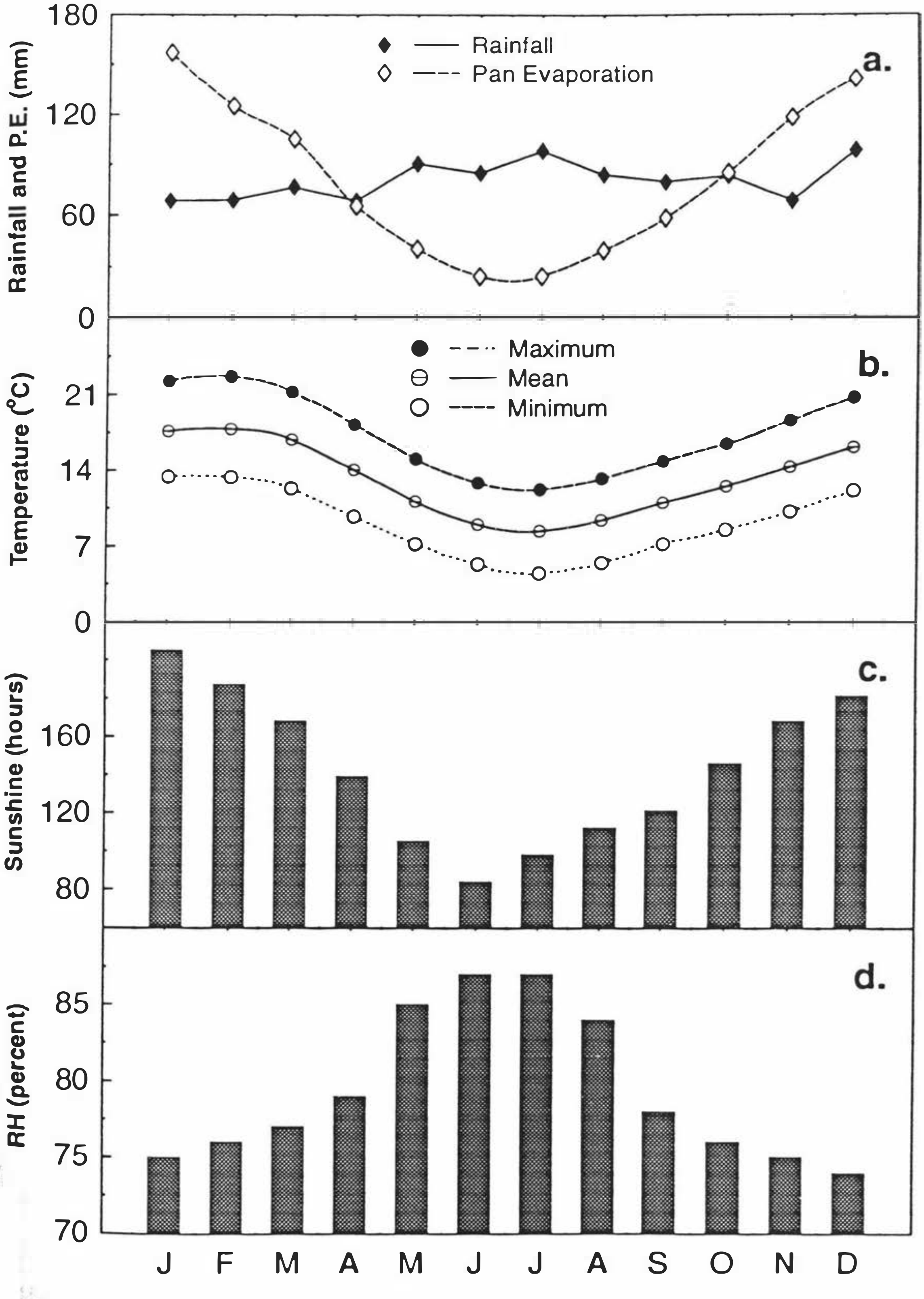


Figure 3.1 The means of 30 years (1964-1994) monthly climatic data at Palmerston North, New Zealand. a). Rainfall and pan evaporation (P.E.); b). Temperature; c.). Sunshine hours; d). Relative humidity.

3.4.1.1 Rainfall and Pan Evaporation

Figure 3.1 a. shows the typical rainfall and evaporation for the area. Rainfall (total of 966 mm) occurs on average on 166 days during the year, ie 45 percent of total year days. The highest (98 mm) and lowest (68 mm) rainfall were recorded in December and April respectively. Annual pan evaporation was 995 mm, the highest occurring in January with 157 mm.

3.4.1.2 Temperature

Monthly minimum temperature 4.5°C and maximum 22.6°C were registered in July and February respectively. The lowest monthly mean temperature 8.4°C occurred in July (Fig. 3.1 b.).

3.4.1.3 Sunshine

The total annual sunshine is 1715 hours. The highest monthly sunshine hours of 205 hours and lowest of 84 hours occurred in January and June respectively (Fig. 3.1 c.).

3.4.1.4 Relative humidity

Relative humidity during the year ranged from 74 to 87 percent (Fig. 3.1 d.).

3.4.2 Soil

The soil type of the experimental plots was a Manawatu fine sandy loam. The soil profile is subdivided into three layers, 0-25 cm fine sandy to silt loam, 25-150 cm fine sand with gravelly coarse sand below 150 cm (Cowie, 1978), but horizon soil depths in the area are markedly variable.

3.5 Duration and layout of the experiments

Two experiments were established, one on close planted trees at 1 m spacing (Plate 3.2 a.) and another on wider spaced trees at 2 m centres (Plate 3.2 b.). The experiments were initiated in July 1990 and conducted for two complete seasons on the close planted trees, but largely discontinued on the wider spaced trees in the second season.

Blocks were arranged along the rows (to allow for possible soil variation) with treatment trees selected for uniformity in trunk girth, selected trees in each block were allocated randomly to treatments.

3.5.1 Close spaced experiment

3.5.1.1 Experimental layout

Treatment blocks were situated in two adjacent rows using 40 trees with 8 blocks. Treatments were arranged in a randomized complete block design with each block containing one three-tree plot of each treatment. Only the centre tree of each plot was used for measurement, the outer trees being guards. In each block the five treatments were:

I. Root-pruning
II. Low rate PBZ (L-PBZ)
III. High rate PBZ (H-PBZ)
IV. Water stress (RDI)
V. Untreated control

3.5.1.2 Root-pruning treatment

Root-pruning was by a chain trench digger machine (Hire Master, Palmerston

North)(Plate 3.3 a.). A trench was dug a distance of 3 m along two sides of each plot 55 cm from the row centre, to 60 cm depth. Trees were root-pruned whilst soil was relatively dry at the beginning of flowering in August 1990 (Plate 3.1 a.) and also 1991.

3.5.1.3 Paclobutrazol treatments

The growth retardant used was PBZ, formulated as 'Cultar' a 250 g/l suspension concentrate (ICI, New Zealand Limited). The chemical and physical properties of PBZ are shown in Appendix 3.1.

3.5.1.3.1 Type of application

PBZ was applied to the soil as a trunk base drench at either of two rates of 0.5 g tree^{-1} and 1.5 g tree^{-1} active ingredient. Both rates were applied in 1 litre of water (Plate 3.2 b.) and poured into a 5 cm depth soil furrow within 25 cm around the trunk.

3.5.1.3.2 Dose and time of application

PBZ was applied at the beginning of flowering.

a). 0.5 g tree^{-1} treatment applied to experimental and guard trees in both 1990 and 1991 seasons (Plate 3.2 b.).

b). 1.5 g tree^{-1} treatment applied to experimental and guard trees in 1990 only.

3.5.1.4 Water stress treatment

This treatment was initiated at the beginning of flowering in August 1990 using wooden sloping frames covered with water proof polyweave plastic to protect the rooting zone of experimental and guard trees from natural rainfall (Plate 3.2 a.). Frames were built with 50 mm × 50 mm tanalised timber to cover an area 3 m × 2.6 m, isolating a 7.8 m^2 area covering both sides of each three tree plot (Plate 3.1 b.). The irrigation supply to these plots was stopped during the period of water stress treatment. On the outer, lower side walls polythene covers were inserted 50 cm into a trench dug into the soil 1.3 metres from the tree to prevent run-off soaking back under the covers (Plate 3.1 a. right). To prevent water ingress around the tree trunk the area was sealed with adhesive tape and silicone sealant.

However, in spite of these precaution's water stress treatments in three blocks were subjected to flooding in the first season from torrential rain and eliminated from the experiment.

Roll up doors in the top of each cover gave access for control of irrigation supply and recording soil moisture (Plate 3.2 a.). In both seasons, trees did not receive any water during the first two growth stages of fruit, but in the third stage of rapid fruit growth, irrigation was supplied as for the untreated control. In the second season water stress trees received between 150-300 litres supplemental water per tree on the 12th., and 17th. of December 1991 to raise soil moisture close to field capacity (FC) in the top 60 cm layer of soil.

Water stress treatment was essentially RDI except as described above, and was so termed. Irrigation was applied in the third phase of fruit growth as to the untreated control in both seasons. However, in the second season water stress trees received some supplemental water in addition to the normal irrigation.

3.5.2 Wide spaced experiment

3.5.2.1 Experimental layout

Only a single row of 2m spaced trees were available for use, and 18 trees

Plate 3.1 'Sundrop' apricot trees in the close planted experiment trained to a Tatura trellis system **a)**. Polyethylene plastic film inserted into a 60 cm depth trench to prevent water run-off access to the water stress treatment; **b)**. Wooden sloping frame before covering with plastic sheet.

Plate 3.2 Application of treatments. **a).** Water stress trees under plastic soil covers, in the close planted experiment; **b).** PBZ, root-pruning and water stress treatments in the wide planted experiment.

Plate 3.3 Root-pruning. **a).** Technician, Giles Russell operating the Trench Digger Machine in the close planted experiment; **b).** Root-pruned trees in the wide planted experiment.

were used. Treatments were arranged in a randomized complete block design with 2 blocks, using four adjacent trees per plot for root-pruning and PBZ treatments and two adjacent trees per plot for water stress and control treatments. The two centre trees only of each 4-tree plot were used for measurement, but in 2-tree plots both were measured.

3.5.2.2 Root-pruning treatment

Trees were root-pruned similarly to the close planted experiment, with the trenches extending a total of 7 m on both sides of trees, to include two experimental and two guard trees. Trees were root-pruned whilst soil was relatively dry at the beginning of flowering in August 1990 and 1991 (Plate 3.3 b.).

3.5.2.3 Paclobutrazol treatments

PBZ was applied at either of two rates (0.5 g $tree^{-1}$ and 1.5 g $tree^{-1}$ a.i.) as described for the close planted experiment (Plate 3.2 b.).

3.5.2.4 Water stress treatment

Treatment was initiated at the beginning of flowering as described in the close planted experiment (see 3.5.1.4). In this experiment the wooden frames were designed to cover an area of 18 m^2 on both sides of two adjacent experimental trees. Treatment was carried out only during the 1990-1991 season, and called RDI as described in the close planted experiment (Plate 3.2 b.).

CHAPTER FOUR

EFFECTS OF TREATMENTS ON VEGETATIVE GROWTH OF 'SUNDROP' APRICOT

4.1 INTRODUCTION

There are differences in size among trees of apricot species and cultivars (Meblenbacher *et al.*, 1991), and 'Sundrop' apricot grows tall, upright and vigorously (Glucina *et al.*, 1990). It is desirable for growers to be able to control vegetative growth and in the absence of a dwarfing rootstock for apricot this may be possible using different vigour control techniques. This experiment was conducted to examine the effects of some of these techniques, namely root-pruning, PBZ application and RDI on close and wide planted 'Sundrop' apricot trees in the humid climate of Palmerston North.

Treatment effects on shoot growth and tree pruning requirements were evaluated. Samples of new seasons shoots and fruit were destructively harvested throughout the season from some close planted non-experimental trees to monitor the pattern of shoot and fruit growth during the season. In the second season, the percentages of available light in the different height regions of the canopy were recorded in the close planted experiment to assess the indirect effect of treatments in the improvement of light penetration within the canopy.

A study of the root system was conducted at the end of 2 year's treatment of the close planted trees to monitor the development of the root system of the experimental trees with respect to position in the soil profile and distance from the tree trunk. This examined possible short term effects of treatments on the rooting density. Richards (1983) suggested there is close coordination between the root and shoot systems in which the root is entirely dependent on the shoot for its carbohydrate supply and the shoot entirely dependent on the root for its supply of mineral nutrients and water. Some difficulties faced in the study of fruit tree root systems have been mentioned by Atkinson (1980). For example, he reported high variability in results obtained by the core sampling method, and that non-normal distribution can complicate analysis and interpretation of data. Rogers and Vyvyan (1934) as cited by Atkinson (1980) suggested that the major roots of some fruit trees are unevenly distributed, so the excavation of only one segment can give misleading results unless replication is adequate. However, recent studies have shown that in older orchards the root-systems can be treated as a layered distribution of root length density (RLD) values rather than as bowl- or ellipsoidal-shapes (Hughes pers. comm., 1994), as shown for kiwifruit by Gandar and Hughes (1988) and for apples by Hughes and Gandar (1993). In young trees root distribution varied with both radial distance from tree trunk and soil depth, but in mature trees the root systems were layered and RLD changed with soil depth.

The emphasis in this orchard study has been on the response of close planted trees. Tree numbers were very low at the wide spacing, and in the second season the number of healthy trees was significantly reduced. The experiment at wide spacing was largely abandoned after the first season (1990-1991).

4.2 MATERIALS AND METHODS

The trees and treatments used have been described in chapter 3.

4.2.1 Measurements

4.2.1.1 Summer pruning

In each of the two seasons of experiment summer pruning was performed to remove excessive vegetative growth after allowing for renewal of fruiting sites. All experimental trees were pruned twice (before and after fruit harvest) as part of normal orchard pruning practice in both seasons. All the new season prunings were collected separately from 3 height zones (section 3.2) in the canopy, cut into short lengths and placed in labelled paper bags. They were oven dried at 89°C for two weeks, and weighed. During oven drying the bags and their contents were turned over twice to ensure that all parts within the paper bags dried completely.

4.2.1.2 Seasonal growth pattern

4.2.1.2.1 Shoot growth

The length of five tagged shoots in the middle region of the canopy on each experimental tree was recorded at intervals with metric tape from 49 days after full bloom (dafb) in the first season in both close and wide spaced trees. Similar growth measurements were made from 33 dafb in the second season on the close planted trees. In addition, at the time of postharvest pruning (March-April) in each season, the lengths of 10 randomly selected new season shoots from the top canopy region of each tree were recorded.

4.2.1.2.2 Destructive shoot and fruit harvest

Eight untreated trees, of similar size and performance to the experimental trees were selected in the close planted rows in the second season. Trees were randomly allocated to 4 groups (two trees per group), and one group was denoted for each time of harvest throughout the season.

At each of 4 harvests (59, 97, 131 and 225 dafb) all the new season shoots and fruits were harvested destructively (Plate 4.1) and kept in plastic bags. Shoots were divided into leaves and stem, and leaf area was measured using a Licor Area Meter, model Li-3100 (Lambda Instruments Corp, USA). Leaf area was determined on six 150 g. random samples from the well mixed total leaf of each harvested tree and total leaf area calculated based on total leaf

Plate 4.1 A close planted 'Sundrop' apricot tree after destructive shoot and fruit harvest, 1991-1992 season.

fresh weight and the average of these random samples. The fresh weight of each plant part (leaves, stem and fruits) was recorded immediately after harvest, and oven dried at 89°C for two weeks to constant weight, to determine dry weight. Mean fruit size was determined by dividing the total fruit fresh weight by fruit number at each harvest. As commercial harvest was carried out at 131 dafb, no fruit sample was available at 225 dafb.

4.2.1.3 Trunk cross sectional area (TCSA)

Five weeks before applying the treatments in 1990, the trunk of each experimental tree was marked with white paint 10 cm above the graft union. The trunk circumference of each tree was measured at this position at intervals throughout both seasons. Data were used to calculate TCSA.

4.2.1.4 Light within the canopy

The light penetration through the canopy was recorded three times (at 54, 95 and 153 dafb) in all experimental trees of the close planted experiment in the 1992 season. A one metre solarimeter tube with sensor was used to measure total incident light, recorded by a Microvolt Integrator, Type Mv2 (Delta-T Devices Ltd, Burwell, Cambridge CB50ES, England), and the data readings expressed as a percentage of open sky light. Two solarimeter tubes were employed to give a simultaneous record of light intensity from the reference point (open sky 3 metres above the ground) and that recorded within the canopy. The solarimeters were kept horizontal, either with a free standing scaffolding frame (open sky) or an adjustable supporting frame attached to the Tatura trellis support system immediately outside the V frame. Incoming radiation was recorded for a 2 min period at each canopy region within 1 hour of solar noon on days with a clear sky. Light was recorded in each of the lower, middle and top regions of the canopy.

4.2.1.5 Root core sampling

This study was carried out on 12 experimental trees, 3 per treatment in 3 adjacent blocks in the close planted experiment in October 1992 after two complete seasons of the experiment. All treatments except L-PBZ were included in the root core sampling.

The method employed for obtaining soil core samples was described by Welbank and Williams (1968) and used by Gandar and Hughes (1988) and Hughes and Gandar (1993). Stainless steel coring tubes were driven into the soil using a modified engine-powered concrete breaker (Plate 4.2 b.) and extracted using a tripod and winch (Plate 4.3 a.).

Eight core samples were collected from around each tree. A coring tube of 46 mm internal diameter was inserted into the soil to sample the top one metre for all of the samples. At the 0.2 and 1.0 m distance from each tree a 2-m long tube of 34 mm internal diameter was driven down the larger diameter hole left by the first tube to collect a further sample from 1.0-1.6 m depth.

Soil core sampling was arranged to cover the interrow region to the midpoint of the alley at one side of the tree trunks at distances of 0.2, 0.4, 0.6, 1.0, 1.4, 1.9, 2.3, and 2.5 m to depths of 1.6, 1, 1, 1.6, 1, 1, 1, and 1 metre respectively. Distances were measured at 90° angle to the tree row to minimize the risk of sampling from guard trees, since trees were spaced only 1 m apart (see 3.2). However, at the 0.2 m distance soil core sampling was influenced by the position of scaffold branches, and the best position for installing the tubes was selected to avoid their damage by equipment (Plate 4.2 a.).

The soil cores were placed horizontally on a bench and divided into 200 mm sections for the top 1 m layer and into 150 mm sections for the 1-1.6 m layer

(Plate 4.3 b.). From each of these sections one 100 mm long sub-sample was taken and the mid-point of this sub-sample defined as the sample depth (5 and 4 sub-samples from 1 m and 1.6 m cores respectively). These sub-samples were collected randomly within the 200 or 150 mm sections. This method ensured all depths were representatively sampled, and a total of 48 sub-samples were collected from each experimental tree.

Roots were washed out of the samples using a commercial version (Gillison's Variety Fabrication, Benzie, Michigan, USA) of the semi-automated root washing machine (Plate 4.4) described by Smucker *et al.* (1982) and used by Hughes and Gandar (1993). An attempt was made to visually distinguish dead from live roots, and discard the former along with roots of grasses and other organic debris.

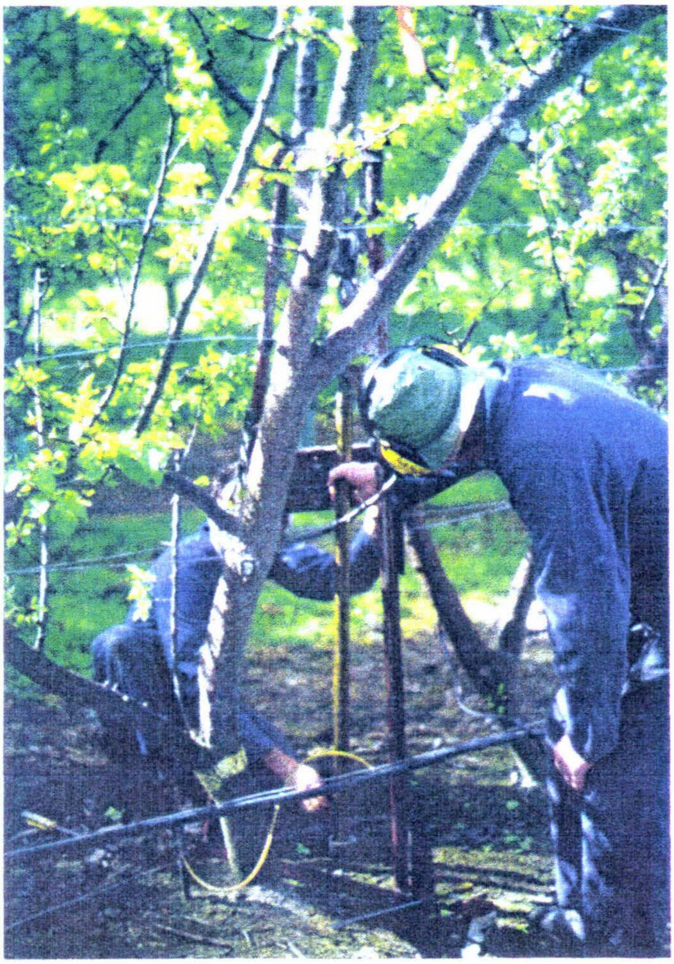
Total root length in each sample was measured using an automatic Comair root-length scanner (Commonwealth Aircraft Corporation Ltd., Melbourne, Australia). Root length density (RLD) for each sample was calculated using the formula:

$$RLD = \frac{\ell}{V}$$

> where: RLD is root-length density measured in cm of roots cm^{-3} of soil; ℓ total root length (cm) and V soil volume (cm^3) in each sample, 166.2 and 90.8 cm^3 for samples within 1 and 1.6 metre cores respectively.

Roots from each sample were then divided into two categories: a). fine roots <1.5 mm diameter and b). roots >1.5 mm diameter. All roots >1.5 mm diameter were woody. Root weight density (RWD) for fine and woody roots was calculated after oven drying samples at 70°C for 2-3 days, to constant weight. The following formula was used for this calculation:

Plate 4.2 Root core sampling from close planted trees in early spring of 1992-1993 season. a). Selecting the best position for installing coring tube and equipment for sample 0.2 m from tree. b). Driving core tube into the soil using a modified engine-powered concrete breaker.



a.

b.

Plate 4.3 Root core sampling from close planted 'Sundrop' apricot. a). Root core extraction using tripod and winch; b). Sectioning the soil column obtained by the coring tube into 10 cm long sub-samples with a knife.

Plate 4.4 Washing out root samples extracted from close planted 'Sundrop' apricot using a semi-automated root washing machine (October 1992).

$$RWD = \frac{Rdwt}{V}$$

where: RWD is root-weight density expressed as g of roots cm^{-3} of soil; Rdwt is dry weight of fine or woody roots (g); and V soil sample volume. The RWD of fine roots were ignored because they make up an insignificant amount of root weight (Hughes pers comm., 1994).

The sample volumes were sufficiently small relative to the rooting volume to treat them as point samples. Therefore each root density was assigned coordinates (r, θ, z) to indicate distance from tree (r), angle relative to row direction (θ) which was usually 90°, and depth to midpoint of sample (z) (Hughes *et al.*, 1986). It was assumed that the root distribution in the resultant 2-dimensional plane at right angles to each tree represented the characteristics of the whole soil volume in each plot.

4.2.2 STATISTICAL ANALYSIS

The data were examined by analysis of variance using the General Linear Model (GLM) procedure (SAS Institute Inc. 1991). The normality of data was confirmed using Proc Univariate (Fernandez, 1992 and Gomez and Gomez, 1984). All data were initially analysed in accordance with the randomized complete block design (RCBD)(Gomez and Gomez, 1984; Steel and Torrie, 1981). For TCSA and shoot growth data (from the middle region of canopy) time was considered as a factor by analysing data using repeated measurements analysis (Fernandez, 1991; Gomez and Gomez, 1984; Mead 1988; Rowell and Walters, 1976).

The RLD data were log transformed to meet the requirements of analysis of variance (Fernandez, 1992; Gandar and Hughes 1993; Hughes *et al.*, 1988; Maindonald, 1992). RWD data were multiplied by the constant 1000 followed by logarithmic transformation. Treatment effects on RLD and RWD values were determined by analysis of variance on the root data obtained from each explored soil sub-volume, as shown in Table 4.1.

Table 4.1. The arrangement for dividing explored soil volumes into sub-volumes for monitoring RLD and RWD of close planted 'Sundrop' apricot trees in October, 1992.

Explored soil region	Depth (m)	Distance from tree trunk (m)
a.	0 - 0.6	0 - 0.55
b.	0 - 0.6	0.55 - 2.5
c.	0.6 - 1	0 - 0.55
d.	0.6 - 1	0.55 - 2.5
e.	1 - 1.6	0 - 1
f.	0 - 1.6	0 - 2.5

4.3 RESULTS: CLOSE PLANTED TREES

4.3.1 Summer pruning

4.3.1.1 1990-1991 season

During the first season of the experiment (Figure 4.1 a.) at the preharvest pruning (89 dafb) the control treatment produced the most pruning weight. There was a trend for control prunings to be greater than L-PBZ, H-PBZ and root-pruning (P=0.06, 0.08 and 0.07, respectively), but the difference from RDI was not significant

These effects were maintained at the time of the postharvest pruning 138 days later (Figure 4.1 a.) when all treatments reduced the quantity of pruned material relative to the control treatment (P<0.001). There tended to be a greater reduction in summer regrowth by H-PBZ than by root-pruning and RDI (P=0.08 and 0.06 respectively).

When both prunings are totalled (Figure 4.1 a.) all treatments reduced vegetative growth (P<0.001) relative to control in terms of the weight of prunings. The analysis of postharvest pruning (Figure 4.2 a.) shows that most pruned material was removed from the top canopy region (52%) of the control treatment. There was more (P<0.05) pruned material in the top canopy region of control than of the other treatments (Figure 4.3 a.). Similarly, all treatments reduced prunings in the lower and middle canopy zones relative to control (Figure 4.3 a., P<0.001 for L-PBZ, H-PBZ and root-pruned and P<0.01 for RDI treatment).

4.3.1.2 1991-1992 season

At the preharvest pruning in December (101 dafb) the amount of material pruned from the control trees was greater than from L-PBZ, H-PBZ and root-pruning treatments (P<0.05). Differences between control and RDI were not significant (Figure 4.1 b.). Both PBZ treatments had more effect in reducing vegetative growth in terms of summer pruning (P<0.05) than the RDI treatment.

The period between the two prunings in this season was 112 days. At the postharvest pruning (213 dafb) shoot regrowth was not influenced by RDI, but both PBZ treatments reduced prunings relative to control (P<0.001 and 0.05 for L-PBZ and H-PBZ respectively). Root-pruning reduced the amount of summer pruning relative to control, although the difference was not significant (Figure 4.1 b.).

When data from both prunings were pooled and treated as total season pruning (Figure 4.1 b.), treatments showed similar statistical differences to the postharvest pruning (213 dafb) as described above. However, the root-pruned treatment produced less regrowth than RDI in this season ($P<0.05$).

Figure 4.3 c. shows that at the preharvest pruning (101 dafb) most growth was removed from the lower and middle regions of the canopy zone. The amount of pruning on control was greater than on both PBZ treatments ($P<0.001$ and 0.05 for L-PBZ and H-PBZ respectively). Differences between control, root-pruned and RDI were not significant in the lower and middle regions of the canopy. At this time (Figure 4.3 c.) the amount of growth in the top region of the canopy was greater on control compared with both PBZ and the root-pruned treatments ($P<0.05$), but control and RDI were not different.

During the time between pre- and postharvest pruning (Figure 4.3 d.) the amount of regrowth in the top zone of the canopy increased dramatically for all treatments. RDI produced more regrowth prunings than the other treatments ($P<0.01$ for both PBZ treatments and 0.05 for root-pruned), although RDI and control were not significantly different. The different in regrowth of control and of both PBZ treatments was not significant in the top zone. At the postharvest pruning (Figure 4.3 d.) the regrowth in the lower and middle zones of the canopy was greatest in the control treatment ($P<0.01$ for L-PBZ, H-PBZ and root-pruned and $P<0.05$ for RDI), with all these treatments producing statistically similar amounts of regrowth.

Figure 4.4 b. also shows that the proportion of preharvest prunings (101 dafb) from the lower and middle regions of the canopy was higher than from the top of the trees for all treatments. At the postharvest pruning (213 dafb) the greater proportion of the prunings came from the top of the canopy, significantly more from the RDI treatment than from control ($P<0.05$). In contrast, the amount of prunings in the lower and middle canopy zones was reduced ($P<0.05$) by RDI relative to control (Figure 4.4 c.).

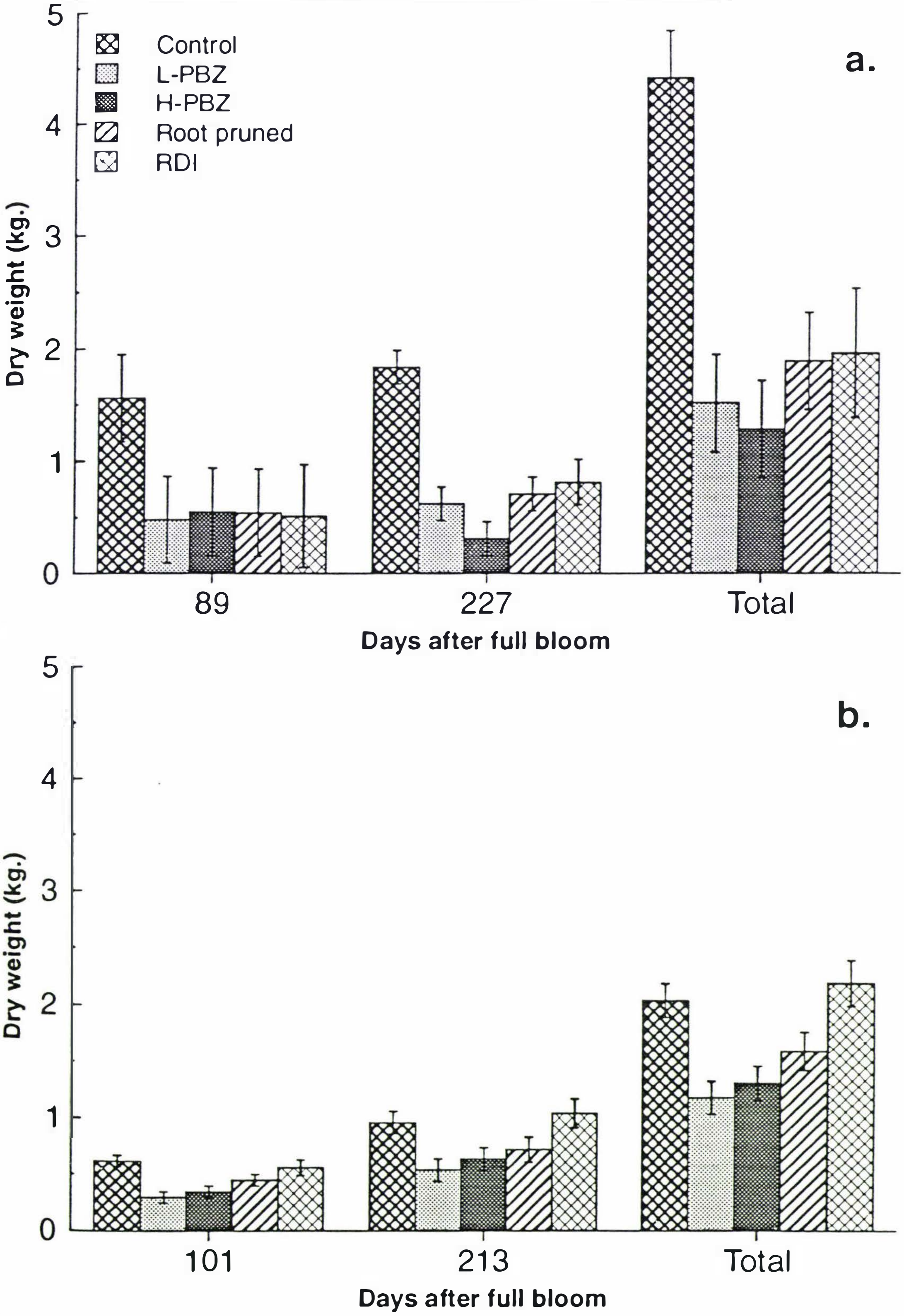


Figure 4.1 The dry weight of shoot regrowth removed in summer pruning by treatment, on close planted 'Sundrop' apricot. a). 1990-1991 season; b). 1991-1992 season. Each data point represents the mean of 8 replicates treatment^{-1} (n=5 for RDI). Tagged shoots removed at the time of fruit harvest added to the total pruning data. Vertical lines indicate the standard error of the means.

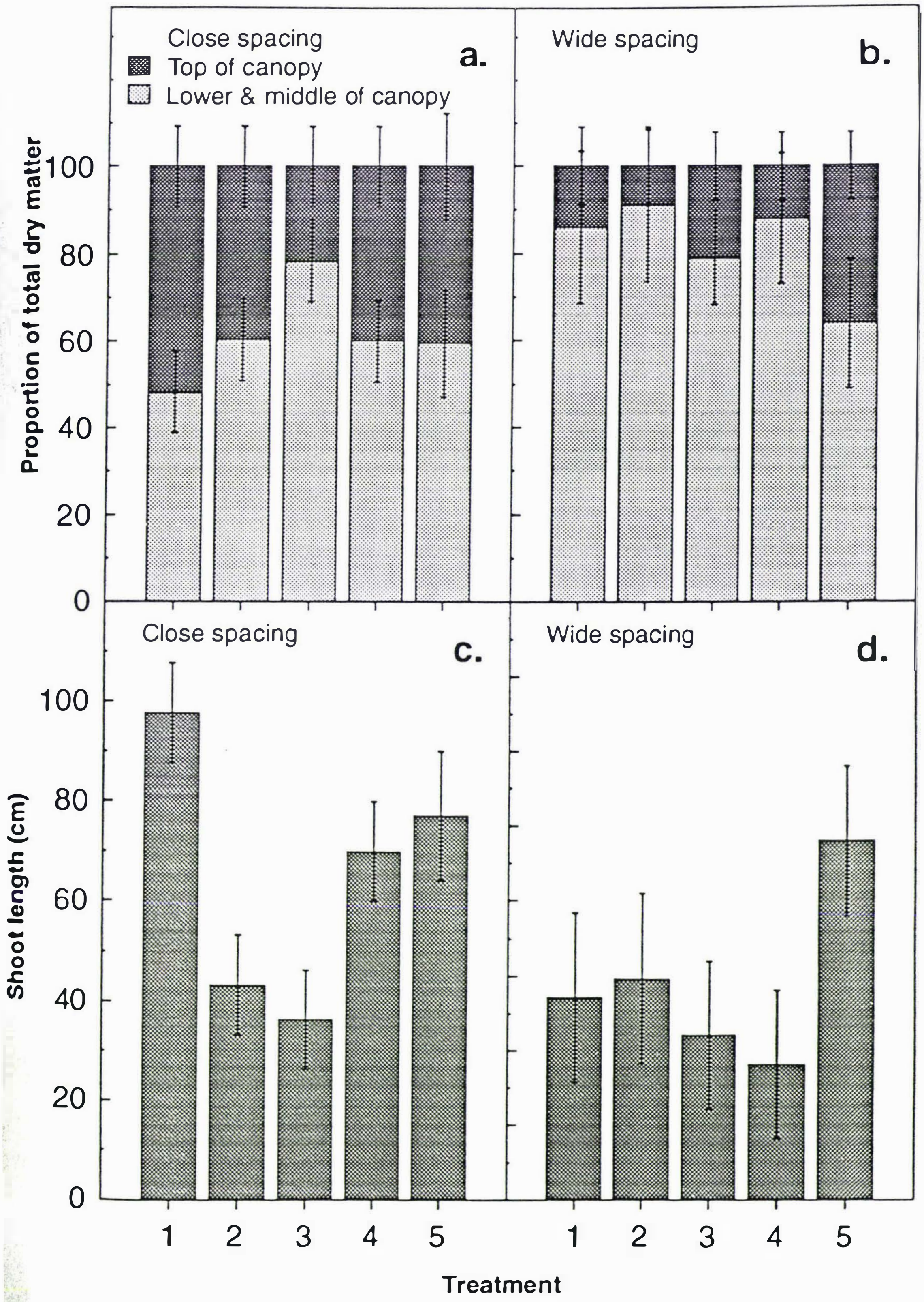


Figure 4.2 The effect of treatment on distribution of summer regrowth and mean shoot length (227 dafb) of 'Sundrop' apricot in 1990-1991 season. a., b). Prunings; c., d). Mean shoot length in top region of canopy. 1=Control; 2=L-PBZ; 3=H-PBZ; 4=Root pruned and 5=RDI. Vertical lines indicate the standard error of the mean.

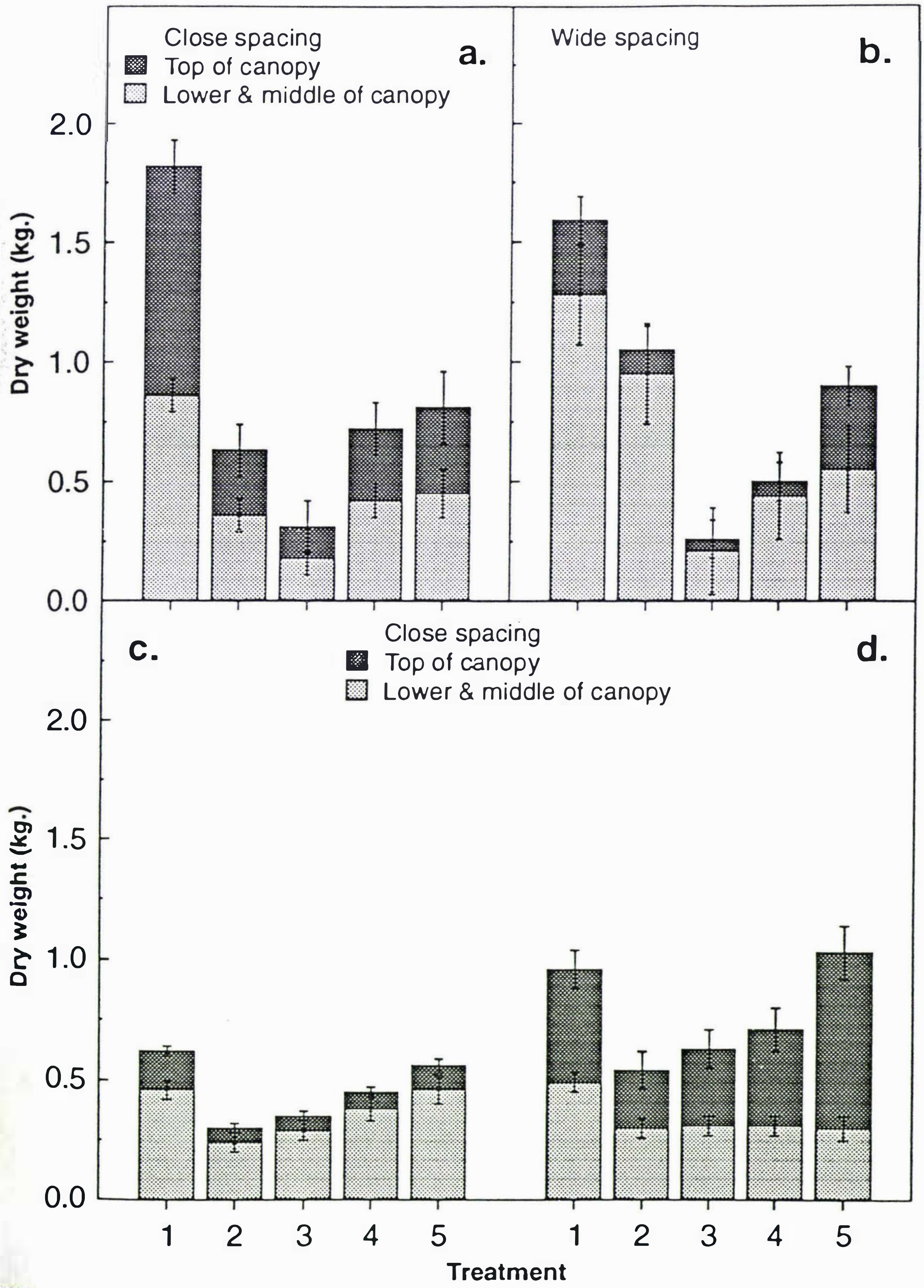


Figure 4.3 The dry weight of shoot regrowth removed in different canopy zones in summer pruning by treatment on 'Sundrop' apricot. a., b). Postharvest pruning (227 dafb) in 1990-1991 season; c., d). Prunings (101 and 213 dafb respectively) in 1991-1992 season. 1=Control; 2=L-PBZ; 3=H-PBZ; 4=Root pruned and 5=RDI. Vertical lines indicate standard error of the mean.

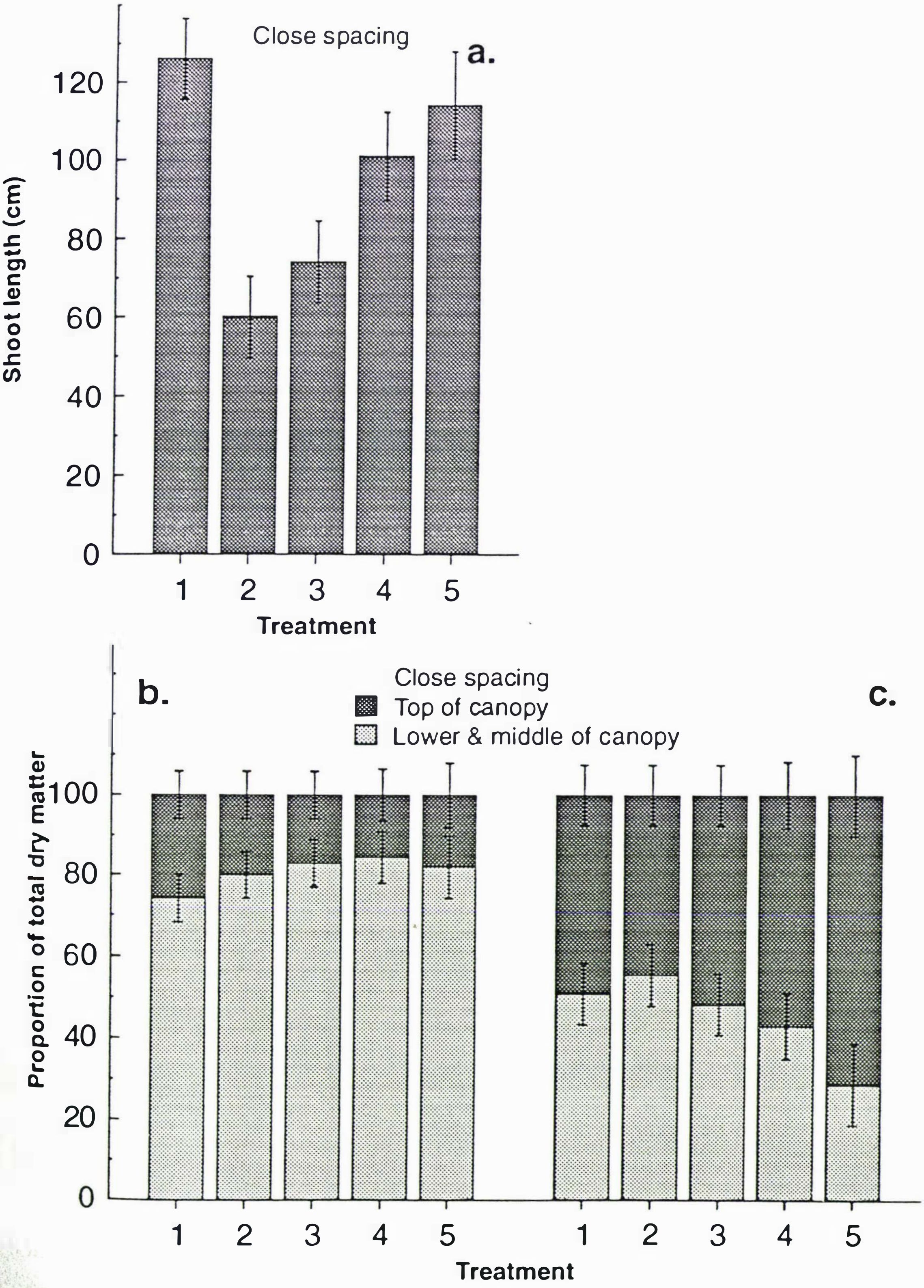


Figure 4.4 The effect of treatments on mean shoot length (213 dafb), and distribution of prunings preharvest (101 dafb) and postharvest (213 dafb) of 'Sundrop' apricot in 1991-1992 season. 1=Control; 2=L-PBZ; 3=H-PBZ; 4=Root pruned and 5=RDI. Vertical lines indicate the standard error of the mean. a). Mean shoot length in top region of canopy; b). Preharvest summer pruning; c). Postharvest summer pruning.

4.3.2 Seasonal growth pattern

4.3.2.1 Shoot growth

4.3.2.1.1 1990-1991 season

The results from randomly sampling and measuring 10 shoots from the top canopy region of each tree at the postharvest pruning are shown in Figure 4.2 c. Mean shoot length was greater on the control trees, while H-PBZ and L-PBZ ($P<0.001$) had the greatest effect in reducing shoot growth. H-PBZ resulted in shorter shoots than root-pruning and RDI ($P<0.05$), and L-PBZ was almost as effective ($P=0.07$ and 0.06 for root-pruned and RDI respectively).

The effect of treatments on the growth shown by 5 shoots per tree in the middle of the canopy measured at intervals in the first season is shown in Figure 4.5 a. The interaction of time × treatments was not significant. More effect was shown by H-PBZ and RDI, both of which reduced shoot elongation. H-PBZ reduced shoot growth throughout the season and differences relative to control were significant at all times of measurement ($P<0.05$ and 0.01 for "49, 56, 62, 98, 105" and "70, 76, 84, 91, 112, 123, 140" dafb respectively). Shoot length increased during the later stages of growth on the RDI treatment, so reductions relative to control were significant only early in the season (49 and 56 dafb with $P<0.01$ and 0.05 respectively). The shoot lengths of control, L-PBZ and root-pruned treatments were not significantly different throughout the season, although L-PBZ appeared to reduce late summer shoot extension.

4.3.2.1.2 1991-1992 season

In the second season on a similar set of mid-canopy shoots (Figure 4.5 b.) the time × treatment interaction was significant ($P<0.001$). In this season RDI did not reduce shoot growth significantly relative to control. However, RDI showed a reduction compared with L-PBZ, H-PBZ and root-pruned during the early season ($P<0.05$ & 0.001 for L-PBZ and H-PBZ respectively at 33 and 38 dafb and $P<0.001$ for root-pruned at 33, 38 & 48 dafb). RDI treatment was similar to both PBZ treatments on 48, 53 and 60 dafb, but later both PBZ treatments gave greater reduction of shoot growth relative to control, root-pruned and RDI ($P<0.001$ for control and root-pruned and $P<0.01$ for RDI after 67 dafb). From about 60 dafb both PBZ treatments strongly inhibited shoot growth ($P<0.01$), but RDI and root-pruning did not.

The randomly sampled, measured shoots in the top portion of the canopy at the postharvest pruning (213 dafb) are recorded in Figure 4.4 a. Shoot length was greatest in the control treatment. Compared with it PBZ treatments reduced growth ($P<0.001$ for L-PBZ and 0.01 for H-PBZ), but root-pruning and RDI treatments did not. L-PBZ showed reductions relative to root-pruned ($P<0.05$) and RDI ($P<0.01$), but was similar to H-PBZ.

4.3.2.2 Destructive shoot and fruit harvest

Figure 4.6 a. and b. show that as a proportion of total top growth, shoots increased significantly from 97 dafb, especially on a dry weight basis. While leaf growth was a major early contributor to new growth, it's relative contribution to total growth declined through the season to only 20% of dry weight at 225 dafb (early April). Figure 4.6 a. and b. also show that on both a fresh and dry weight basis shoots represented the lowest proportion of new growth until the time of fruit harvest (131 dafb). The proportion of shoots to leaves changed in favour of shoots at the last date.

The fruit as a proportion of new top growth increased on both a fresh and dry weight basis, until the time of fruit harvest (Figure 4.6 a. & b.), and fruits showed a sharp increase in size up to the time of harvest (Figure 4.6 d.), but this increase was influenced by fruit number. Leaf area increased markedly between 59 and 97 dafb (Figure 4.6 c.).

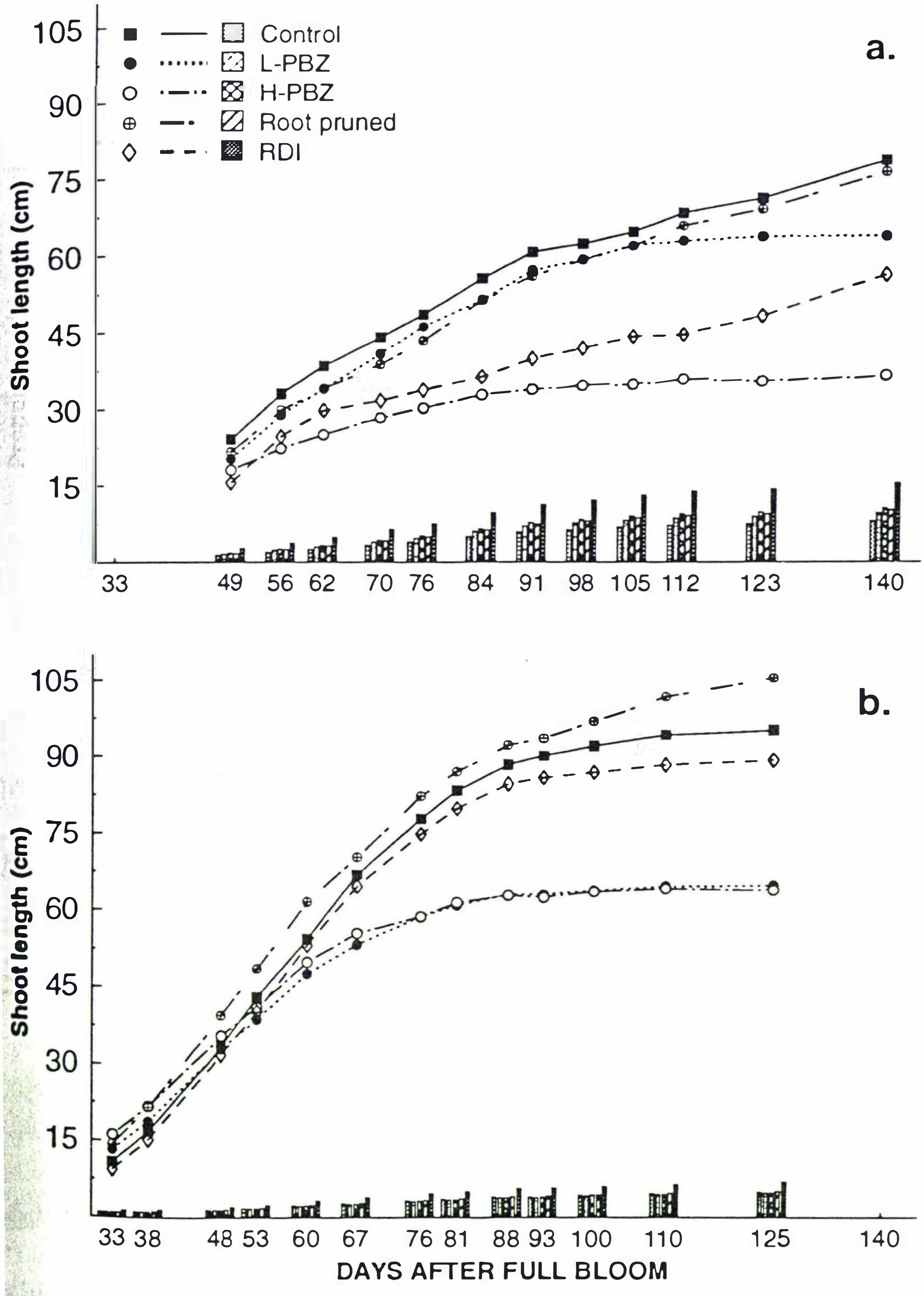


Figure 4.5 The effect of different vigour control techniques on cumulative shoot growth, on close planted 'Sundrop' apricot. a). 1990-1991 season; b). 1991-1992 season. Bars show the standard error of means.

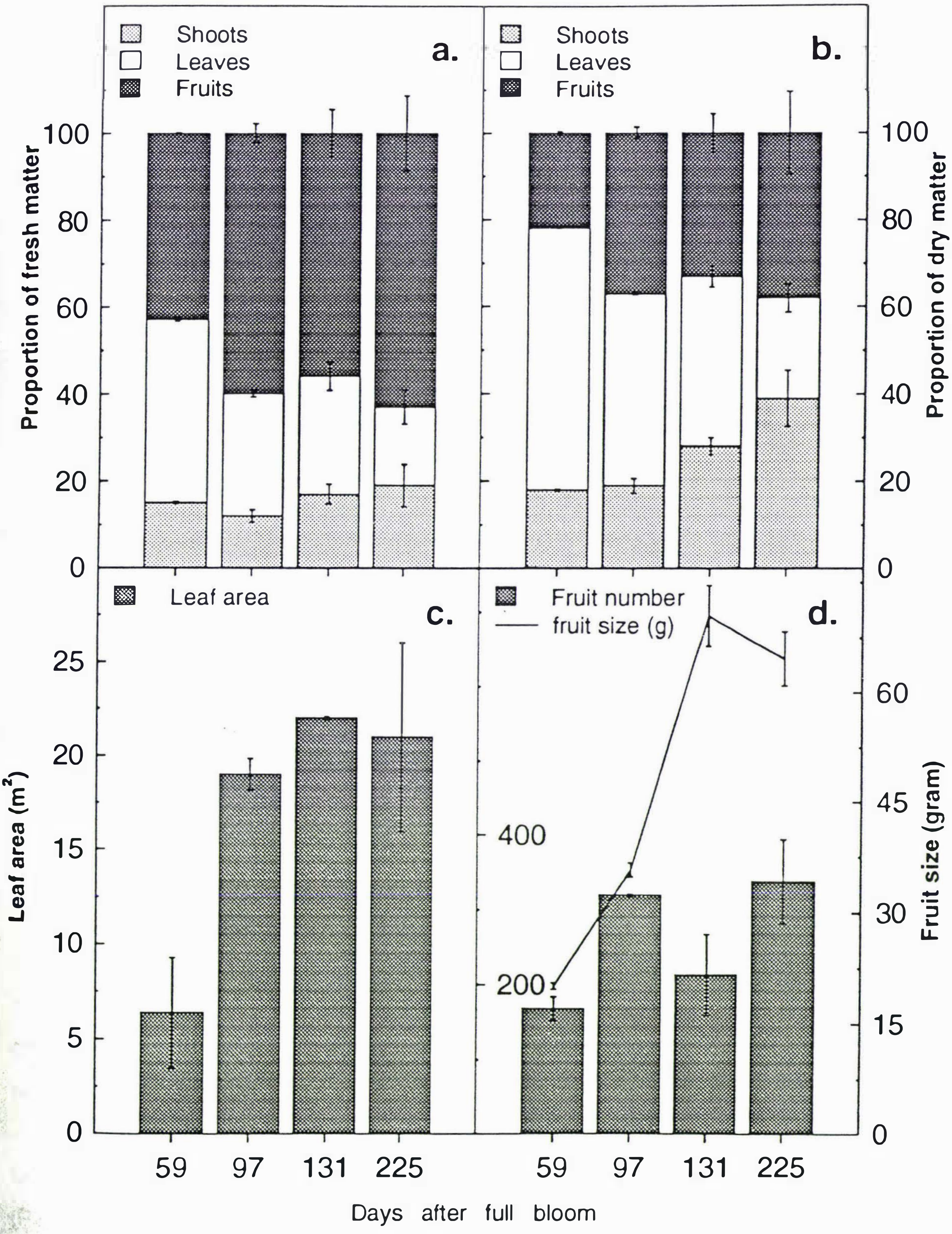


Figure 4.6 The pattern of new growth (shoots and fruits) throughout the 1991-1992 season on close planted "Control" 'Sundrop' apricot, determined by destructive harvests. Each data point denotes the average of two trees and vertical lines indicate the standard error of the mean. a). The distribution of fresh matter; b). The distribution of dry matter; c). The changes in leaf area and d). Fruit load at harvest.

4.3.3 Trunk cross sectional area (TCSA)

The influence of treatments on the annual increase of TCSA will be discussed in chapter 5 (see Figure 5.11). Changes in TCSA throughout both seasons for all treatments are shown in Table 4.2 a. The interaction of time × treatment was significant ($P<0.001$).

In the first season there was no treatment effect on TCSA. In the second season there was a trend for L-PBZ to reduce growth relative to control shortly after applying the treatments ($P=0.06$), and trunk size increase was smaller than on control later in the season ($P<0.05$ at January and $P<0.01$ at April, 1992). The trunk size was greater in RDI than L-PBZ at the end of this season ($P<0.05$ April). There was also a trend for H-PBZ to reduce TCSA increase ($P=0.06$) relative to that of control at the end of the second season.

4.3.4 Light within the canopy

In general light intensity within the tree framework decreased with increasing depth from the top to the lower region of the canopy at the first two measurement dates (54 and 95 dafb) and the interaction of canopy depth × treatments was significant for the first two light measurements. However, there was no interaction between canopy depth and treatment for the third measurement date (153 dafb) and the amount of incoming light for all treatments at all canopy levels was similar (Figure 4.7 a., b. and c.). The differences between treatments on incoming light at the lowest canopy level was not significant throughout the season.

L-PBZ when compared with control treatment showed greater light level ($P<0.05$) at the first two measurements (54 and 95 dafb) in the top canopy region. At the second time of measurement (95 dafb) incoming light was improved in the middle canopy zone by L-PBZ and root-pruning treatments as

Table 4.2. *The effect of treatments on trunk Cross Sectional Area (TCSA) of 'Sundrop' apricot during 1990-1991 and 1991-1992 seasons.* **a).** close planted trees, and **b).** wide planted trees.

Date of record	**a).** TCSA (cm²) for close-planted trees					Significance
	Control	L-PBZ	H-PBZ	Root pruned	RDI	of F values
15/07/90	61.6 ± 4.72[z]	59.4 ± 4.72	65.3 ± 4.72	63.2 ± 5.15	63.8 ± 6.27	ns
23/01/91	76.1 ± 5.47	67.7 ± 5.47	76.7 ± 5.47	77.3 ± 5.97	77.9 ± 7.26	ns
03/05/91	82.8 ± 5.90	70.9 ± 5.90	80.4 ± 5.90	82.0 ± 6.44	86.3 ± 7.83	ns
10/09/91	87.9 ± 5.72	72.0 ± 5.72	80.4 ± 5.72	82.9 ± 6.24	86.4 ± 7.59	ns
03/01/92	96.7 ± 5.65	76.4 ± 5.65	85.0 ± 5.65	89.7 ± 6.17	93.9 ± 7.50	*
13/04/92	105.5 ± 6.28	78.9 ± 6.28	88.3 ± 6.28	95.9 ± 6.85	106 ± 8.34	**
	b). TCSA (cm²) for wide-planted trees					
15/07/90	72.3 ± 7.36	84.0 ± 7.36	71.6 ± 6.32	73.3 ± 6.32	76.8 ± 6.32	ns
23/01/91	91.1 ± 9.36	104 ± 9.36	90.7 ± 8.03	89.5 ± 8.03	101 ± 8.03	ns
03/05/91	100 ± 10.9	112 ± 10.9	94.2 ± 9.36	92.9 ± 9.36	109 ± 9.36	ns
10/09/91	101 ± 10.9	113 ± 10.9	94.2 ± 9.37	93.6 ± 9.37	109 ± 9.37	ns
03/01/92	112 ± 12.1	122 ± 12.1	101 ± 10.4	103 ± 10.4	120 ± 10.4	ns
13/04/92	121 ± 12.7	126 ± 12.7	104 ± 10.9	111 ± 10.9	132 ± 10.9	ns

[z] ± Standard error of the mean.

ns,*,**,*** Nonsignificant or significant at $P < 0.05$, 0.01 or 0.001 respectively.

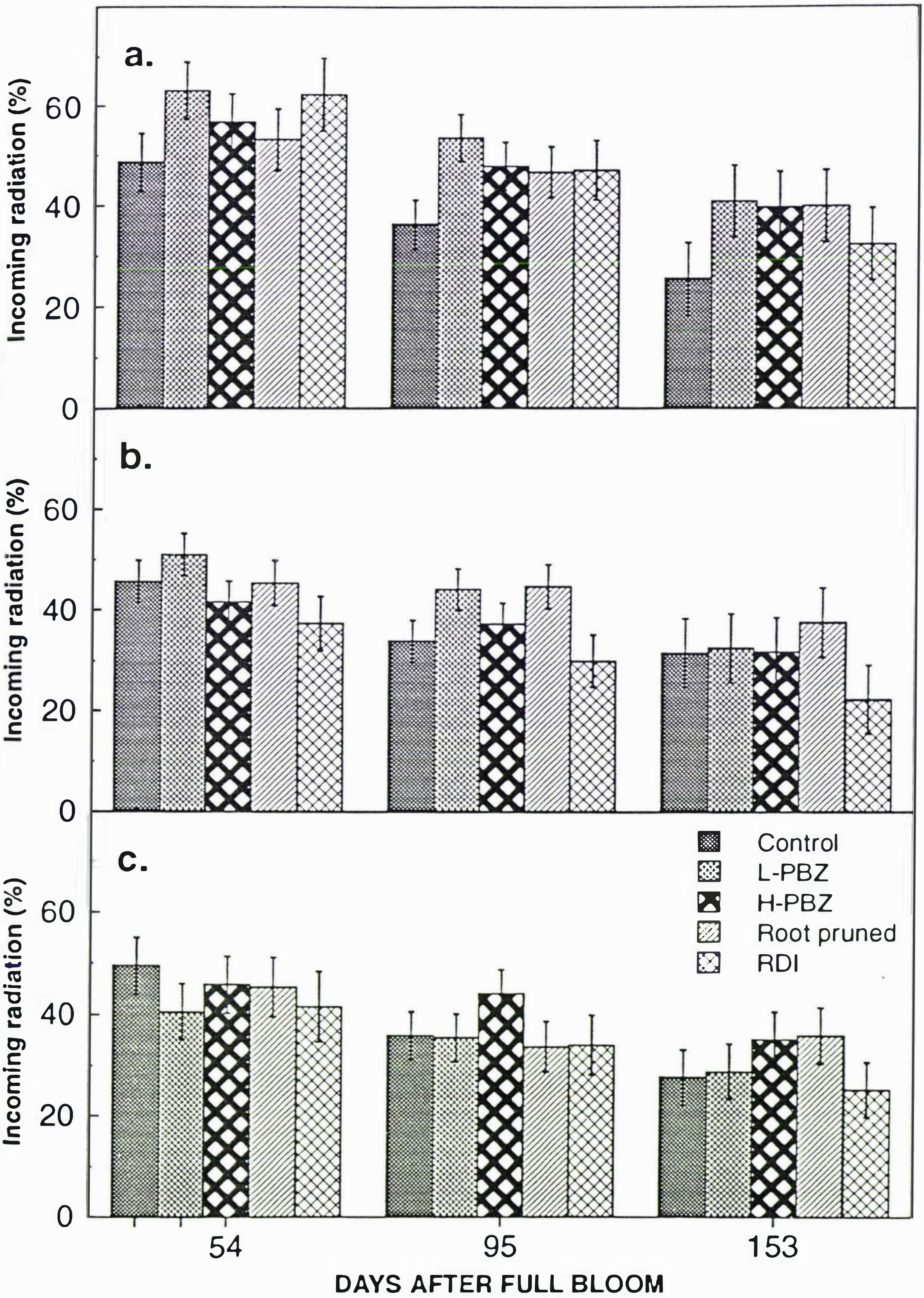


Figure 4.7 The relative light intensity in different regions down the canopy of close planted 'Sundrop' apricot in 1991-1992 season. Vertical lines indicate the standard error of the least square means. a). Top zone; b). The middle zone; and c). The lower zone of canopy.

compared with RDI ($P<0.05$), and there was a trend for improvement by these two treatments compared with control treatment ($P=0.09$, 0.08 for L-PBZ and root-pruned respectively). No other differences between treatments and control were significant.

4.3.2 Root characteristics

4.3.2.1 Root-length density (RLD)

Positions of core samples categorized for RLD (Figure 4.8) or RWD (Figure 4.11) were plotted using the coordinates r and z. This method gave patterns estimating root-occupied and un-occupied soil volume within the whole explored soil volume. A volume is defined (Gandar and Hughes, 1988) as "occupied" if it contains roots, "unoccupied" if it does not, and "explored" if it contains roots anywhere within it. There was little evidence of the ellipsoidal geometry characteristic of young root systems (Gandar and Hughes, 1988), therefore it was assumed that RLD distribution is 'layered' as described in Gandar and Hughes (1988) and Hughes and Gandar (1993). RLD declined with increasing depth in all treatments, and was more concentrated for root-pruned trees closer to the tree trunk (Figure 4.8 c.). This is demonstrated by the relatively high concentration of samples in the >0.2 cm. cm^{-3} within 60 cm of the tree in that treatment.

The numbers of core sub-samples containing no roots (RLD=0) increased with depth, although this tends to be obscured in Figure 4.9 because data from all treatment replicates are superimposed. In all treatments, about 80% of the volume of soil sampled was occupied (RLD>0) and 20 percent was un-occupied by roots (Figure 4.9 f.).

Cumulative distributions of RLD volumes for samples with RLD>0 and categorized for selected regions (Table 4.1) of soil are shown in Figure 4.10.

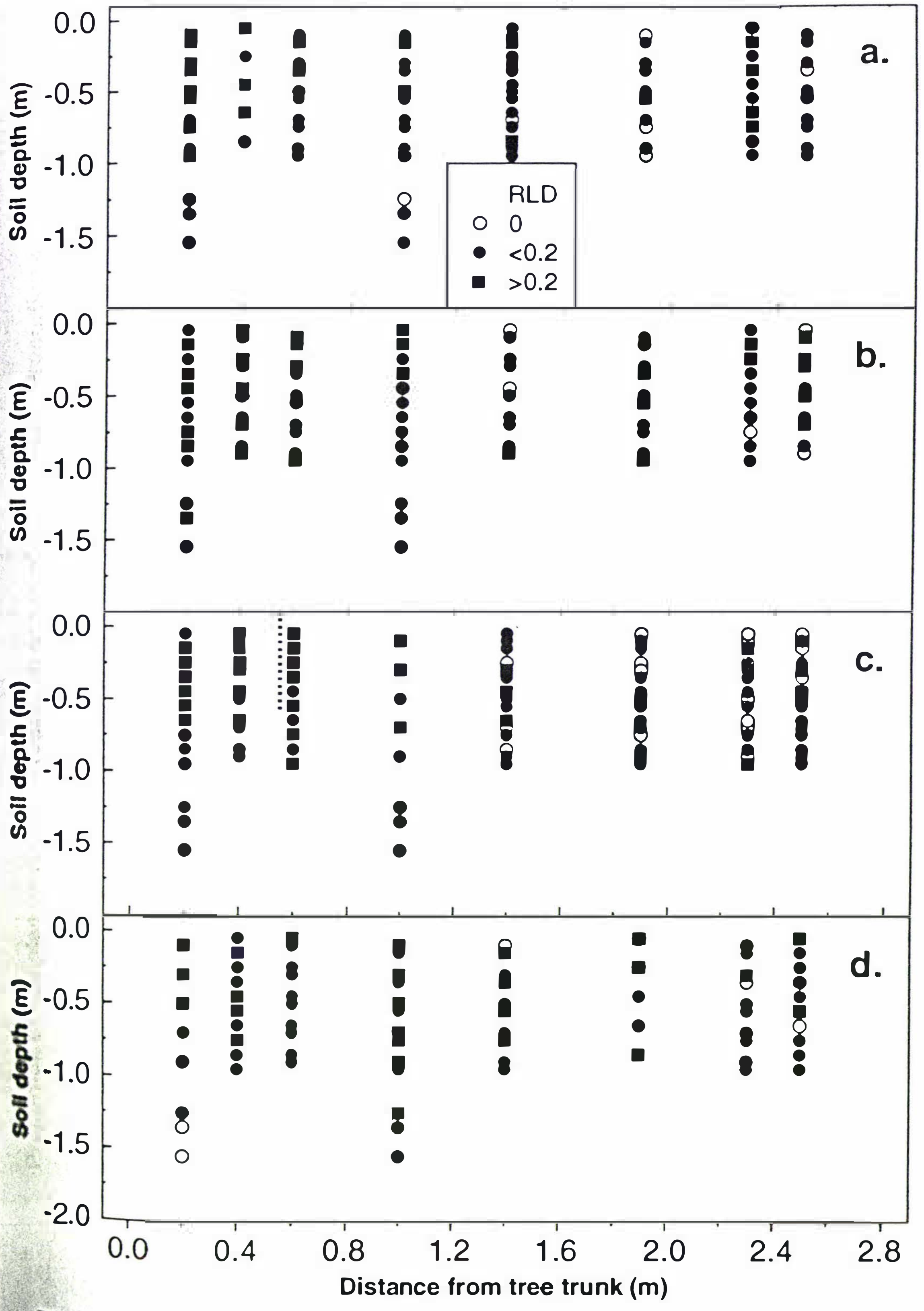


Figure 4.8 Coordinates of fine root (<1.5 mm diam.) samples from close planted 'Sundrop' apricot classified by RLD (0, <0.2 and >0.2 cm/m^3 of soil). Each data point denotes the position of a core sample relative to the tree trunk at zero. a). Control; b). H-PBZ; c). Root pruned with position of root-pruning slit shown. and d). RDI.

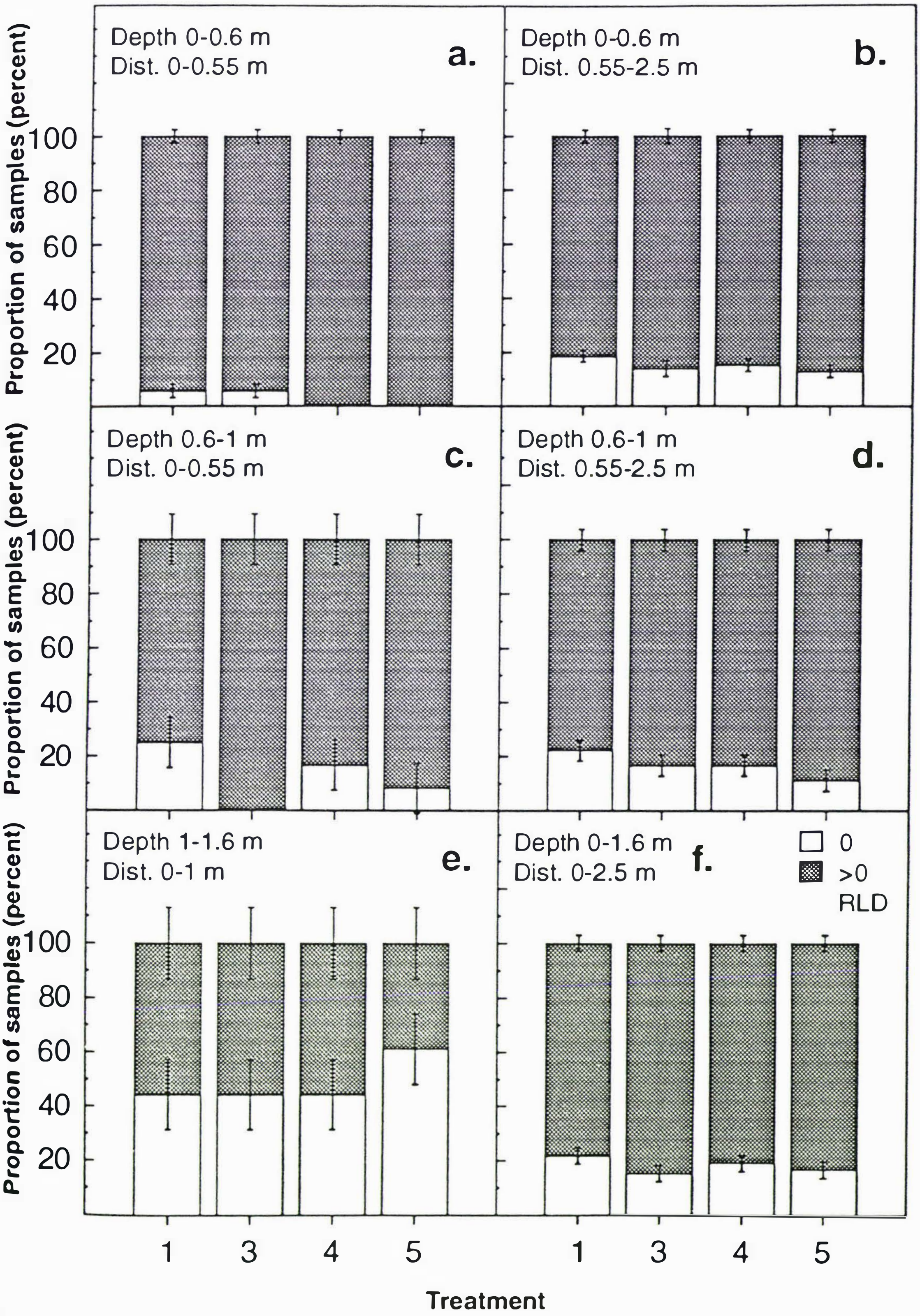


Figure 4.9 Proportion of samples from close planted 'Sundrop' apricot within selected regions of the root system which contained fine root (<1.5 mm diam.), based on RLD data. Treatments were: 1 = Control; 3 = H-PBZ; 4 = Root pruned and 5 = RDI.

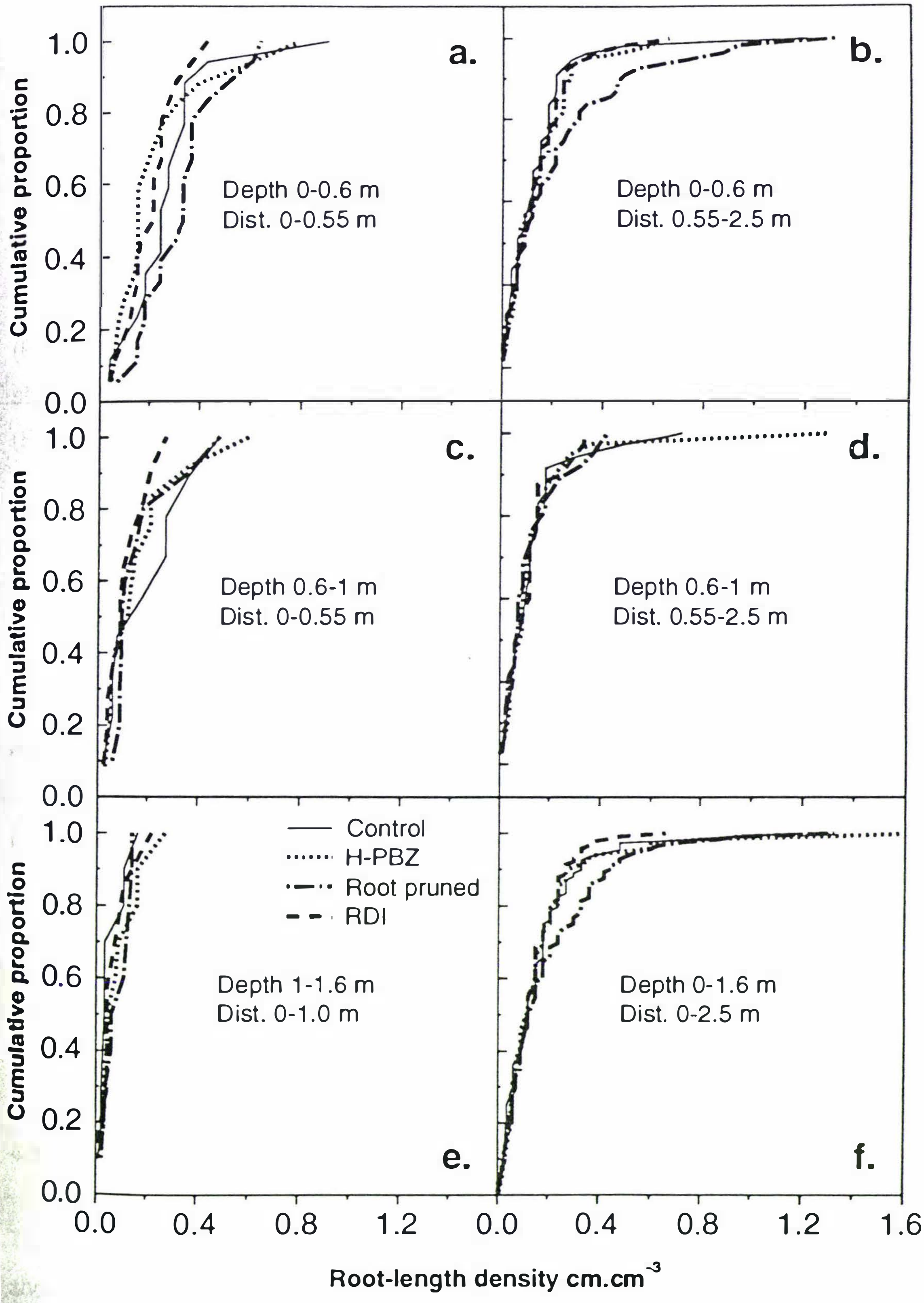


Figure 4.10 Effect of treatments on cumulative proportions of sample cores by RLD (cm. cm^{-3}) for close planted 'Sundrop' apricot. Samples at depth and distance from tree trunk as shown.

The basis for categorization was the position of the root-pruning slit. There was a trend for the root-pruning treatment to have more core samples with high RLDs in the surface soil close to the tree, when compared to the other treatments (P=0.09, 0.06 and 0.10 relative to control, H-PBZ and RDI respectively)(Figure 4.10 a.). Similarly samples with high RLD were more numerous in the root-pruned treatment in the top 0.6 m of soil outside the pruning slit. This response was significantly different from the other treatments (P<0.05 from control and H-PBZ, and P<0.001 from RDI). There were no statistically significant differences among the other treatments in this region (Figure 4.10 b.).

At soil depths greater than 0.6 m within 0.55 m from the tree trunks (Figure 4.10 c.) RDI trees had lower numbers of samples with high RLD, significant at P<0.05 compared to the control treatment. However, at this soil depth but further from the tree trunk RLD for all treatments was not significantly different (Figure 4.10 d.). All treatments at the lowest soil depth sampled (e., below 1 m) were also similar (Table 4.1 & Figure 4.10 e.).

In general, root-pruned trees had more samples with high RLD (P<0.001) compared with the other treatments (Figure 4.10 f.). However, when treatments were analysed after pooling all samples, both those with RLD>0 and those with RLD=0, from the whole there were no statistically significant differences between means (Table 4.3). This illustrates the advantage of comparing treatments using selected categories of data (Figure 4.10).

4.3.2.2 Root-weight density (RWD)

The coordinates of samples containing woody roots classed for RWD are shown in Figure 4.11. Figure 4.11 c. indicates that in the root-pruned treatment there were more woody roots within 0.6 m of the trunk, and fewer outside the root-pruning slit at 0.55 m.

Table 4.3. Root length density (RLD) and root weight density (RWD) for the whole explored and un-occupied soil volume from close planted 'Sundrop' apricot recorded in October, 1993 after 2 years treatment.

Explored soil region^{y}	RLD (cm of root·cm^{-3} of soil)				
	Control	H-PBZ	Root-pruned	RDI	
a.	0.25 ± 0.05^{z}	0.21 ± 0.05	0.32 ± 0.04	0.20 ± 0.02	ns^{x}
b.	0.12 ± 0.20	0.16 ± 0.03	0.19 ± 0.04	0.13 ± 0.02	ns
c.	0.15 ± 0.05	0.17 ± 0.05	0.14 ± 0.04	0.10 ± 0.02	ns
d.	0.11 ± 0.02	0.13 ± 0.04	0.10 ± 0.02	0.11 ± 0.02	ns
e.	0.03 ± 0.01	0.05 ± 0.02	0.05 ± 0.01	0.03 ± 0.01	ns
f.	0.12 ± 0.01	0.14 ± 0.02	0.16 ± 0.02	0.12 ± 0.01	ns
	RWD (g of root·cm^{-3} of soil)				
a.	3.72 ± 1.32	3.59 ± 2.12	3.53 ± 1.20	3.19 ± 1.47	ns
b.	0.85 ± 0.54	1.04 ± 0.51	0.32 ± 0.11	0.41 ± 0.18	ns
c.	0.17 ± 0.12	0.10 ± 0.04	4.98 ± 3.69	0.08 ± 0.05	ns
d.	0.53 ± 0.25	0.16 ± 0.05	0.26 ± 0.14	0.27 ± 0.13	ns
e.	0.11 ± 0.05	0.04 ± 0.02	0.02 ± 0.02	0.02 ± 0.01	ns
f.	0.99 ± 0.29	0.93 ± 0.35	1.09 ± 0.38	0.66 ± 0.22	ns

^{z}± Standard error of the mean.

^{y} a.= 0-0.6 m depth & 0-0.55 m distance; b.= 0-0.6, 0.55-2.5; c.= 0.6-1, 0-0.55; d.= 0.6-1, 0.55-2.5; e.= 1-2, 0-1; f.= 0-2, 0-2.5 (see Table 4.1).

^{x} ns Nonsignificant.

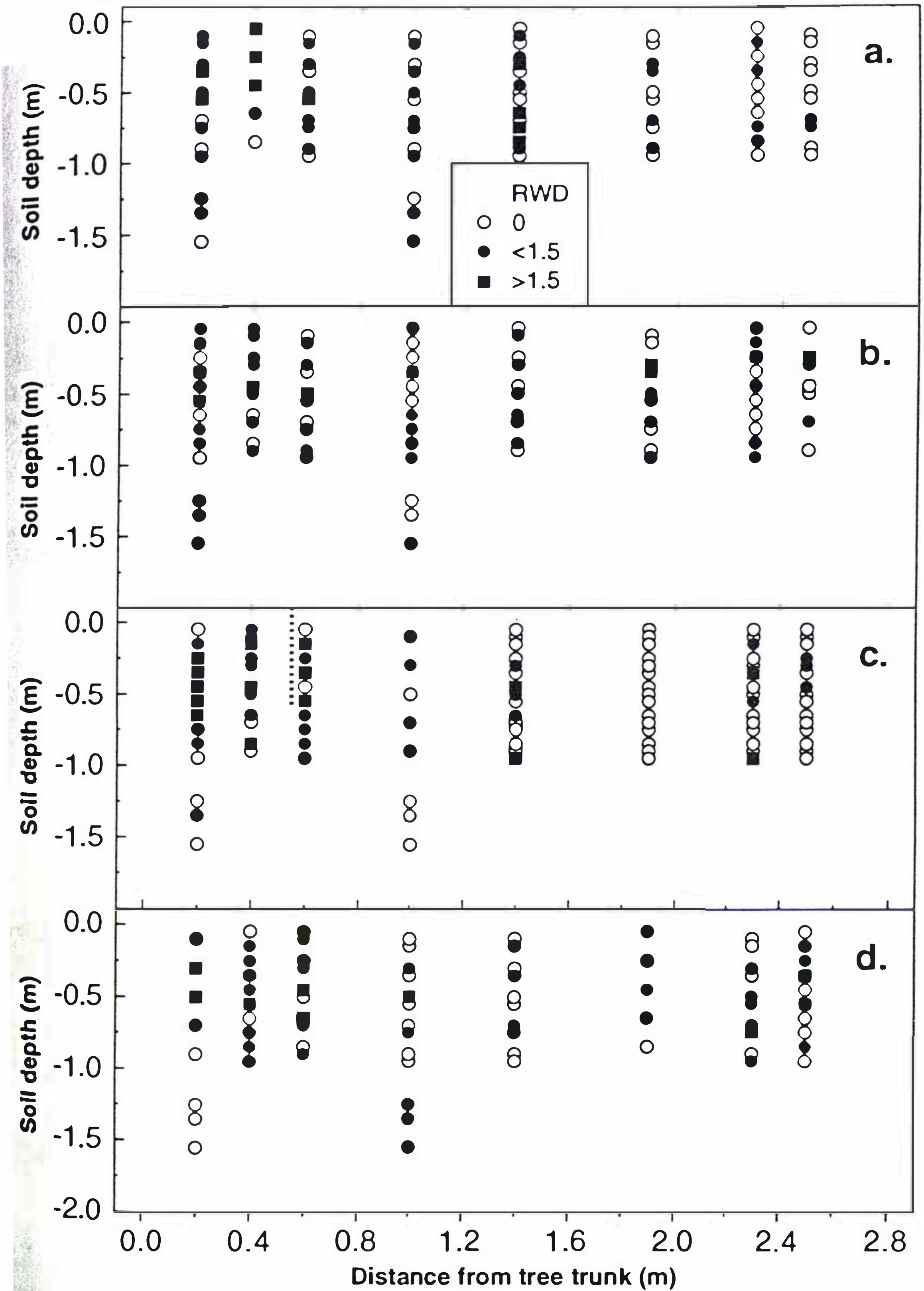


Figure 4.11 Coordinates of woody root (>1.5 mm diam.) samples from close planted 'Sundrop' apricot classified by RWD (0, <1.5 and >1.5 gram/cm^3 of soil). Each data point denotes the position of a core sample relative to the tree trunk at zero. a). Control; b). H-PBZ; c). Root pruned with position of root-pruning slit shown. and d). RDI.

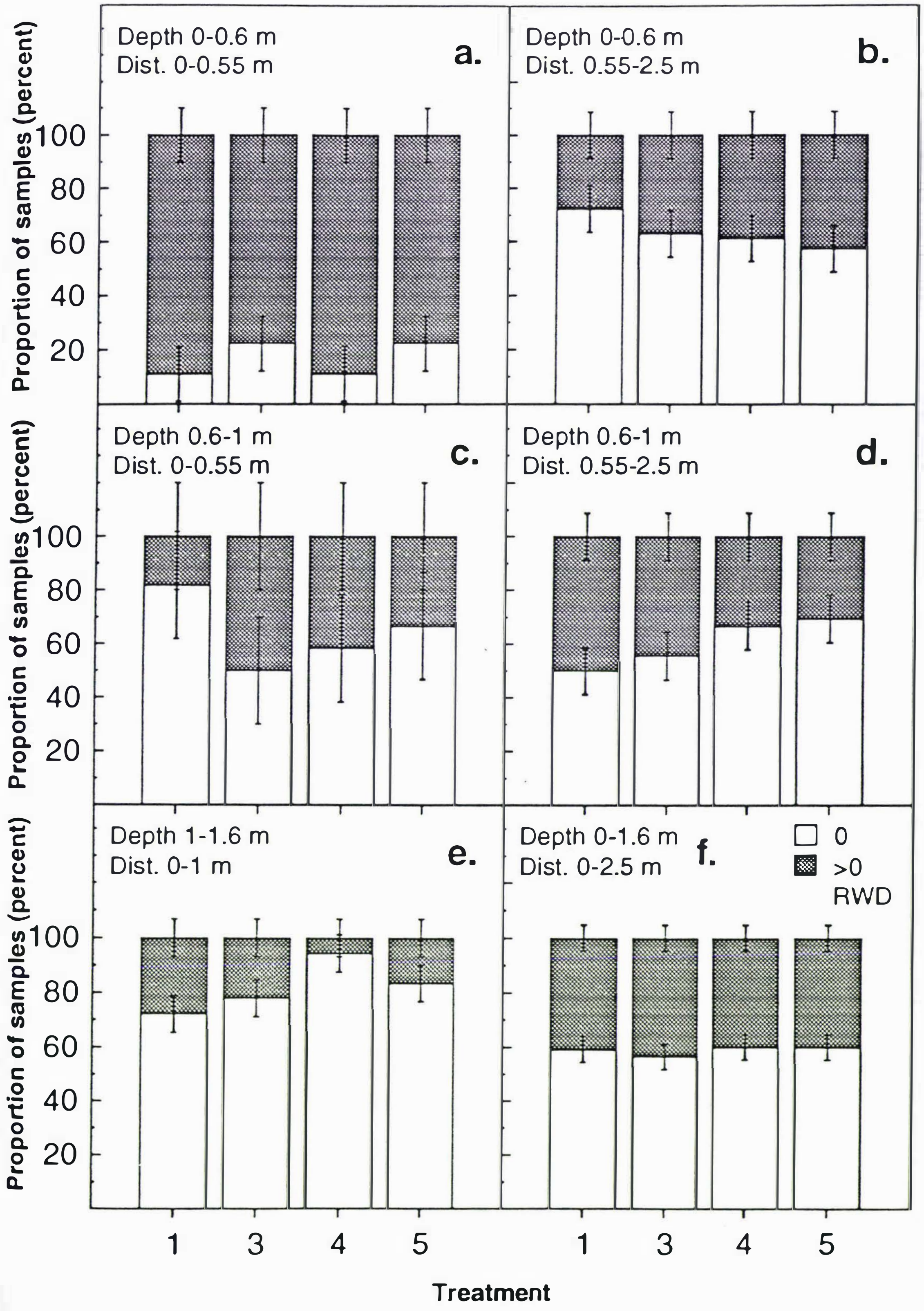


Figure 4.12 Proportion of samples from close planted 'Sundrop' apricot within selected regions of the root system which contained woody root (>1.5 mm diam.), based on RWD data. Treatments were: 1 = Control; 3 = H-PBZ; 4 = Root pruned and 5 = RDI.

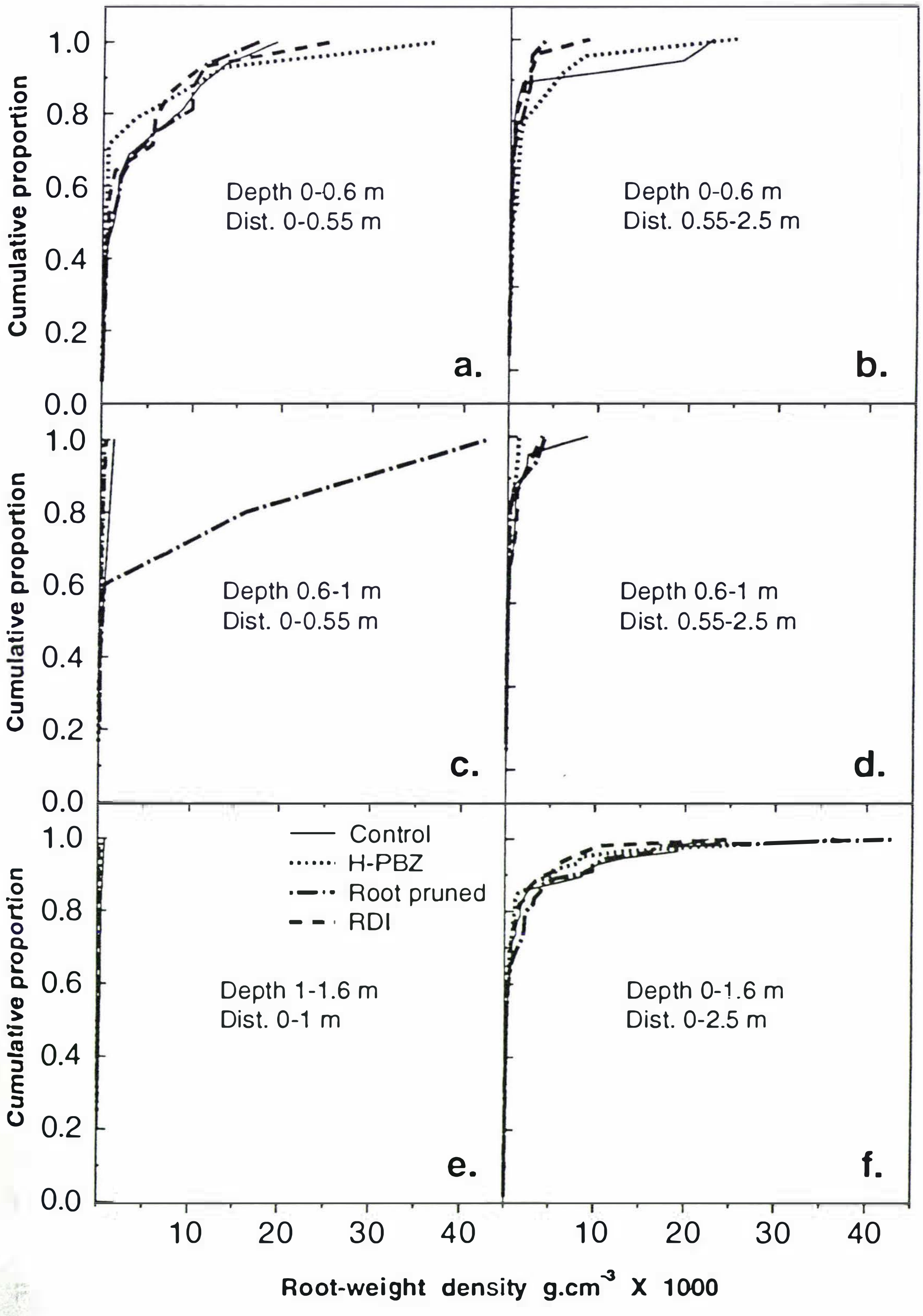


Figure 4.13 Effect of treatments on cumulative proportions of sample cores by RWD (g. cm^{-3}) of woody roots for close planted 'Sundrop' apricot. Samples at depth and distance from tree trunk as shown.

The numbers of samples containing woody roots (RWD>0) was not significantly different among the treatments in the a, b, c, d and f regions (Figure 4.12 a., b., c., d. and f.). However, below 1 m the root-pruned treatment had significantly ($P<0.05$) fewer samples with RWD>0 than the control treatment (Figure 4.12 e.). The other treatments were not significantly different from the control.

The RWD cumulative proportion (Figure 4.13 b.) shown that in root-pruned trees there were fewer woody roots in the top layer of soil and outside the root-pruning cut. This was significant ($P<0.05$) against the H-PBZ treatment, but not relative to control. Close to the trunk at the 0.6-1m depth (Figure 4.13 c.) the proportion of samples with woody roots was greater in the root-pruning treatment relative to the H-PBZ treatment ($P<0.05$). When all samples were pooled from each treatment there were no statistically significant differences between treatments (Figure 4.13 f.). Similarly, no treatment differences were significant within soil regions for data shown in Figure 4.13 a., d. and e.

4.4 RESULTS: WIDE PLANTED TREES (1990-1991 SEASON)

4.4.1 Summer pruning

The distribution of pruning dry matter between the top and lower zones of the canopy are shown in Figure 4.2 b. The proportion of growth in the lower and middle part of the canopy on the RDI treatment was lower than on L-PBZ and root-pruned treatments ($P<0.05$), and trended toward a reduction relative to control ($P=0.08$). The proportions of regrowth by zone for control and other treatments were not significantly different.

Figure 4.3 b. shows that the weight of postharvest summer prunings from control was greater than from the other treatments in both the top and lower zones of the canopy. The difference between control and L-PBZ was not significant, but control was significantly greater than the other treatments ($P<0.01$ for H-PBZ and 0.05 for root-pruned and RDI treatments). H-PBZ had more effect than RDI in reducing growth in all parts of the canopy ($P<0.05$).

Preharvest pruning showed little difference between treatments, but at the postharvest pruning all treatments tended to reduce growth (Figure **4.14** b.). The only significant effect was that H-PBZ reduced prunings compared with control ($P<0.05$).

4.4.2 Shoot growth

The data from randomly sampled shoots in the top zone of the canopy measured at the postharvest pruning are shown in Figure 4.2 d. Shoot length was greatest on the RDI treatment, although all treatments were statistically similar. Figure 4.14 a. shows the effect of treatments on the growth of mid-zone shoots measured at intervals through the season. The data show that all experimental treatments resulted in lower shoot lengths throughout the season than control ($P<0.01$ for RDI and $P<0.05$ for H-PBZ, root-pruned and L-PBZ). These treatments were not statistically different from each other during the season except between RDI and L-PBZ up to 62 dafb.

4.4.3 Trunk cross sectional area (TCSA)

The changes in trunk cross sectional area are shown in Table 4.2 b. There were no significant differences among the treatments.

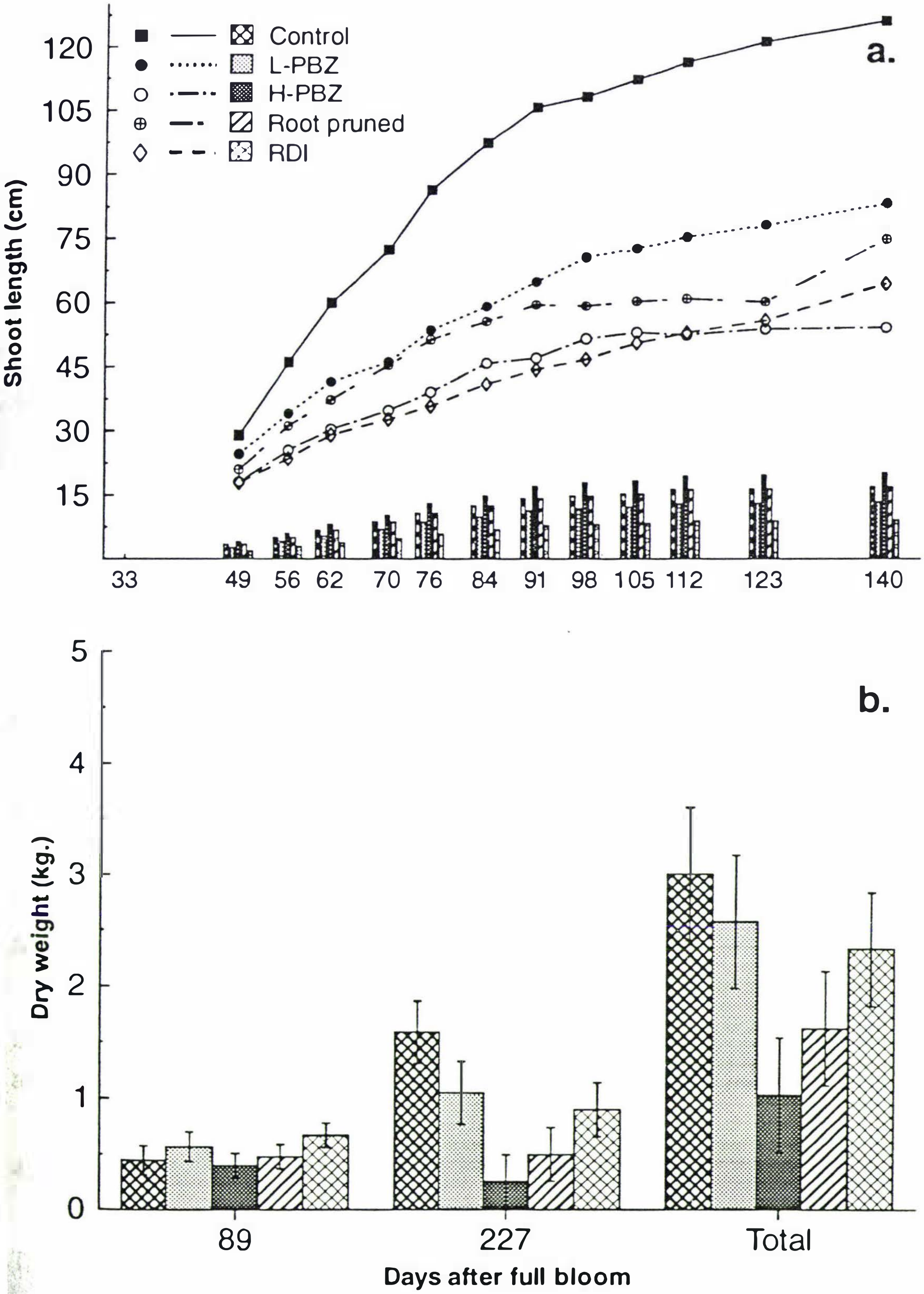


Figure 4.14 The effect of treatment on shoot length (middle canopy zone) and pruning on wide planted 'Sundrop' apricot in 1990-1991 season. a). The cumulative shoot lengths. Bars show the standard error of means; b). Regrowth in terms of summer pruning. Dry weight of tagged shoots removed at the time of fruit harvest added to total pruning data. Vertical lines indicate the standard error of the means.

4.5 DISCUSSION

In this study, the response of apricot trees to a number of treatments applied to reduce vegetative growth was monitored on close spaced trees during two seasons, and in one season on wide spaced trees. The shoot growth data of close spaced trees showed that without vigour reduction strong upright summer growth occurred. The Tatura trellis system trains main shoots and branches in a V shape at 30° to the vertical. Laterals must be encouraged to furnish the underneath of the V shape and not to result in excessive shading within the canopy. Summer pruning contributes to this result (van den Ende and Chalmers, 1978; van den Ende, 1984), but other management techniques may also play a role.

The highest proportion of early season top growth was in shoots with leaves, but from about 90 dafb this proportion decreased in favour of fruits until harvest (Figure 4.6 a. & b.). There was a large increase in fruit size during stage III of fruit growth (from 97 dafb), while shoot growth was most rapid during 60-90 dafb. Usually at about this time (89 and 101 dafb in first and second season respectively) preharvest summer pruning was performed as part of normal management practice to remove excess vegetative growth and allow more light to penetrate into the tree canopy. Chalmers *et al.* (1984 & 1985) described a similar relationship between the timing of shoot and fruit growth, and showed that by using it to determine the timing of RDI it provided an opportunity to reduce vegetative vigour without reducing fruit growth.

4.5.1 Tree response to PBZ

The results from close spaced trees showed that both PBZ treatments reduced summer pruning and shoot extension growth during the season. A similar result was reported using soil applied PBZ on ‘Starkrimson Delicious’ apple (Lehman *et al.*, 1990a), ‘Bing’ sweet cherry (Jacyna *et al.*, 1989) and nectarine and peach (Blanco, 1986). Loreti *et al.* (1989) found a soil or spray application of PBZ reduced vegetative growth of peach cv. 'San Pedro' for 2-3 years depending on dose of application. In the present study, in the season of their application to both close and wide spaced trees H-PBZ (1.5g a.i. tree^{-1}) had a greater effect than L-PBZ (0.5g a.i. tree^{-1}). For example, cumulative shoot length was reduced 54% in close planted trees by H-PBZ and only 19% by L-PBZ treatments in the first season. The total year's prunings was reduced 71% by H-PBZ and 66% by L-PBZ treatments in the first season. A similar result was obtained in wide planted trees and cumulative shoot length was reduced 57% by H-PBZ and 34% by L-PBZ treatments. Also at this spacing, total year pruning was reduced 66% by H-PBZ and 14% by L-PBZ treatments. In the second season following a repeat application, L-PBZ inhibited vegetative growth 42% (total year pruning) whereas there was a 36% reduction from the residual H-PBZ. The inhibitory effect of the H-PBZ treatment in the second season after it's single initial application was greatly reduced, for example it's inhibitory effect in reducing total year pruning was only about half of it's effect in the first season. Ogata *et al.* (1989) found that soil applied PBZ reduced peach and cherry growth in the season of application and the degree of inhibition reduced over time, and in the third year the trees tended to grow more strongly than untreated trees. They considered that annual application was better than a single treatment for controlling tree size. Multiple low-rate applications of PBZ for controlling vegetative growth of fruit trees was also recommended by Quinlan and Richardson (1986).

In the present study, the decrease in the effect of H-PBZ treatment over time may have been caused by its increasing the root:shoot ratio during the first year of experiment as a result of reducing shoot growth combined with a low PBZ residue in the soil in the second season. According to Anon (1982), Lever (1986) and Reed *et al.* (1989) the movement of soil-applied PBZ within the plant is acropetal in the xylem to leaves and buds with no phloem mobility. After such a PBZ increase in root:shoot ratio in the 1990-1991 season, top growth may occur in the year following treatment as the tree reestablishes its root:shoot ratio. The root study carried out after two years of study showed that H-PBZ tended to increase the amount of woody roots in the top soil layer and further from the trunk, although the difference from control was not significant (Figure 4.13 b.). Davis *et al.* (1988); Grossmann (1992) and Hodairi and Canham (1990a) suggested that in general PBZ reduced shoot growth and changed the root:shoot ratio in favour of the roots. The reduction of shoot growth was associated with redistribution of assimilate, so that roots received more, increasing the root:shoot ratio and hence root dry weight (Hodairi and Canham, 1990a). Also, microscopic study of root tip cross-sections of peach seedlings by Williamson *et al.* (1986) following PBZ application showed that root tip diameter increased and growth development of the root cortex and stele was modified by PBZ.

In the first season, of the two PBZ rates H-PBZ had the greater effect in reducing summer pruning and shoot growth of trees at both spacings. L-PBZ did not reduce preharvest summer pruning and shoot growth in wide spaced trees relative to control. This presumably was due to higher shoot growth in early stage I of fruit growth when possibly L-PBZ had little effect in reducing shoot growth, and this contributed to more preharvest pruning. Trees at wide spacing were the same age as those at close spacing, but usually had bigger canopy size (ie. bigger TCSA, Table 4.2) and possibly a larger root system. Webster (1989) suggested that rootstock, scion cultivar, soil type and tree size influenced the response of plum trees to soil applied PBZ using dosage rates 0.4 to 3.2 g active ingredient. Also, Gaash (1986) reported PBZ reduced vegetative growth of apple, plum and apricot, with dose response curves being related to species, clone, rootstock and tree size. Larger trees with longer shoots and greater trunk girth needed a greater amount of chemical. In close spaced trees, TCSA was significantly reduced only when L-PBZ had been used for two consecutive seasons. However, the annual increase in TCSA was reduced by both PBZ rates in both seasons (see chapter 5, Figure 5.11 b. &

d.). An indirect effect of L-PBZ treatment appeared in the increased light levels in the top (54 and 95 dafb) and middle canopy zones (95 dafb) in the second season. The analysis of the spatial distribution of pruning material in the first season (Figure 4.2 a.) revealed that control trees produced 52% of their annual regrowth in the top canopy zone, while the PBZ treatments reduced annual regrowth in the top canopy zone by 10% and 20% (L-PBZ and H-PBZ), and this pattern continued to a lesser degree in the second season. This possibly explains the indirect effect of L-PBZ increasing light penetration in the second season of study. The effect of H-PBZ in reducing vegetative growth (described earlier in this section, also Figure 4.2 a.) was higher in the first season, although there is no light measurement data from the first season of study to illustrate any higher indirect effect on light penetration. Lever (1986) reported that PBZ reduced vegetative growth of fruit trees which had an indirect effect in improving light penetration through the canopy.

4.5.2 Tree response to root-pruning

The amount of summer prunings both preharvest and postharvest was reduced by root-pruning in the first year (Figure 4.1 a.). In the second season only the preharvest prunings dry weight was reduced by this treatment (Figure 4.1 b.). The pruning data revealed that the effect of root-pruning in reducing prunings reduced from the first season to the end of the second season, when postharvest pruning was not significantly affected by root-pruning. Harris *et al.* (1971) as cited by Geisler and Ferree (1984a) reported vegetative growth was reduced soon after root-pruning trees, but the effect diminished as time elapsed after pruning, and 1 year after pruning there were no differences in shoot growth between root-pruned and unpruned trees. The present result on close spaced trees showed that while measuring extension growth of shoots did not show a significant effect of root-pruning treatment (Figure 4.2 c. & 4.5), growth was reduced as determined by total summer pruning in both the first ($P<0.001$) and second season ($P=0.068$) of the experiment (Figure 4.1). This is also reflected in a small increase in light in the middle canopy zone (Figure 4.7 b. P=0.084 at 95 dafb). In the wide planted trees the effect of root-pruning increased during the season. At the preharvest pruning (89 dafb) the treatment showed little effect, but later at the postharvest pruning there was more reduction of shoot growth. This may have resulted from the wide tree spacing modifying the shape and form of root distribution within the soil. Atkinson *et al.* (1976) and Atkinson (1976 and 1978) reported that the distance between trees had an effect on root distribution. For instance in wide spaced apple trees the root system was composed mainly of horizontal roots with relatively few vertical sinkers. However, at closer spacing, the horizontal portion was reduced and the root system was composed mainly of vertical sinkers. The results from both close and wide spaced trees presently described (shoot growth and summer pruning data) are in agreement with such types of root system. Thus it might be possible to conclude that root-pruning at the same distance and depth had a more severe effect on wide spaced trees than on the close spaced, possibly removing a larger proportion of the root system. The canopy of the wide spaced trees was bigger, increasing the demand for water and other materials from the root system. The time needed for the trees to restore the balance of the root:shoot ratio may have been greater. Geisler and Ferree (1984b) reported that in general as the severity of root-pruning increased, the rate of shoot growth decreased. They reported that root-pruned trees resumed shoot growth in the third or fourth week following root-pruning, and suggested that to restrict tree size by root-pruning roots would have to be removed repeatedly at intervals of several weeks. Schumacher (1975) as cited by Schupp and Ferree (1988b) found that distance from the tree trunk had an effect on the response of 4-year-old 'Maigold' apple trees to root-pruning. Trees pruned at 40 cm distance showed reduced shoot growth, whereas pruning at 60 cm increased shoot growth by 60%. Schupp and Ferree (1988b) noted that distance of root-pruning, but not pruning depth influenced shoot growth and concluded most roots were located in the top 25 cm of soil.

Hence two years after the first of 2 root-prunings, the trees had a greater RLD (Figure 4.10 f.), due to increased root growth in the top 60 cm of the soil profile. There was increased RLD both within the area of pruning, and also beyond the point at which roots had been cut (Figure 4.10 a., b. & f.). The RWD was not changed by root-pruning relative to control. This indicates the trees responded rapidly to replace the lost part of the root system, and the treatment increased the intensity of soil exploration. Some of the regenerated roots were removed by the second season's pruning, which was performed in the same position as in the first season. This may explain why root-pruning was able to reduce early season vegetative growth in the second season and it's inhibitory effect was less than in the first season. Remaining regenerated roots (in the a. region) from the first season treatment, and the extra roots regenerated after the second season root-pruning, together with removal of harvested fruits, may have allowed greater assimilate partitioning to the shoots and resulted in more shoot growth after the harvest period. This contributed to strong postharvest shoot growth and high second season prunings which were similar to control.

4.5.3 Tree response to RDI

Before discussing the vegetative growth response of trees to the RDI treatment it is useful to note (see chapter 3) this study was carried out in a deep soil and the humid climate of Palmerston North. In addition, as will be discussed in chapter six, the climatic conditions of the second season of study were somewhat different from the first year. This year was slightly cooler, but was more humid with more rainfall during the main growing season. However, the objective of RDI was to impose a mild water stress during the first two stages of fruit growth and resume full irrigation during stage III of fruit growth. The general difficulty in obtaining desired levels of water stress in this environment was compounded in the close planted experiment by the loss of three RDI plots due to flooding (see 3.5.1.4). This has meant that many results from this treatment are based on only 5 replicates (8 in control and other treatments).

Only in the first season a mild water stress during the first two stages of fruit growth reduced shoot growth and prunings. Under the cooler and damper climatic conditions of the second season it appears the degree of stress was insufficient to reduce vegetative growth. The RDI treatment did not reduce TCSA increase. Huslig *et al.* (1993) reported that RDI did not reduce growth of TCSA of peach trees under conditions of fine-loamy soil in the climatic condition of Perkins, Oklahoma.

In the first season RDI of close spaced trees reduced both pre- (P=0.07) and post-harvest (P<0.001) summer pruning. The total dry weight of summer pruning was reduced by RDI (Figure 4.1 a.), and extension growth of tagged shoots was reduced by an average 26%. In 1990-1991 RDI reduced shoot growth early in the season, and especially from 62 dafb (Figure 4.5), but it was not as inhibitory as H-PBZ.

While the overall effect of RDI treatment was to reduce shoot vigour by an average across both seasons of about 18% and 36% (cumulative shoot growth and total year prunings respectively) on close spaced trees, and by 49% and 22% in the first season on wide spaced trees, the effect was not as great as that recorded by Higgs and Jones (1991). They found a 62% reduction in the weight of shoots removed by summer pruning following RDI on apple trees. Vegetative growth of peach trees was reduced by 80 and 70% when their daily water replacement was reduced to 1/8 and 1/4 respectively of the evapotranspiration from a class A pan during the early stage of fruit growth (Chalmers *et al.*, 1984). In the present study, the RDI reduced vegetative growth less in the second season. This suggests there may have been seasonal differences in the implementation of the RDI treatment. As will be discussed in chapter 6, in the second season more rainfall occurred early in the season (eg. in September). The covers were sealed and RDI started at full bloom (end of August). During early stage I of fruit growth soil moisture was still high and would not have suppressed shoot growth. This could explain the high level of preharvest summer pruning, which was not significantly different from control. Low evaporation from the soil surface under the plastic covers used for RDI treatment may also have contributed to the slow drying out of soil moisture. At early stage I the temperature was low and the covers by preventing evaporation may have retained soil moisture. Such an effect under black plastic mulching on apple trees was reported by Bacon (1974).

Jerie *et al.* (1989a) suggested that maintaining a high density orchard without using dwarfing rootstocks required control of the wetted root zone by water stress with RDI. They concluded there was good potential for achieving this in shallow but well drained soils and/or in a dry climate during periods of rapid shoot growth. However the humid and wet climate of Palmerston North with low spring temperatures made it difficult to practise RDI (see chapter 6). In the 1991-1992 season RDI did not reduce pruning or shoot growth, which may reflect an inadequate level of water stress. Root core sampling of close spaced trees showed the existence of roots down to 1.6 m soil depth, so trees were able to extract water from deep in the soil, which it was difficult to dry during the second season (see chapter 6). Reduction in vegetative growth using RDI (inducing plant water deficit by applying less water than the plant uses) has been reported previously, for example Boland *et al.* (1993); Chalmers *et al.* (1981 and 1986); Mitchell and Chalmers (1982); Mitchell *et al.* (1984). Chalmers *et al.* (1984) suggested that as shoot growth is integrally linked with root growth, restricting root growth will tend to restrict shoot growth. Root restriction was achieved in their experiment by using very shallow soil, limiting the wetted volume with trickle irrigation and by high tree density. The present results of close spaced trees in the second year agree with the results of Girona *et al.* (1993) who applied RDI to peach trees under deep soil conditions. They reported that RDI produced a small reduction of TCSA (8%), and there were no clear indications of decreased shoot growth relative to control. They concluded that the minor effect on vegetative growth was possibly a consequence of the relatively long time that it took to achieve moderate water stress in the RDI treatments on a deep soil.

SUMMARY

'Sundrop' apricot trees tended to produce a high proportion (≈ 50%) of their growth in the top canopy zone in the absence of any growth controlling treatment (control). All treatments reduced this tendency giving ≈10 to 40% reduction of regrowth in the top of tree in the first season on close spaced trees.

In the first season both PBZ treatments significantly reduced vegetative growth at both tree spacings, with a higher inhibitory effect by H-PBZ. However, in the second season reapplying the L-PBZ treatment gave a higher inhibitory effect than the carry-over from the first year applied H-PBZ treatment. Although the total PBZ dose over two years was lower, repeating L-PBZ treatment in the second season gave greater effects.

The response to root-pruning in both close and wide spaced trees reflected the spacing. In close spaced trees in the first season root-pruned trees showed a more immediate response to root-pruning than in the second year in terms of reduced vegetative growth. However, the effect of root-pruning declined through the season and disappeared in the second season in spite of reapplied root-pruning. The result from the first season suggested that root-pruning of wide planted trees had a longer term effect in reducing shoot growth than on close planted trees.

RDI resulted in a reduction of shoot growth and prunings in both close and wide spaced trees in the first season, but had no effect in reducing vegetative growth in the second season of the close spacing experiment. This was possibly caused by lower temperature and evaporation and higher rainfall and higher early season soil moisture in the second season, which possibly produced an insufficient level of water stress in the second season.

Root core sampling on close spaced trees revealed roots in the deeper layer of explored soil, but the root responses to treatments were mostly in the top 1 m of soil. Root-pruning tended to increase RLD closer to the tree trunk and particularly in the pruning cut region.

The results suggested that among the applied treatments the most effective was the use of PBZ. Other treatments were short-lived or variable in response in terms of reducing vegetative growth. So, based on these results and under the conditions of this experiment RDI and root-pruning could not be recommended in commercial application. However, as will be discussed later (chapter 8), the combination of root-pruning and RDI might offer a new area of investigation to increase the efficiency of both these treatments in reducing vegetative growth.

CHAPTER FIVE

EFFECTS OF TREATMENTS ON REPRODUCTIVE GROWTH OF 'SUNDROP' APRICOT

5.1 INTRODUCTION

Managing vegetative and reproductive growth, and controlling tree size are important aspects of high-density orchard culture. Forshey and Elfving (1989) suggested that the ultimate objective of all pomological practices in any orchard management system is the manipulation of the vegetative growth-fruiting relationship.

Any orchard management should aim to produce high quality fruits, this being particularly important in New Zealand where much of the fruit industry is export dependent. 'Sundrop' apricot produces good quality fruits, and it has been suggested it was their bright orange colour and good size that attracted considerable grower attention in the apricot growing areas in New Zealand (Noiton *et al.*, 1993).

One of the objectives of this study was to evaluate the effects of PBZ, root-pruning and RDI on vegetative and fruit growth and quality of 'Sundrop' apricot. The effects of the different growth control techniques on vegetative growth were discussed in the previous chapter. This chapter will evaluate the effects of the treatments on fruit growth and quality of close and wide spaced 'Sundrop' apricot trees under the humid climate of the experimental site. Data on the wide planted trees was restricted to the 1990-1991 season as previously described.

Seasonal changes in fruit size based on destructive fruit data were discussed in the previous chapter (Section 4.3.2.2). In this chapter seasonal changes in fruit growth based on non-destructive fruit measurements are presented. The yield, stage of maturity and fruit quality factors such as colour and total soluble solids (TSS) were assessed at the time of each commercial fruit harvest.

Robinson (1992) suggested that partitioning index (P-index) indicates how well the tree partitioned its resources into fruit. It is the ratio of annual fruit produced to annual amount of vegetative growth. Partitioning index of close spaced trees based on yield and annual increase in TCSA was calculated, to allow an estimate of how efficient the applied treatments were in reducing vegetative growth and partitioning the saved carbohydrates into fruit.

Fruit growth is a function of volume (or weight) increase, and diameter by itself is generally a poor measure of fruit growth because it is not linearly related to volume or weight (Westwood, 1993). Apricot is not a sphere-shaped fruit, and destructive fruit sampling throughout both seasons produced a model to predict fruit volume and fresh weight from non-destructive fruit diameter data (Chalmers, 1990 pers. comm.). Only fruit diameter and estimated fruit volume are presented.

The flowering period for all treatments was monitored at the beginning of the second year of study to evaluate the effect of first year treatments on the time and duration of flowering. The time of full bloom in both seasons was used as a reference date for most assessments in the orchard experiment. The effect of treatments on flower and fruit density and on fruit set was also examined on the close spaced trees.

This chapter will report and discuss some factors related to reproductive aspects, growth, yield and quality of the fruit of experimental trees, and the effects of the treatments on these during the orchard experiment.

5.2 MATERIALS AND METHODS

5.2.1 Measurements

5.2.1.1 Flowering period, flower and fruit density and fruit set

Flowering period was determined by estimating the percentage of open flower each 2-3 days from 27th. August 1991 on the close spaced trees. Two weeks before full bloom on each experimental tree 2 similar size shoots from the middle canopy zone were selected and labelled on each of the east and west sides of the tree. The total number of flower buds on each shoot was counted at the time of floral bud burst and the shoot length recorded. Fruit number on each marked shoot was recorded 59 dafb and fruit set as a percentage of total flower bud number calculated. At this time the number of fruits on each experimental tree was also counted.

5.2.1.2 Fruit growth

5.2.1.2.1 Fruit volume-diameter relationship

Because of the limitation of insufficient uniform non-experimental trees, this study was performed on some experimental trees of the close planted experiment in both seasons. Fruit samples were obtained from 24 (Control, H-PBZ and RDI) and 32 (Control, H-PBZ, Root-pruned and RDI) close spaced experimental trees in the first and second seasons of study respectively. On each tree two fruits from the middle canopy zone were randomly sampled at weekly intervals from 30 dafb until the time of fruit harvest in both seasons of study. The fruits collected from each tree were kept separately in labelled plastic bags and parameters recorded immediately after harvest. Fruit fresh weight was recorded and fruit diameter measured across the suture line using a Digital Caliper (Mitutoyo Corporation, Japan). Fruit volume was recorded by a water displacement technique or weighing fruits under water as described by Westwood (1993). Data was used to build up a model for predicting fruit volume and fruit fresh weight from non-destructive fruit diameter measurements.

5.2.1.2.2 Seasonal fruit growth

Five fruits of uniform size were tagged on the east side of the middle canopy zone of all experimental trees (Plate 5.1). Fruit diameter was measured at weekly intervals across the suture line of fruit using a Digital Caliper from 44 (dafb) on both close and wide spaced trees in 1990-1991 and from 33 dafb on close spaced trees in 1991-1992 season. In addition, in the first season on close spaced trees this measurement was performed in the 3 canopy regions (upper, middle and lower) on control and H-PBZ treatments.

Non-destructive fruit growth data were expressed as fruit diameter, volume and fresh weight. Growth rate was calculated using the following formula (Hunt, 1978):

$$FGR = \frac{FT_2 - FT_1}{T_2 - T_1}$$

where FGR is fruit growth rate (mm, g. or cm^3 day^{-1}); FT_2, fruit size at time 2 (diameter in mm, volume in cm^3 or fruit fresh weight in g.). FT_1, fruit size at time 1 (mm, cm^3 or g.). T_2, time 2 of record in days and T_1, time 1.

Tagged fruits were harvested at the time of commercial fruit harvest and fresh weight recorded. Fruits were separated into mesocarp and stone, then fresh weight of each part recorded. Dry weights of mesocarp and stone were recorded after one week drying at 89°C to constant weight. Total fruit weight and proportion of mesocarp and stone were calculated from fresh and dry weight data.

5.2.1.3 Yield and fruit quality

Fruit were harvested at commercial maturity based on a golden yellow background colour and were picked on 5 dates in the first season and 4 dates in the second season (Plate 5.2). The number and total fresh weight of harvested fruits in each canopy zone (lower, middle and upper) at each picking date was recorded. Thus total fruit number and yield were calculated in each canopy zone and for the whole tree canopy. Mean fruit size, yield efficiency (g. of fruit cm^{-2} TCSA) and crop densiy (fruit number cm^{-2} TCSA) were calculated based on yield, fruit number and TCSA data. Yield efficiency was calculated using the formula:

$$YE = \frac{TY}{TCSA}$$

where YE is yield efficiency (g. of fruit cm^{-2} TCSA); TY, total harvested fruit per tree and TCSA is trunk cross sectional area in cm^2 at the time of fruit harvest (Table 4.2, TCSA used were recorded at 23/01/1991 and 03/01/1992 during first and second season respectively).

Crop density was calculated using the formula:

$$CD = \frac{NF}{TCSA}$$

where CD is crop density (fruit number cm^{-2} TCSA); NF is total number of fruits at the time of fruit harvest and TCSA is as described above.

At each picking date, 2 fruits from the middle canopy zone of each tree were randomly selected for background colour and TSS assessment. Colour measurements were made on each cheek of each fruit using a Minolta Chromameter CR-100 (Minolta Camera Co., Ltd, Japan). The meter was calibrated at illuminant condition C using the manufacturer's white standard reflector plate. Skin fruit colour was expressed in Commission Internationale d'Eclairage L^*, a^*, b^* colour-space coordinates (Francis, 1980; Voss, 1992 & Lancaster *et al.*, 1994). Hue angle values decrease as skin colour changes from green to yellow (Little, 1975). Thus high hue angle values indicate an intense green colour. The lightness (L^*) value factor defines the lightness or darkness of the colour, so high L^* value indicates a light colour (closer to white) and a low value indicates a greater degree of darkness (Voss, 1992).

Total soluble solids (TSS) was recorded using a hand-held temperature compensating Atago N-20 refractometer (Model N, McCornick Fruit Tech., brix range from 0-20% at 20°C). Juice was squeezed using a garlic press from pieces of flesh cut from each cheek of the fruit, and two readings were made for each fruit (4 values recorded for each experimental tree at each picking date). The refractometer was zeroed using distilled water. The prism surface and the light plate were thoroughly washed and dried with a clean soft tissue between each reading.

5.2.1.4 Partitioning index

Partitioning index (P-index) was calculated according to method described by Robinson (1992) using the formula:

$$P\text{-}index = \frac{kg\ yield}{TCSA\ GR}$$

where P-index is partitioning index (kg yield cm^{-2} TCSA $year^{-1}$); TCSA GR is growth rate of trunk cross sectional area (cm^2 $year^{-1}$).

Annual increase of TCSA (TCSA GR) was calculated based on the following formula (Hunt, 1978):

$$TCSA\ GR = \frac{TCSA_{t2} - TCSA_{t1}}{T_2 - T_1}$$

where $TCSA_{t2}$ and $TCSA_{t1}$ are TCSA recorded at time 2 (T_2) and time

Plate 5.1. Non-destructive fruit growth measurement on close spaced 'Sundrop' apricot trees during 1990-1991 season. **a).** Tagging fruits prior to fruit measurement. **b).** Fruits at 40 days after full bloom.

Plate 5.2. Wide planted 'Sundrop' apricot trees in 1990-1991 season. **a).** Picking fruit of RDI treatment using hydraulic ladder in January 1991 (127 dafb). **b).** General view of wide planted trees before fruit harvest.

1 (T_1) respectively (TCSA recorded at July 1990, May 1991 and April 1992 were used in this calculation; see Table 4.2).

5.2.2 STATISTICAL ANALYSIS

The data were analysed as described previously (section 4.2.2). In experimental work to evaluate changes in fruit growth and size the transformation of non-destructive fruit diameter data to fruit volumes is desirable (Mitchell, 1986; Chalmers, pers. comm.; Jerie, pers. comm.). Fruit sampling data from both seasons were used to establish a model for predicting fruit volume and fresh weight from non-destructive fruit diameter measurements. Data were examined for differences between the two seasons and between treatments. Data from the two seasons of sampling and for all sampled treatments did not show any significant differences, thus both sets of data were pooled. The model was fitted according to the method described by Myers (1990). Data was pooled, and randomly split into two sub-samples: a fitting sample (70 percent of data) and a validation sample (30 percent of data). This method (data splitting) allowed for cross validation of the model. Because of the cubic relationship between fruit diameter and fresh weight or volume, data was logarithmically (base e) transformed. The relationships between fruit diameter and both fruit volume and fruit fresh weight were obtained on fitting sample data (Figure 5.2 a. & b.). The goodness of these fits and the regression equations was examined on the validation data (Figure 5.3 a. & b.).

In the comparison of seasonal fruit growth, fruit number at the time of harvest was used as a covariate for fruit growth data (Steel & Torrie, 1981), as treatments had different fruit numbers per tree during the experiment (Tables 5.1, 5.2 a., 5.3 a., 5.7 and 5.8 a.). Data adjusted for fruit number were used for comparison of the treatments. Time was considered as a factor by analysing seasonal fruit growth data as repeated measurement analysis (RM).

5.3 RESULTS: CLOSE PLANTED TREES

5.3.1 Flowering period, fruit set, flower and fruit density

Both L-PBZ and H-PBZ advanced flowering by 2-4 days (Figure 5.1 b.) in the following season relative to control, root-pruned and RDI treatments ($P<0.001$). The first of September was adopted as the time of full bloom for use as the reference date for all treatments during both seasons of study (This date was also valid for the first season, but data are not presented).

The trend was for flower and fruit densities (m^{-1} of shoot) to be higher on trees of the RDI treatment in the second season (Figure 5.1 a), although differences were not significant. L-PBZ and RDI enhanced fruit set in the second season of study relative to control and root-pruned treatments ($P<0.05$). Similarly the total number of fruits in the second season was increased following L-PBZ and RDI (Table 5.1 b.) and differences relative to control and root-pruned trees were significant ($P<0.01$ and $P<0.001$ respectively).

5.3.2 Seasonal fruit growth

There were positive linear relationships ($r^2 = 0.98$) between fruit diameter and both fruit volume and fruit fresh weight (Figure 5.2 a. & b.).

The interaction between time and treatment for all fruit data in both seasons was significant ($P<0.001$). The changes in fruit growth rate based on fruit volume computed from diameter data are shown in Figures 5.5 a. and 5.6 a. These graphs show there were three stages of fruit growth throughout the season: in both seasons stage I ended by 61 dafb, stage II was between 61 and 91 dafb and stage III of fruit growth started from 91 dafb and continued until harvest.

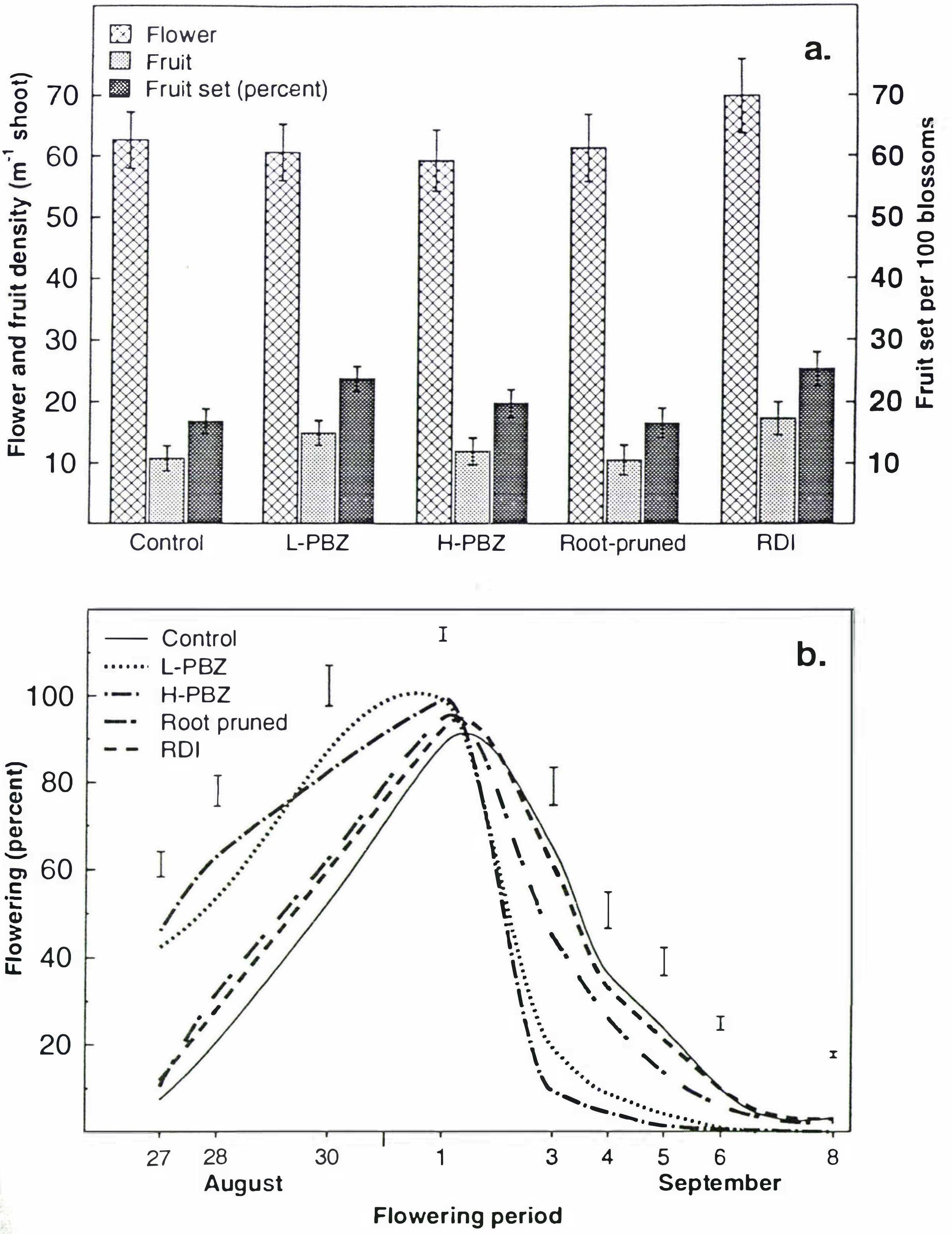


Figure 5.1 Influence of first season treatments on second season flowering and fruiting of close spaced 'Sundrop' apricot. a). Flower and fruit density and percentage fruit set; b). Flowering period in 1991-1992 season. Bars show the standard error of the treatment means.

Table 5.1. The total number of fruit on all treatments of close spaced 'Sundrop' apricot trees recorded 59 days after full bloom.

	a). Fruit number in 1990-1991 season					Significance
	Control	L-PBZ	H-PBZ	Root pruned	RDI	of F values
East^z	115 ± 20^x	130 ± 20	100 ± 20	91 ± 20	118 ± 27	ns
West^y	156 ± 22	155 ± 22	155 ± 22	132 ± 22	146 ± 29	ns
Total	271 ± 29	285 ± 29	255 ± 29	223 ± 29	264 ± 39	ns
	b). Fruit number in 1991-1992 season					
East	126 ± 13	156 ± 13	105 ± 14	117 ± 13	173 ± 17	**
West	130 ± 14	173 ± 14	124 ± 15	113 ± 14	192 ± 18	**
Total	256 ± 19	329 ± 19	229 ± 21	230 ± 19	365 ± 26	***

^{z}East side of tree canopy.
^{y}West side of tree canopy.
^{x} ± Standard error of the mean.
^{ns},*,**,*** Nonsignificant or significant at P = 0.05, 0.01 or 0.001 respectively.

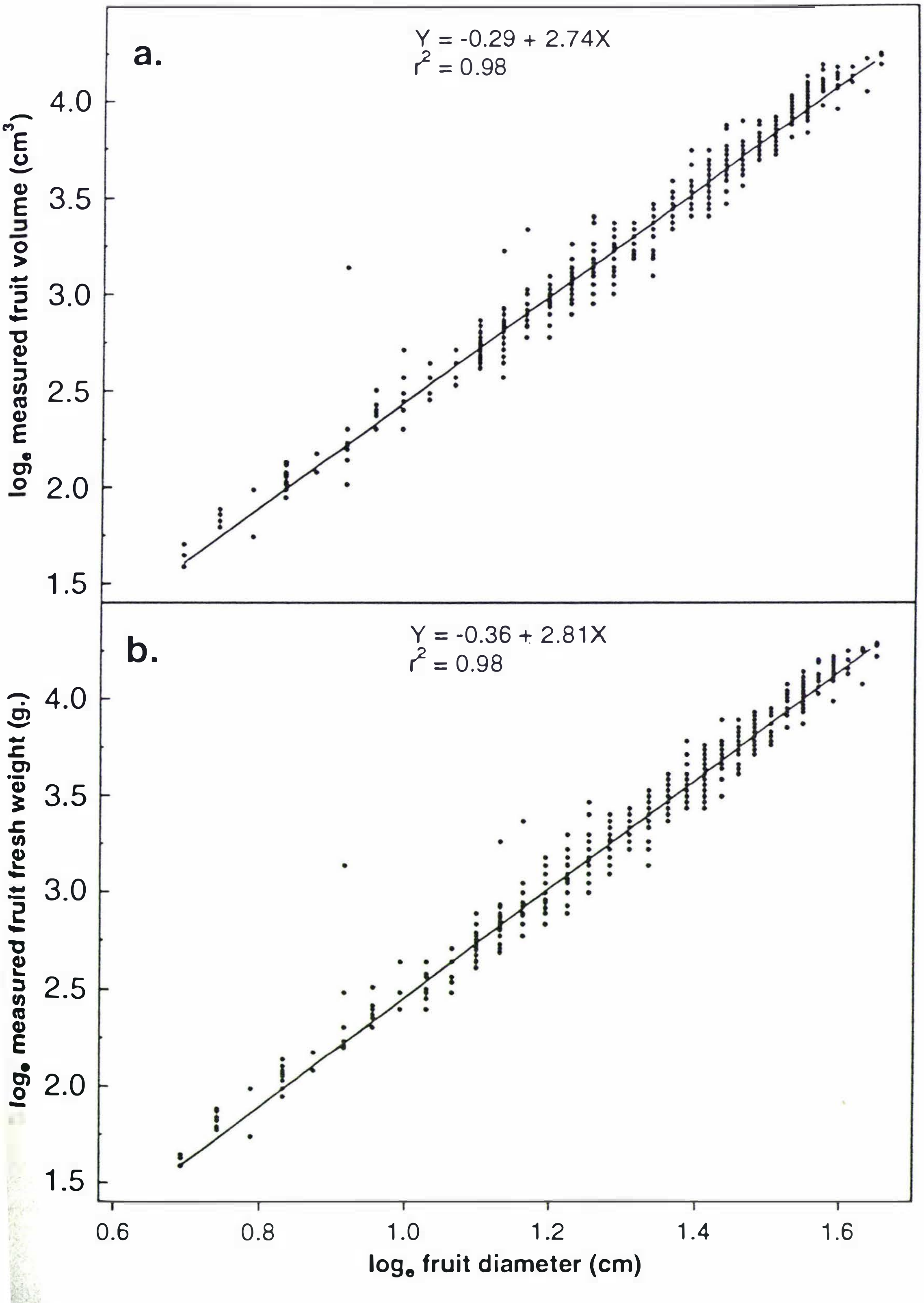
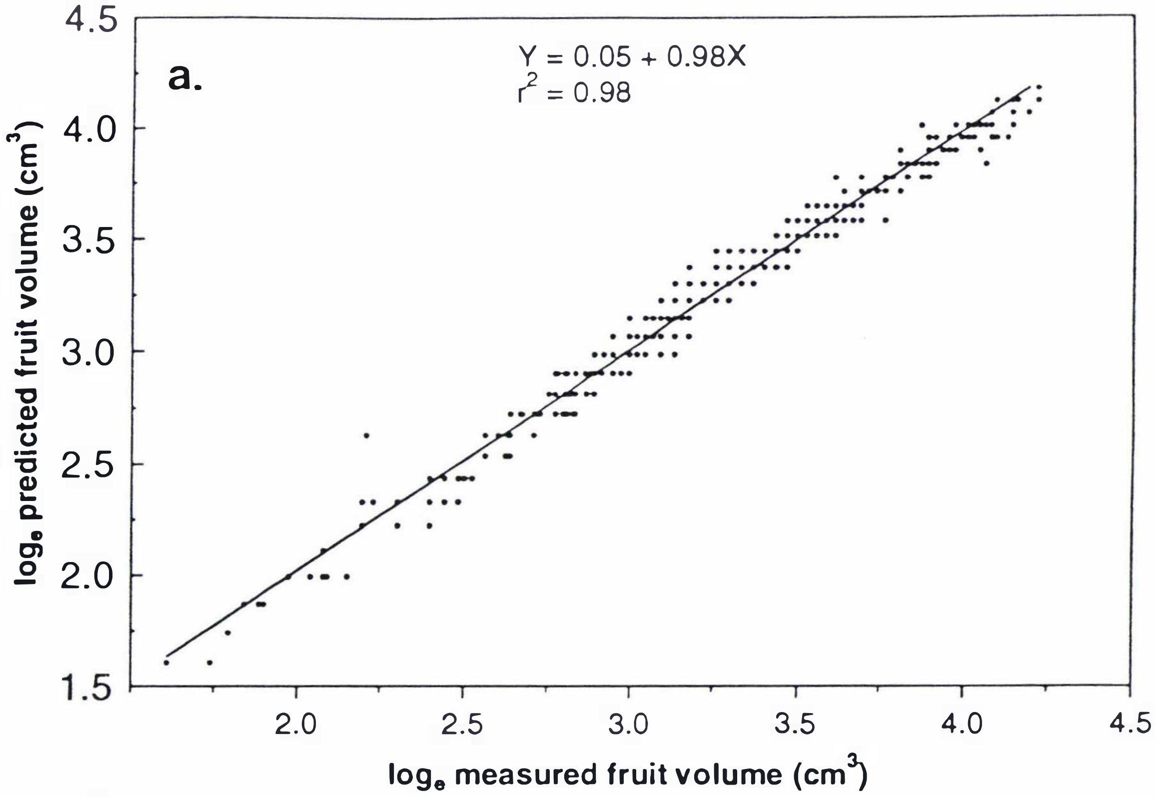


Figure 5.2 Relationships (log$_e$ transformed fitting data) between a). fruit diameter and measured fruit volume, and b). fruit diameter and measured fruit fresh weight on close planted 'Sundrop' apricot trees. Data was pooled from two seasons measurements.



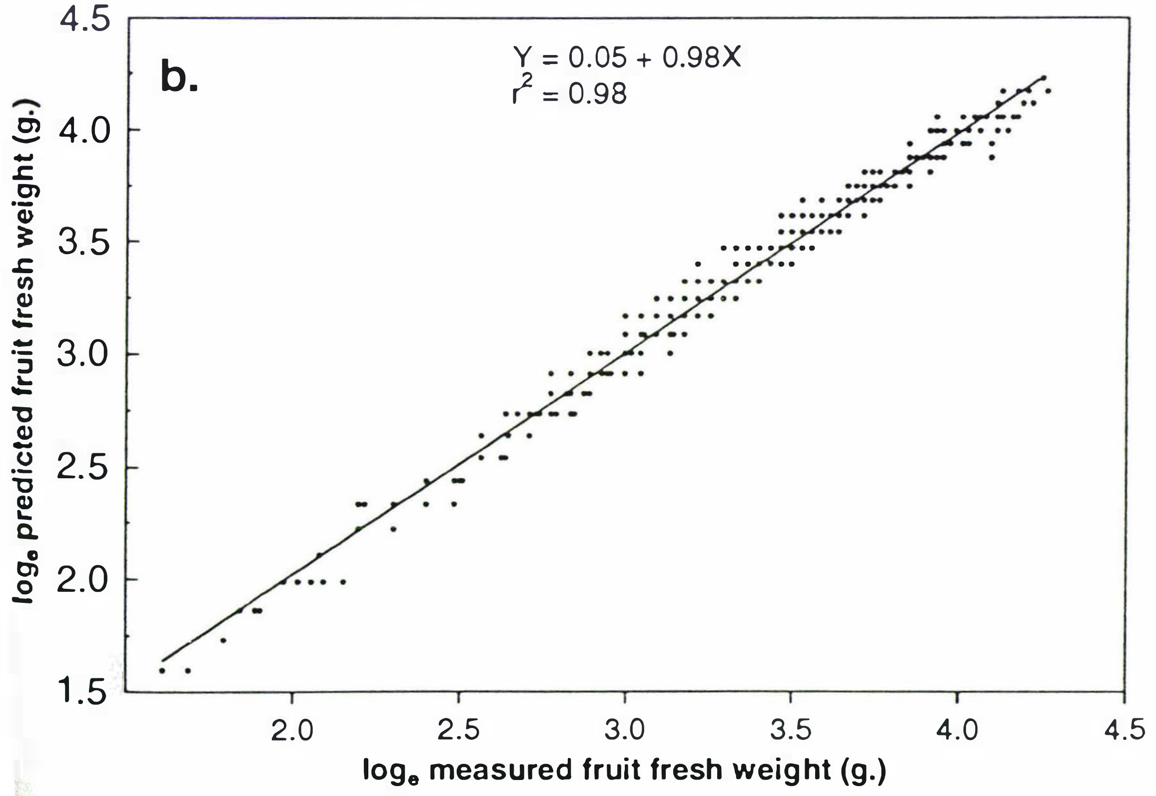


Figure 5.3 Relationships (log$_e$ transformed validation data) between a). measured fruit volume and predicted fruit volume and b). measured fruit fresh weight and predicted fruit fresh weight (g.) on close planted 'Sundrop' apricot trees during the first and second season.

5.3.2.1 Season 1990-1991

The changes in fruit diameter throughout the season are shown in Figure 5.4 a. L-PBZ treatment had larger fruit diameter throughout the season relative to control and root-pruning treatments ($P<0.05$ for stages I and II of fruit growth and $P<0.01$ for early stage III). H-PBZ had greater fruit diameter than control at early stage III of fruit growth ($P<0.05$), however the difference disappeared by the end of fruit harvest. Both PBZ treatments had greater fruit diameter throughout the season relative to the root-pruned treatment ($P<0.001$ for L-PBZ and $P<0.01$ for H-PBZ at 63 dafb, $P<0.01$ for L-PBZ and $P<0.05$ for H-PBZ at 87 dafb and $P<0.001$ for both PBZ treatments at 128 dafb). Root-pruned and control were similar throughout the season until harvest when fruit size was significantly reduced by root-pruning treatment ($P<0.05$). Stage I fruit growth of RDI and root-pruned were similar, but from 63 dafb until the time of harvest RDI treatment had greater fruit diameter ($P<0.05$) and at harvest this treatment produced larger fruit than root-pruning ($P<0.01$). RDI tended to greater fruit diameter throughout the season than control, although the differences were usually not significant.

The rate of fruit growth in terms of fruit volume (cm^3 day^{-1}, Figure 5.5 a.) at the end of stage II and beginning of stage III increased significantly on L-PBZ treated trees relative to the control ($P<0.01$), and H-PBZ treatment showed a greater growth rate prior to fruit harvest ($P<0.001$). However, the rates of RDI (at 128 dafb) and root-pruning (123 and 128 dafb) declined relative to control ($P<0.05$).

L-PBZ increased fruit volume relative to control treatment (Figure 5.5 b.), the difference being significant throughout the season ($P<0.05$ at the end of all 3 stages of fruit growth). L-PBZ showed greater fruit volume relative to the root-pruned treatment throughout the season ($P<0.01$, $P<0.05$ and $P<0.001$ for 53, 101 dafb and the other days respectively). H-PBZ also increased fruit size relative to root-pruned throughout the season ($P<0.001$ for 115-128 dafb, $P<0.01$ for 44, 63 and 79-94 dafb and $P<0.05$ for the other dates). This treatment also showed greater fruit volume than control during stage III of fruit growth ($P<0.05$), although difference at harvest was not significant. RDI increased fruit volume relative to control ($P<0.05$) on 94, 101 and 109 dafb. Figure 5.7 a. & b. show the effect of H-PBZ on fruit size in the upper and lower of the three canopy zones. H-PBZ produced larger fruit than control in both these zones. The treatment differences in the lower canopy zone were significant at 125 dafb ($P<0.05$) and in the top canopy zone during stages II and III of fruit growth ($P<0.05$ for 72 and 105 dafb and $P<0.01$ for 94, 115 and 125 dafb).

The fresh and dry weight proportions of stone and mesocarp of tagged fruit from the middle canopy zone are shown in Figure 5.8 a., b. & c. On both fresh and dry weight bases, mesocarp constituted the larger part of the fruit. There were no significant differences between treatments in proportional composition of the fruit. Figure 5.8 c. shows that both PBZ treatments produced larger amounts of fruit dry matter (mesocarp and stone) relative to control and root-pruned treatment ($P<0.05$ for L-PBZ and $P<0.01$ for H-PBZ), which almost entirely were due to differences in mesocarp dry weight. Stone dry weight among all the treatments was similar, although both PBZ treatments resulted in significantly larger stone dry weights than the root-pruned treatment ($P<0.01$).

5.3.2.2 Season 1991-1992

Both PBZ treatments increased fruit diameter (Figure 5.4 b.). The differences between L-PBZ and control, root-pruned and RDI were significant throughout the season ($P<0.001$ at 60, 93 and 128 dafb). H-PBZ had greater fruit diameter relative to control and root-pruned throughout the season (both $P<0.001$ at 60 dafb, $P<0.01$ relative to control and $P<0.001$ to root-pruned at

93 dafb, and $P=0.07$ relative to control and $P<0.001$ to root-pruned at 128 dafb). Also, H-PBZ had greater fruit diameter relative to RDI until 67 dafb ($P<0.01$), although this difference disappeared during the third phase of fruit growth. Control, RDI and root-pruned treatments had similar fruit diameters during the second season of the experiment.

Final dry weights of tagged fruits in the second season are shown in Figure 5.8 d. Control, L-PBZ, H-PBZ and root-pruned treatments differences were not significant, however RDI reduced final fruit dry matter relative to control, L-PBZ and H-PBZ ($P<0.05$), but was not significantly different from the root-pruned treatment.

At the end of stage II (88 dafb) fruit volume increase (cm^3 day^{-1}) on the RDI treatment was lower than on H-PBZ and root-pruned ($P<0.05$, Figure 5.6 a.). However the growth rate of RDI dramatically increased at the beginning of stage III (93 dafb) relative to these two treatments ($P<0.05$ relative to control and $P<0.01$ to root-pruned). All treatments had similar fruit growth rates at the end of stage III of fruit growth.

Figure 5.6 b. shows the cumulative increase in fruit volume throughout the season for all treatments. Control, root-pruned and RDI had similar fruit volumes during the season. L-PBZ treatment significantly increased fruit volume relative to control, root-pruned and RDI throughout the season ($P<0.001$ at 60, 93 and 123 dafb). Differences occurred between H-PBZ and RDI during stage I, early and late stage II (67 and 88 dafb) and before harvest in stage III (110 dafb) of fruit growth ($P<0.05$). H-PBZ also showed increased fruit volume during the season relative to control and root-pruned ($P<0.001$ at 60 and 93 dafb and $P<0.05$ against control on 123 dafb and $P<0.001$ against root-pruned on 123 dafb).

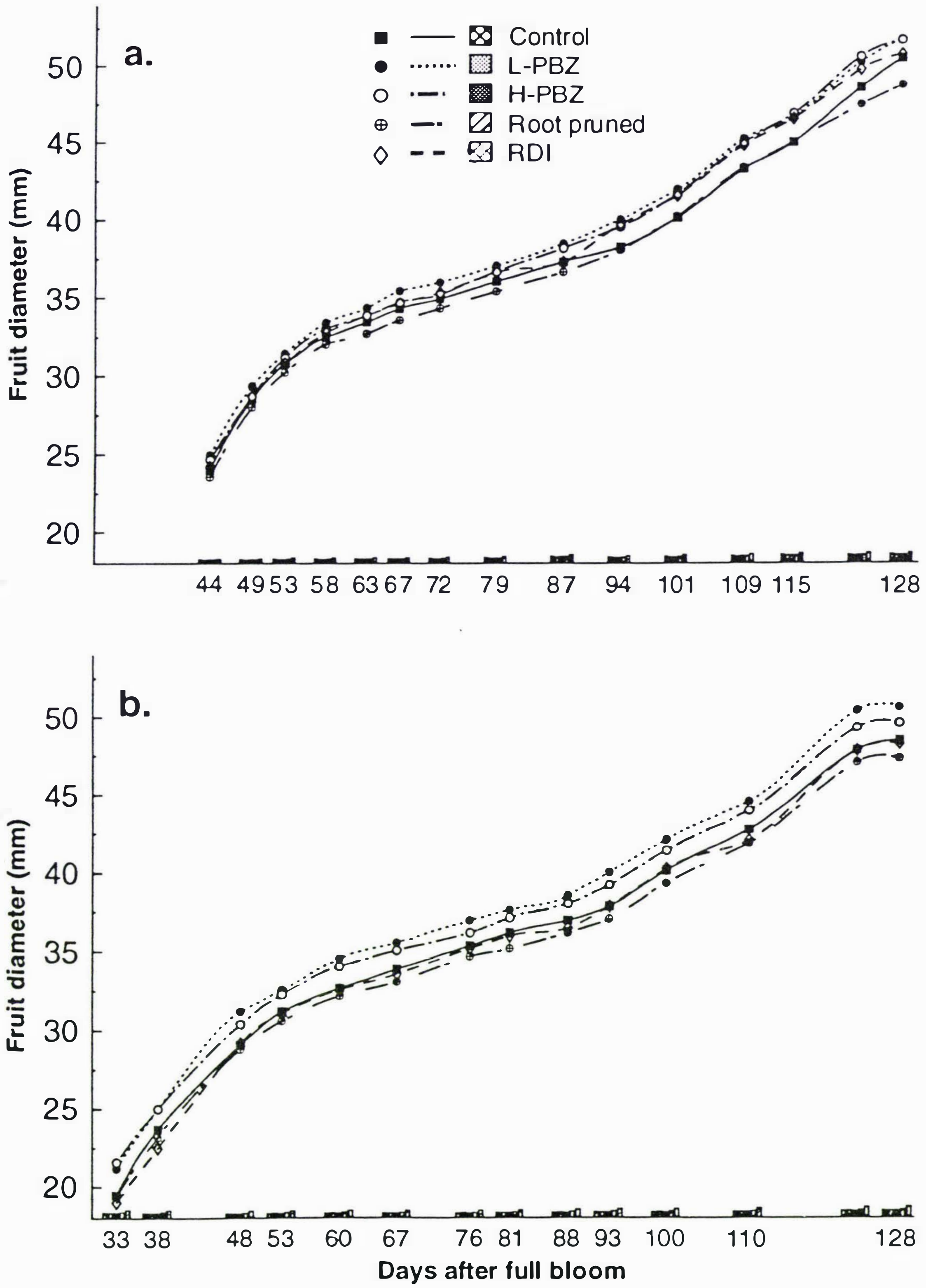


Figure 5.4 Cumulative increase in fruit diameter on close spaced 'Sundrop' apricot throughout the growing season. a). 1990-1991, and b). 1991-1992 season. Bars show the standard error of means. Fruit number at the time of harvest was used as a covariate for data.

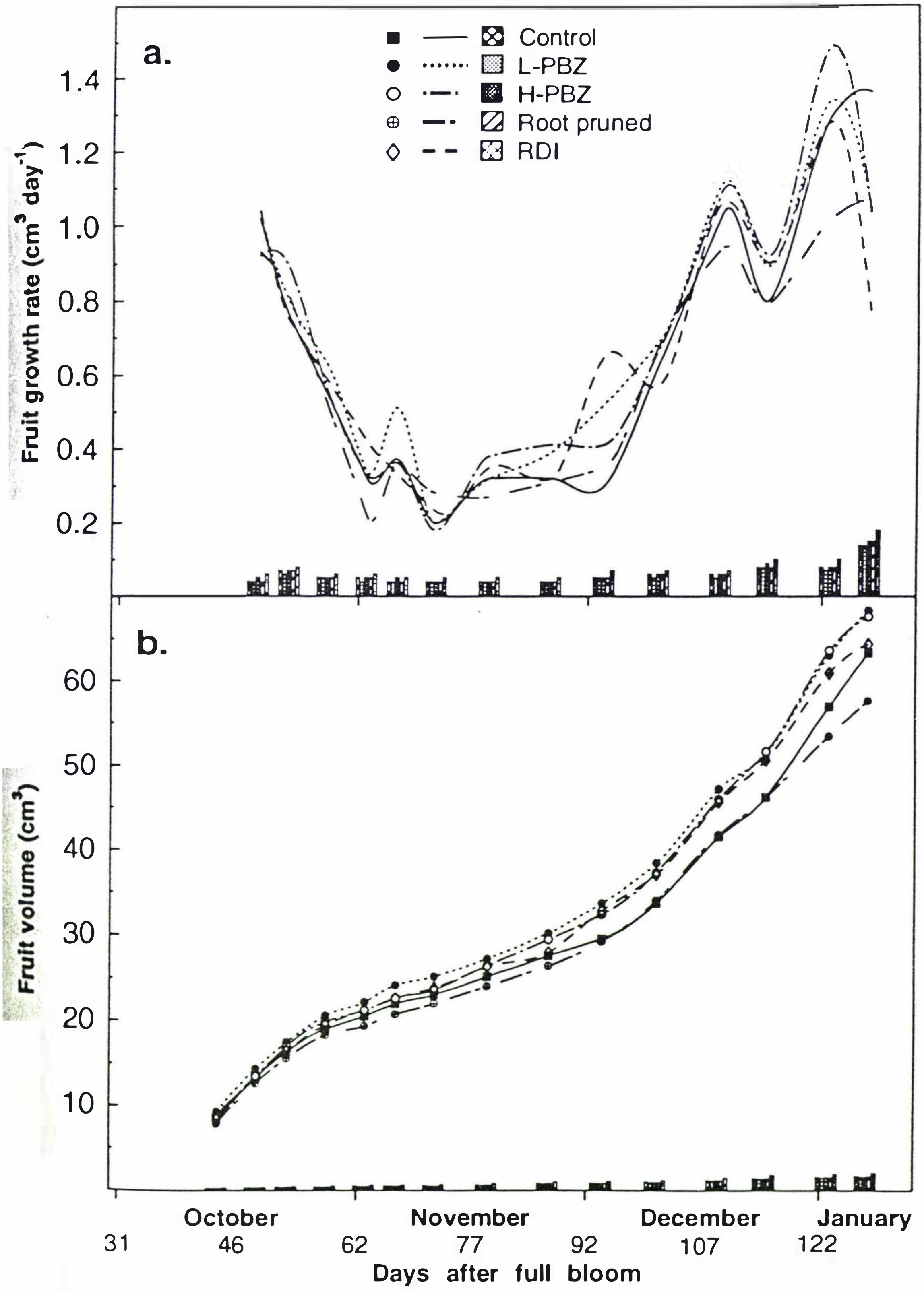


Figure 5.5 Influence of treatments on fruit volume (cm^3) of close planted 'Sundrop' apricot throughout the 1990-1991 Season. a). Daily rate of increase of fruit volume (cm^3 day^{-1}) and b). Fruit volume (cm^3). Bars show the standard error of the treatment means. Fruit number at the time of harvest was used as a covariate for data.

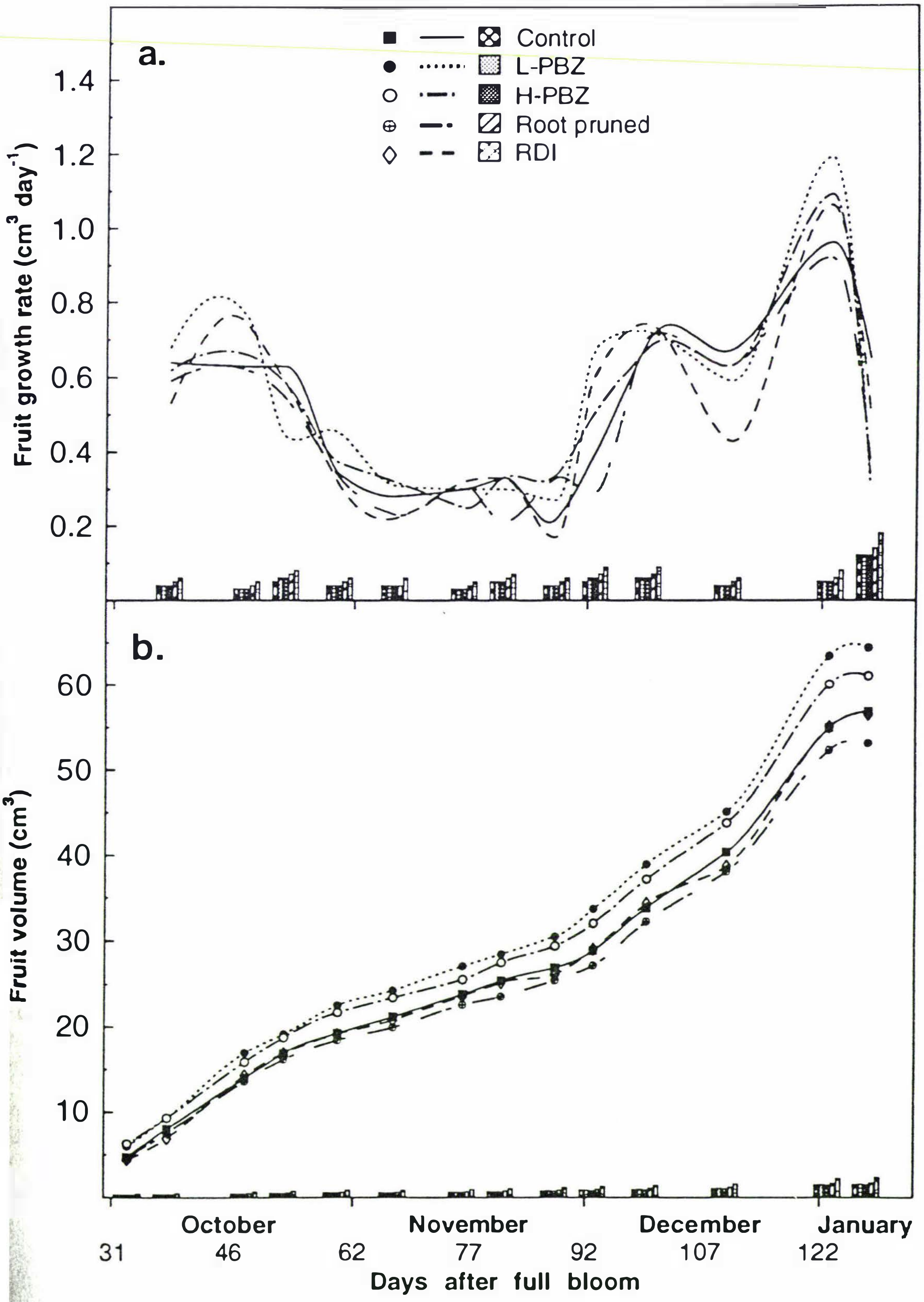


Figure 5.6 Influence of treatments on fruit volume (cm^3) of close planted 'Sundrop' apricot throughout the 1991-1992 Season. a). Daily rate of increase of fruit volume (cm^3 day^{-1}) and b). Fruit volume (cm^3). Bars show the standard error of the treatment means. Fruit number at the time of harvest was used as a covariate for data.

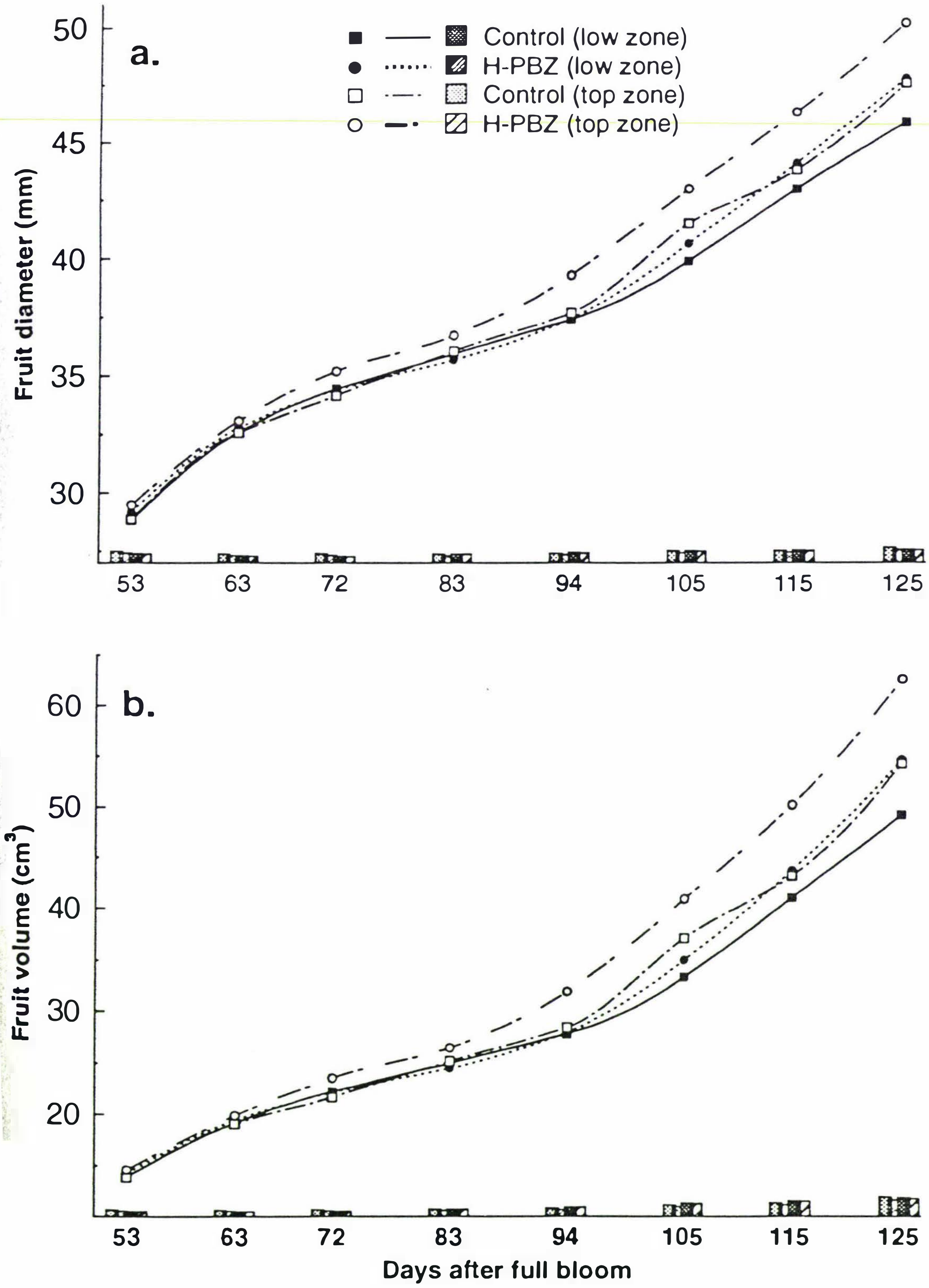


Figure 5.7 Seasonal changes in fruit size of close planted 'Sundrop' apricot on the upper and lower canopy zone during 1990-1991 season. Bars show the standard error of means. a). Fruit diameter (mm); b). Computed fruit volume (cm^3). Fruit number at the time of harvest was used as a covariate for data.

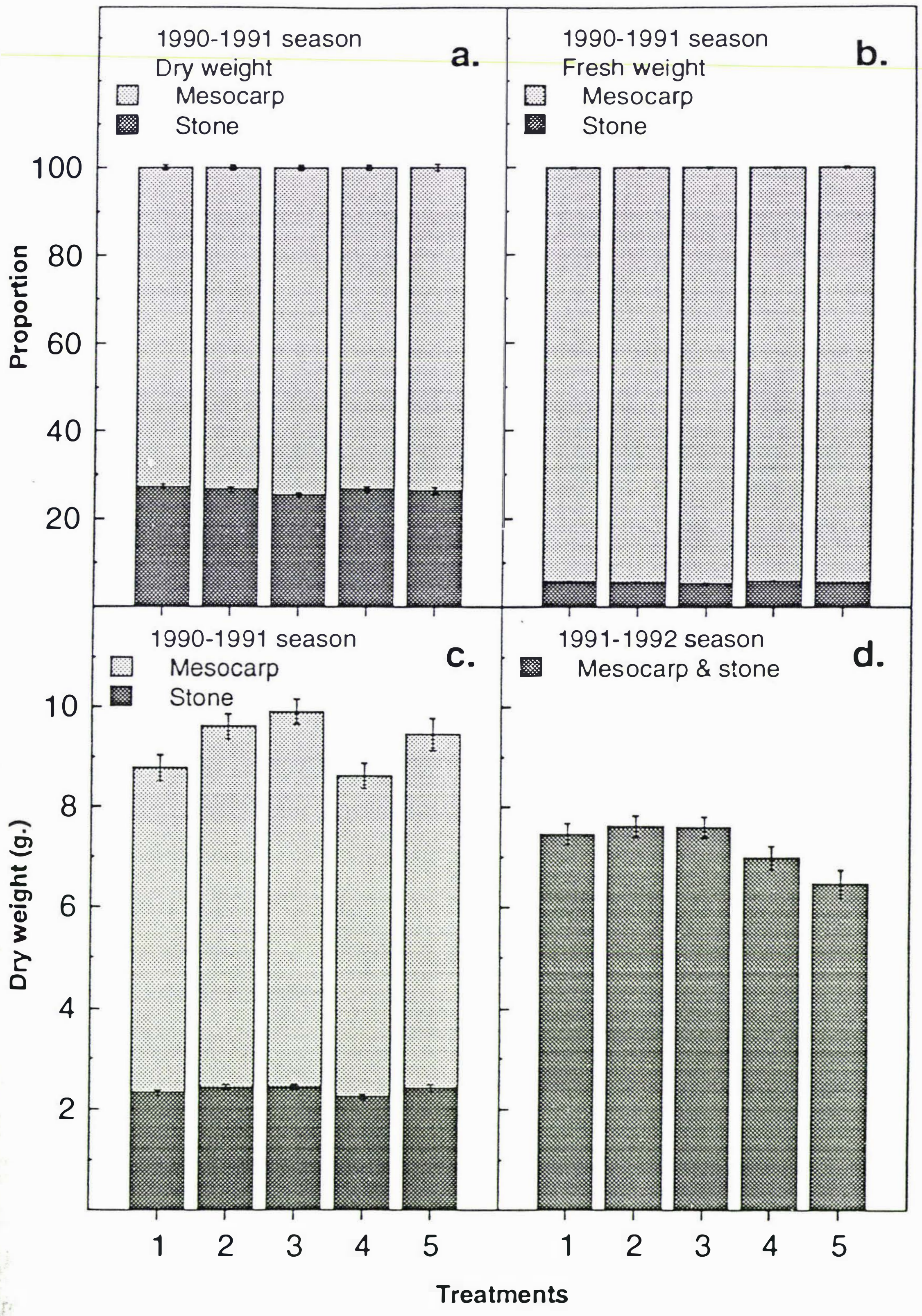


Figure 5.8 The influence of treatments on close planted 'Sundrop' apricot fruit (tagged fruit from middle canopy zone) recorded at the time of fruit harvest. a). Proportion of dry matter; b). Proportion of fresh weight; c). Mesocarp and seed dry matter, and d). Fruit dry matter. 1=Control; 2=L-PBZ; 3=H-PBZ; 4=Root pruned and 5=RDI. Vertical lines represent the standard error of mean.

5.3.3 Yield and fruit quality

5.3.3.1 Season 1990-1991

The number and weight of fruits harvested from the different canopy zones are shown in Table 5.2 a. & b. More fruits were carried in the middle canopy zone (Table 5.2 a.), the differences between treatments for any canopy zone were not significant. All treatments statistically had similar yield on a total canopy basis (Table 5.2 b. & Figure 5.9 b.). Whilst there were trends for PBZ treatments to produce bigger fruit and root-pruning to reduce fruit size and yield, these differences were not significant (Table 5.2 c. & Figure 5.9 d.).

Figure 5.9 b. shows the yields at each harvest date. At the first pick (127 dafb) H-PBZ ($P=0.052$), root-pruned ($P=0.07$) and RDI ($P=0.09$) tended to yield more than control. At the second pick (131 dafb) RDI produced more harvested yield than control ($P<0.05$). At the third picking date (142 dafb) L-PBZ produced more than root-pruned ($P<0.05$). At the final picks (146 and 149 dafb) root-pruned yielded less than control ($P<0.05$). No other differences were significant. Crop density of L-PBZ (Figure 5.9 c.) was higher than control ($P<0.05$), the differences among the other treatments were not significant. Similarly (Figure 5.9 a.) L-PBZ yield efficiency was significantly greater than both root-pruned and control trees ($P<0.05$).

Table 5.4 a. shows that Hue angle of sampled fruits at the first picking date (127 dafb) was higher (greener) for the RDI treatment relative to control ($P<0.05$) and to H-PBZ ($P<0.01$). The lightness value of H-PBZ (Table 5.5 a.) at this date (127 dafb) was higher (lighter in colour) than control and RDI ($P<0.05$). The hue angle of control at the third pick was higher than the other treatments, with differences relative to H-PBZ and root-pruned treatments being significant ($P<0.05$).

In this first season H-PBZ fruit harvested (Table 5.6 a.) at the second picking date (131 dafb), showed the highest TSS, this being significant relative to control (P<0.01), L-PBZ (P<0.001) and RDI (P<0.05). At the third picking date (142 dafb) control had the lowest TSS, significant against all other treatments (P<0.05, 0.001, 0.001 and 0.01 relative to L-PBZ, H-PBZ, root-pruned and RDI treatments respectively). The TSS at the last picking date (149 dafb) was significantly higher (P<0.05) for root-pruned than for control fruit.

5.3.3.2 Season 1991-1992

In the second season, RDI and L-PBZ produced more fruits per tree at harvest (Table 5.3 a.) than control and H-PBZ (P<0.05) and root-pruned (P<0.01 for RDI and P<0.05 for L-PBZ). Table 5.3 b. shows that RDI and L-PBZ gave significantly higher yield per tree than control (P<0.01 for L-PBZ and P<0.05 for RDI), and root-pruned (P<0.05).

Fruit size at harvest was improved by both PBZ treatments, the difference between H-PBZ and root-pruned treatments being significant (P<0.05), but no other differences were significant (Table 5.3 c. & Figure 5.10 d.).
Figure 5.10 c. shows that L-PBZ and RDI showed higher crop density than control (P<0.001 for L-PBZ and P<0.01 for RDI), H-PBZ (P<0.01 for L-PBZ and P<0.05 for RDI) and root-pruned treatments (P<0.001 for L-PBZ and P<0.05 for RDI). Similarly, the yield efficiencies of L-PBZ and RDI were higher (Figure 5.10 a.) than control (P<0.001 for L-PBZ and P<0.01 for RDI), while L-PBZ was also more yield efficient than H-PBZ (P<0.01) and root-pruned (P<0.001).

The breakdown of yield over the four picking dates of the second season is shown in Figure 5.10 b. There was a trend toward increase in harvested yield at the first pick (130 dafb) for L-PBZ relative to control (P=0.08). The differences among the other treatments were not significant at this date. At the second harvest (136 dafb) all treatments tended to yield more than control, although only L-PBZ was significantly greater than control ($P<0.05$), and RDI trended ($P=0.07$) similarly. At the third harvest (140 dafb) only L-PBZ showed a positive trend towards a difference relative to the root-pruned treatment ($P=0.055$).

Hue angle (Table 5.4 b.) and lightness (Table 5.5 b.) at the first 3 picking dates were similar for all treatments. However, at the final harvest hue and L value of L-PBZ fruit were significantly lower than those of root-pruned ($P<0.05$), and the RDI lightness value was higher than L-PBZ ($P<0.05$).

Treatments had a significant effect on fruit TSS only at the first harvest (Table 5.6 b.). The H-PBZ mean of 10.6 °Brix was higher than root-pruned, RDI ($P<0.05$) and L-PBZ ($P<0.001$). Total soluble solids recorded for RDI at 140 dafb (12.29 °Brix) was the highest TSS value in the second season, although the differences among treatments at this time were not significant.

5.3.4 Partitioning index

Figure 5.11 shows the P-index and TCSA growth rate (cm^2 $year^{-1}$) in both seasons of close spaced trees. TCSA growth rate (Figure 5.11 b. & d.) of the PBZ treatments was greatly reduced in both seasons relative to control ($P<0.01$ and $P<0.05$ for L-PBZ and H-PBZ respectively in the first season and $P<0.001$ for both PBZ treatments in the second season). Root-pruned treatment's TCSA increase in the second season was lower than control ($P<0.01$). In general , as TCSA growth rate decreased P-index increased, although the reduction in TCSA growth of root-pruned trees in the second season did not significantly affect P-index compared with control. Both PBZ treatments showed increased P-indices relative to control in both season of study ($P<0.05$), whilst in either season were root-pruned or RDI P-indices significantly different from control (Figure 5.11 a. & c.).

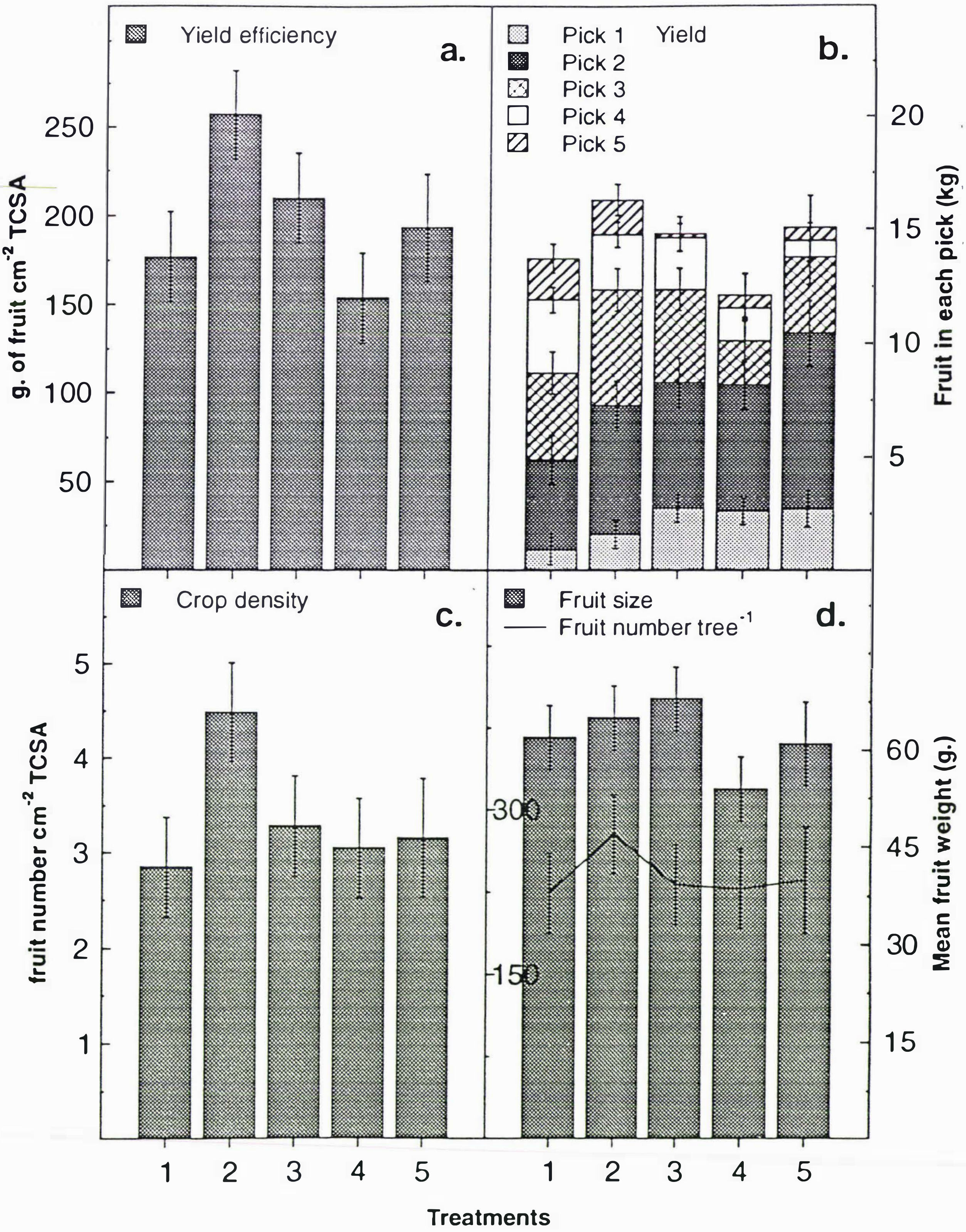


Figure 5.9 The influence of treatments on yield of close planted 'Sundrop' apricot in 1990-1991 season. a). Yield efficiency; b). Early harvest and fruit maturity; c). Crop density and d). Mean fruit weight and mean total number of harvested fruits. 1 = Control; 2 = L-PBZ; 3 = H-PBZ; 4 = Root pruned and 5 = RDI. Vertical lines represent standard error of the mean.

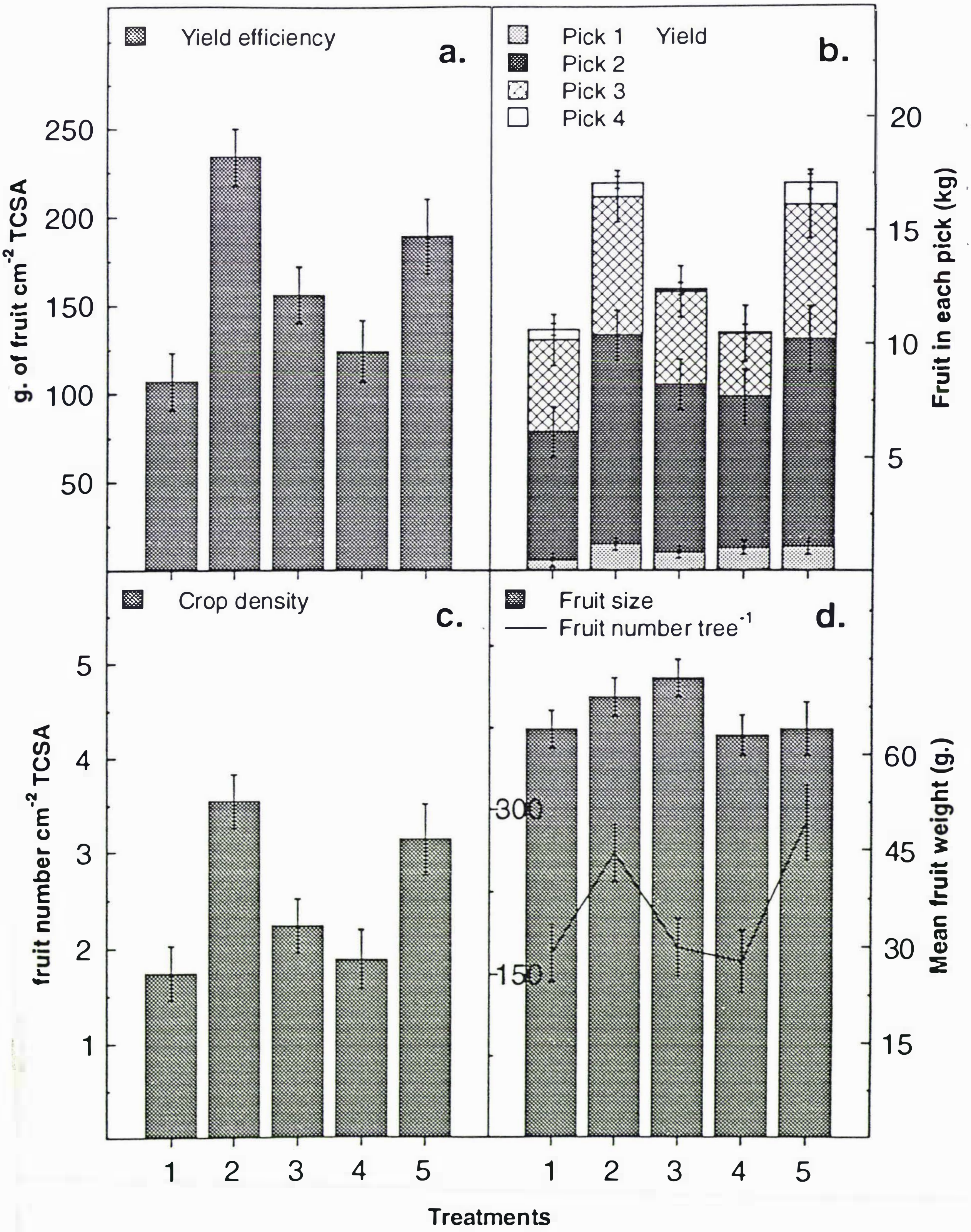


Figure 5.10 The influence of treatments on yield of close planted 'Sundrop' apricot in 1991-1992 season. a). Yield efficiency; b). Early harvest and fruit maturity; c). Crop density and d). Mean fruit weight and mean total number of harvested fruits. 1 = Control; 2 = L-PBZ; 3 = H-PBZ; 4 = Root pruned and 5 = RDI. Vertical lines represent standard error of the mean.

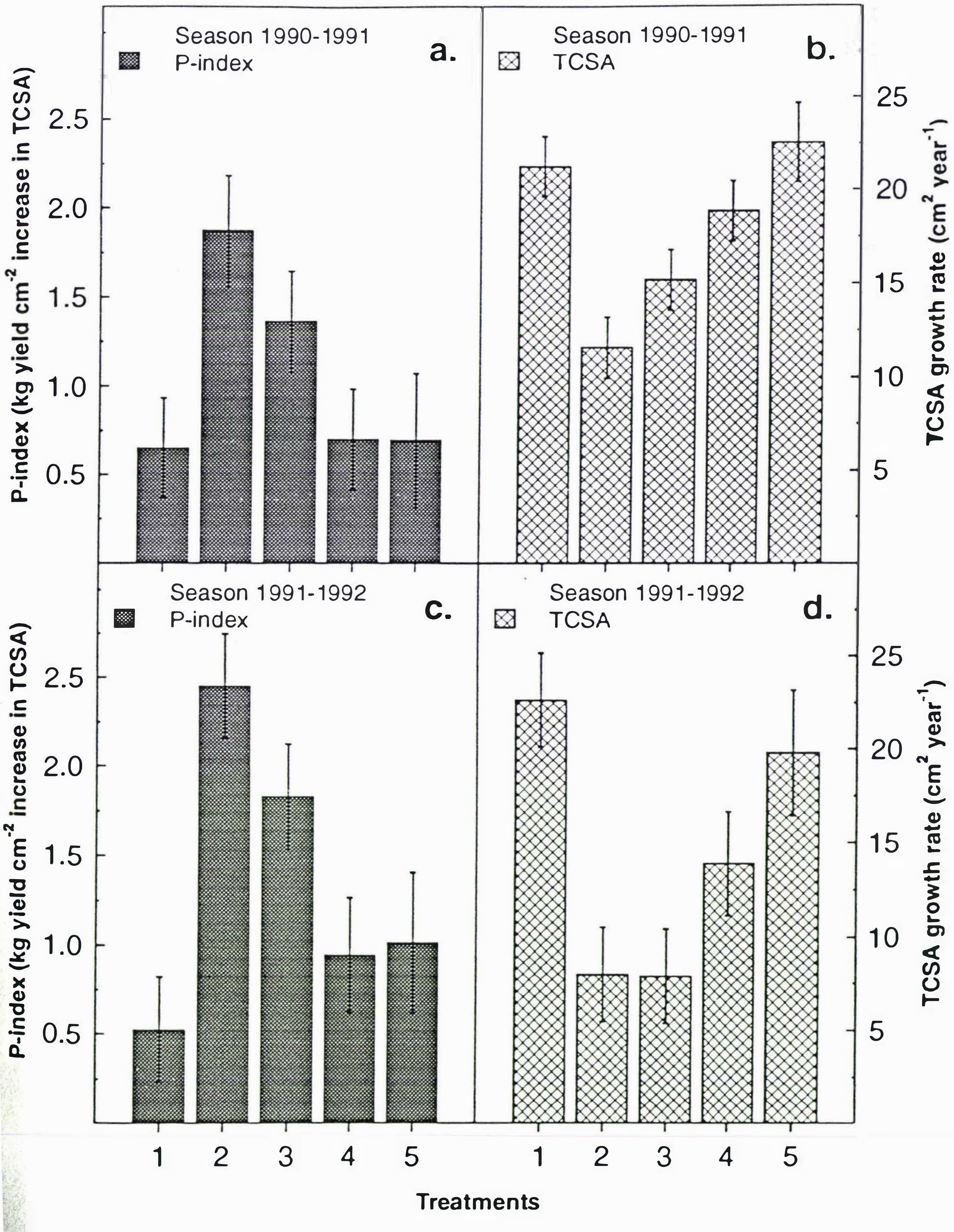


Figure 5.11 The effect of treatments on close spaced 'Sundrop' apricot trees. a). & c). Partitioning index (P-index); b). and d). Annual increase in trunk cross sectional area recorded in winter (TCSA). 1 = Control; 2 = L-PBZ; 3 = H-PBZ; 4 = Root pruned and 5 = RDI. Each column represents the mean of 8 trees (5 in RDI). Vertical lines represent standard error of the mean.

Table 5.2. Fruit number, yield and adjusted fruit size of harvested fruit by treatment and canopy position of close spaced 'Sundrop' apricot trees in January 1991.

Canopy zone[z]	a). Fruit number					Significance of F values
	Control	L-PBZ	H-PBZ	Root pruned	RDI	
Zone 1	12 ± 7[y]	29 ± 7	16 ± 7	13 ± 7	8 ± 8	ns
Zone 2	124 ± 25	198 ± 25	147 ± 25	142 ± 25	132 ± 34	ns
Zone 3	87 ± 16	51 ± 16	69 ± 16	73 ± 16	96 ± 21	ns
Tree canopy	223 ± 37	278 ± 37	232 ± 37	228 ± 37	236 ± 48	ns
	b). Yield fresh weight (kg)					
Zone 1	0.75 ± 0.37	1.70 ± 0.37	1.51 ± 0.45	0.64 ± 0.37	0.71 ± 0.56	ns
Zone 2	7.52 ± 1.24	10.9 ± 1.24	8.48 ± 1.24	6.50 ± 1.24	9.57 ± 1.64	*
Zone 3	5.42 ± 0.98	3.72 ± 0.98	5.25 ± 0.98	4.51 ± 0.98	4.98 ± 1.30	ns
Tree canopy	13.7 ± 2.03	16.3 ± 2.03	15.2 ± 2.03	11.6 ± 2.03	15.3 ± 2.69	ns
	c). Average fruit size (g.)					
Tree canopy	62 ± 4.93	65 ± 5.01	68 ± 4.92	54 ± 4.92	61 ± 6.50	ns

[z] Lower, middle and upper regions of tree canopy for zone 1, zone 2 and zone 3 respectively.
[y] ± Standard error of the mean.
ns,*,**,*** Nonsignificant or significant at P = 0.05, 0.01 or 0.001 respectively.

Table 5.3. Fruit number, yield and adjusted fruit size of harvested fruit by treatment and canopy position of close spaced 'Sundrop' apricot trees in January 1992.

Canopy zone[z]	a). Fruit number					Significance of F values
	Control	L-PBZ	H-PBZ	Root pruned	RDI	
Zone 1	12 ± 5[y]	39 ± 5	25 ± 5	19 ± 5	26 ± 6	**
Zone 2	89 ± 17	183 ± 17	104 ± 17	100 ± 19	212 ± 23	***
Zone 3	68 ± 11	38 ± 11	46 ± 11	43 ± 12	50 ± 14	ns
Tree canopy	169 ± 26	260 ± 26	175 ± 26	162 ± 28	288 ± 34	**
	b). Yield fresh weight (kg)					
Zone 1	0.71 ± 0.30	2.21 ± 0.30	1.23 ± 0.30	1.10 ± 0.33	1.51 ± 0.40	**
Zone 2	6.38 ± 1.17	12.2 ± 1.17	7.62 ± 1.17	6.54 ± 1.28	12.2 ± 1.55	**
Zone 3	3.53 ± 0.57	2.74 ± 0.57	3.60 ± 0.57	2.88 ± 0.62	3.49 ± 0.76	ns
Tree canopy	10.6 ± 1.62	17.1 ± 1.62	12.4 ± 1.62	10.5 ± 1.77	17.1 ± 2.15	**
	c). Average fruit size (gram)					
Tree canopy	64 ± 2.95	69 ± 3.05	72 ± 2.95	63 ± 3.25	64 ± 4.16	*

[z] Lower, middle and upper regions of tree canopy for zone 1, zone 2 and zone 3 respectively.
[y] ± Standard error of the mean.
ns,*,**,*** Nonsignificant or significant at P = 0.05, 0.01 or 0.001 respectively.

Table 5.4. The influence of treatments on mesocarp colour (Hue angle[z]) of close planted 'Sundrop' apricot fruit recorded at different dates during January 1991 and 1992.

dafb[y]	a). Hue angle (degrees) 1990-1991 season					Significance of F values
	Control	L-PBZ	H-PBZ	Root pruned	RDI	
127	88.42 ± 4.64[x]	94.41 ± 4.06	83.38 ± 3.68	90.77 ± 3.68	101.84 ± 4.93	**
131	71.29 ± 1.89	72.74 ± 2.06	67.77 ± 1.89	71.14 ± 1.86	66.48 ± 2.80	ns
142	74.15 ± 1.64	71.35 ± 1.50	68.62 ± 1.64	70.33 ± 1.64	73.04 ± 2.00	*
146	72.35 ± 1.51	75.39 ± 1.64	74.01 ± 2.03	71.25 ± 1.90	72.61 ± 2.03	ns
149	76.78 ± 1.54	74.54 ± 1.77	73.58 ± 1.95	73.81 ± 2.54	75.49 ± 3.54	ns
	b). Hue angle (degrees) 1991-1992 season					
130	74.04 ± 1.85	70.67 ± 1.54	71.25 ± 1.96	69.84 ± 1.53	70.99 ± 2.29	ns
136	70.38 ± 1.21	70.43 ± 1.14	70.94 ± 1.14	70.17 ± 1.23	72.21 ± 1.49	ns
140	72.33 ± 0.92	71.21 ± 0.92	71.37 ± 0.92	72.44 ± 1.00	70.96 ± 1.22	ns
147	72.11 ± 1.02	69.87 ± 1.23	71.18 ± 1.26	75.15 ± 1.78	71.45 ± 1.38	*

[z] Higher hue angles indicate greener colour.

[y] Fruits harvested at 5 and 4 different picking times in 1991 and 1992 season respectively.

[x] ± Standard error of the mean.

[ns,*,**,***] Nonsignificant or significant at P = 0.05, 0.01 or 0.001 respectively.

Table 5.5. The lightness^z (%) of fruit colour of close planted 'Sundrop' apricot recorded at each harvest date during January 1991 and 1992.

dafb^y	a). Lightness (%) 1990-1991 season					Significance of F values
	Control	L-PBZ	H-PBZ	Root pruned	RDI	
127	52.53 ± 1.31^x	53.89 ± 0.99	55.91 ± 0.90	54.01 ± 0.90	52.80 ± 1.20	ns
131	56.67 ± 1.02	56.08 ± 1.11	55.51 ± 1.02	56.57 ± 1.00	54.84 ± 1.51	*
142	58.18 ± 1.28	56.03 ± 1.17	55.61 ± 1.28	55.70 ± 1.28	60.04 ± 1.57	*
146	58.43 ± 0.89	59.99 ± 0.97	60.20 ± 1.20	60.77 ± 1.12	57.57 ± 1.19	ns
149	59.10 ± 0.82	58.91 ± 0.95	58.79 ± 1.04	59.86 ± 1.36	59.10 ± 1.89	ns
	b). Lightness (%) 1991-1992 season					
130	55.53 ± 1.03	55.18 ± 0.86	56.26 ± 1.09	53.81 ± 0.85	53.40 ± 1.27	ns
136	56.90 ± 1.25	56.03 ± 1.18	53.96 ± 1.18	55.13 ± 1.27	57.52 ± 1.54	ns
140	57.18 ± 0.78	58.45 ± 0.78	57.19 ± 0.78	57.85 ± 0.85	56.39 ± 1.03	ns
147	56.69 ± 0.67	55.13 ± 0.81	56.60 ± 0.83	58.32 ± 1.17	57.96 ± 0.90	*

^{z}A higher value indicating a lighter colour and lower value indicating a darker colour.
^{y}Fruits harvested at 5 and 4 different dates in 1991 and 1992 season respectively.
^{x} ± Standard error of the mean.
^{ns,*,**,***} Nonsignificant or significant at P = 0.05, 0.01 or 0.001 respectively.

Table 5.6. 'Sundrop' apricot fruit total soluble solids at harvest on the close planted experiment as influenced by different vigour control treatments recorded during January 1991 and 1992.

dafb[z]	Control	L-PBZ	H-PBZ	Root pruned	RDI	Significance of F value
	a). TSS (°Brix) for 1990-1991 season					
127	10.36 ± 0.68[y]	11.49 ± 0.60	11.43 ± 0.54	12.08 ± 0.56	11.86 ± 0.72	ns
131	11.74 ± 0.38	11.41 ± 0.38	13.31 ± 0.39	12.46 ± 0.37	11.77 ± 0.56	**
142	10.02 ± 0.38	11.18 ± 0.35	12.92 ± 0.38	12.26 ± 0.38	11.91 ± 0.47	***
146	11.33 ± 0.45	10.49 ± 0.48	12.75 ± 0.59	12.44 ± 0.58	10.34 ± 0.61	**
149	9.16 ± 0.46	9.74 ± 0.55	10.25 ± 0.62	11.04 ± 0.75	10.82 ± 1.14	*
	b). TSS (°Brix) for 1991-1992 season					
130	9.13 ± 0.60	7.61 ± 0.50	10.59 ± 0.64	8.91 ± 0.49	7.95 ± 0.74	**
136	10.34 ± 0.57	10.77 ± 0.57	10.50 ± 0.57	11.14 ± 0.63	10.84 ± 0.76	ns
140	12.61 ± 0.69	10.85 ± 0.69	11.99 ± 0.69	10.91 ± 0.75	12.29 ± 0.92	ns
147	11.16 ± 0.71	9.31 ± 0.97	11.26 ± 0.88	8.08 ± 1.82	9.61 ± 0.97	ns

[z] Fruits harvested at 5 and 4 different dates in 1991 and 1992 season respectively.

[y] ± Standard error of the mean.

ns, *, **, *** Nonsignificant or significant at P = 0.05, 0.01 or 0.001 respectively.

5.4 RESULTS: WIDE PLANTED TREES

5.4.1 Fruit growth

In the first season there were significantly ($P<0.05$) more fruit on the L-PBZ trees (Table 5.7 a.) than on the RDI. In the second season the number of fruit on all treatments was greater, with higher numbers of fruit on the PBZ treatments, but the only significant difference ($P<0.05$) was between L-PBZ and RDI.

The changes in fruit size, recorded only during the first season (1990-1991), in both fruit diameter and volume are shown in Figure 5.12 a. & b. The interaction between time and treatments for both fruit diameter and fruit volume were significant ($P<0.001$). Final fruit size was higher on both PBZ treatments, but never becomes significantly greater that control. RDI significantly reduced fruit volume and diameter in late stage III compared with H-PBZ ($P<0.01$ at 109, 115 dafb and $P<0.05$ at 123 dafb). RDI fruit volume in stage I was lower than H-PBZ ($P<0.05$ at 53 dafb), and both its fruit diameter and volume during later stage I and early stage II (49-67 dafb) were significantly smaller than control ($P<0.05$). At early stage I (44 dafb) the root-pruned treatment had higher fruit diameter and volume than RDI ($P<0.05$), however later RDI fruit were larger than root-pruned fruit ($P<0.05$ at 63-72 dafb and 94-101 dafb). Both PBZ treatments showed increase in fruit diameter and volume from 49 dafb relative to the root-pruned treatment, the differences remaining significant until harvest ($P<0.05$ and 0.01 for fruit diameter at 53 dafb for L-PBZ and H-PBZ respectively, $P<0.01$ at 67 dafb, $P<0.001$ at 94 and 123 dafb for both PBZ treatments). From later stage I (53 dafb) until harvest the fruit diameter and volume of the root-pruned treatment were smaller than control ($P<0.01$, 0.001 and 0.01 at 53, 67 and 94-123 dafb respectively). However, at harvest (128 dafb) final size of control fruits was not significantly different from that of the other treatments.

Fruit composition of tagged fruits (mesocarp and stone) on both fresh and dry weight bases are shown in Figure 5.13. As a proportion of fresh weight (Figure 5.13 c.) the mesocarp of H-PBZ, root-pruned and RDI fruits was less ($P<0.05$) than that of control. Figure 5.13 a. shows that both PBZ treatments gave high fruit dry weight, with H-PBZ being significantly greater than both control ($P<0.05$) and root-pruned ($P<0.001$) while L-PBZ was significantly greater only than root-pruned ($P<0.05$). The root-pruned treatment had lower stone dry weight compared with the other treatments ($P<0.01$ relative to control and L-PBZ, $P<0.001$ to H-PBZ and RDI), these being the only significant differences among the treatments in this parameter. In fruit mesocarp dry weight both PBZ treatments were significantly greater than root-pruned ($P<0.01$) and RDI ($P<0.05$), but neither PBZ treatment was significantly different from control. As a proportion of dry weight (Figure 5.13 b.) L-PBZ had more mesocarp than RDI ($P<0.05$), and RDI had a higher proportion of stone than L-PBZ ($P<0.05$). The differences among the other treatments were not significant.

5.4.2 Yield and fruit quality (1991 data only)

Table 5.8 a. & b. shows that the only significant differences between treatments in the number and total weight of fruit harvested in each of the 3 canopy zones was that root-pruned and RDI treatments had lower fruit number in the lower canopy zone than control ($P<0.05$). The average fruit size at harvest was higher for L-PBZ treatment (Table 5.8 c. and Figure 5.14 d.) than root-pruned ($P<0.01$) and RDI ($P<0.05$). H-PBZ had larger fruit than root-pruned ($P<0.01$), with no other significant differences between the treatments.

At the fourth picking date (146 dafb Figure 5.14 b.) the weight of harvested fruit from the control treatment was higher than from the other treatments (L-PBZ, root-pruned and RDI $P<0.001$ and H-PBZ $P<0.05$). The differences among the treatments at other picking dates were not significant.

Table 5.7. The total number of fruit on all treatments of wide planted 'Sundrop' apricot trees recorded 59 days after full bloom.

	a). Fruit number in 1990-1991 season					Significance
	Control	L-PBZ	H-PBZ	Root pruned	RDI	of F values
East[z]	57 ± 36[x]	139 ± 36	81 ± 31	118 ± 31	57 ± 31	ns
West[y]	123 ± 47	205 ± 47	84 ± 40	152 ± 40	63 ± 40	*
Total	180 ± 73	344 ± 73	165 ± 63	270 ± 63	120 ± 63	*
	b). Fruit number in 1991-1992 season					
East	202 ± 47	341 ± 47	314 ± 40	209 ± 40	196 ± 40	ns
West	271 ± 45	288 ± 45	198 ± 38	215 ± 38	189 ± 38	*
Total	473 ± 87	629 ± 87	512 ± 74	424 ± 74	385 ± 74	*

[z] East side of tree canopy.
[y] West side of tree canopy.
[x] ± Standard error of the mean.
ns,*,**,*** Nonsignificant or significant at P = 0.05, 0.01 or 0.001 respectively.

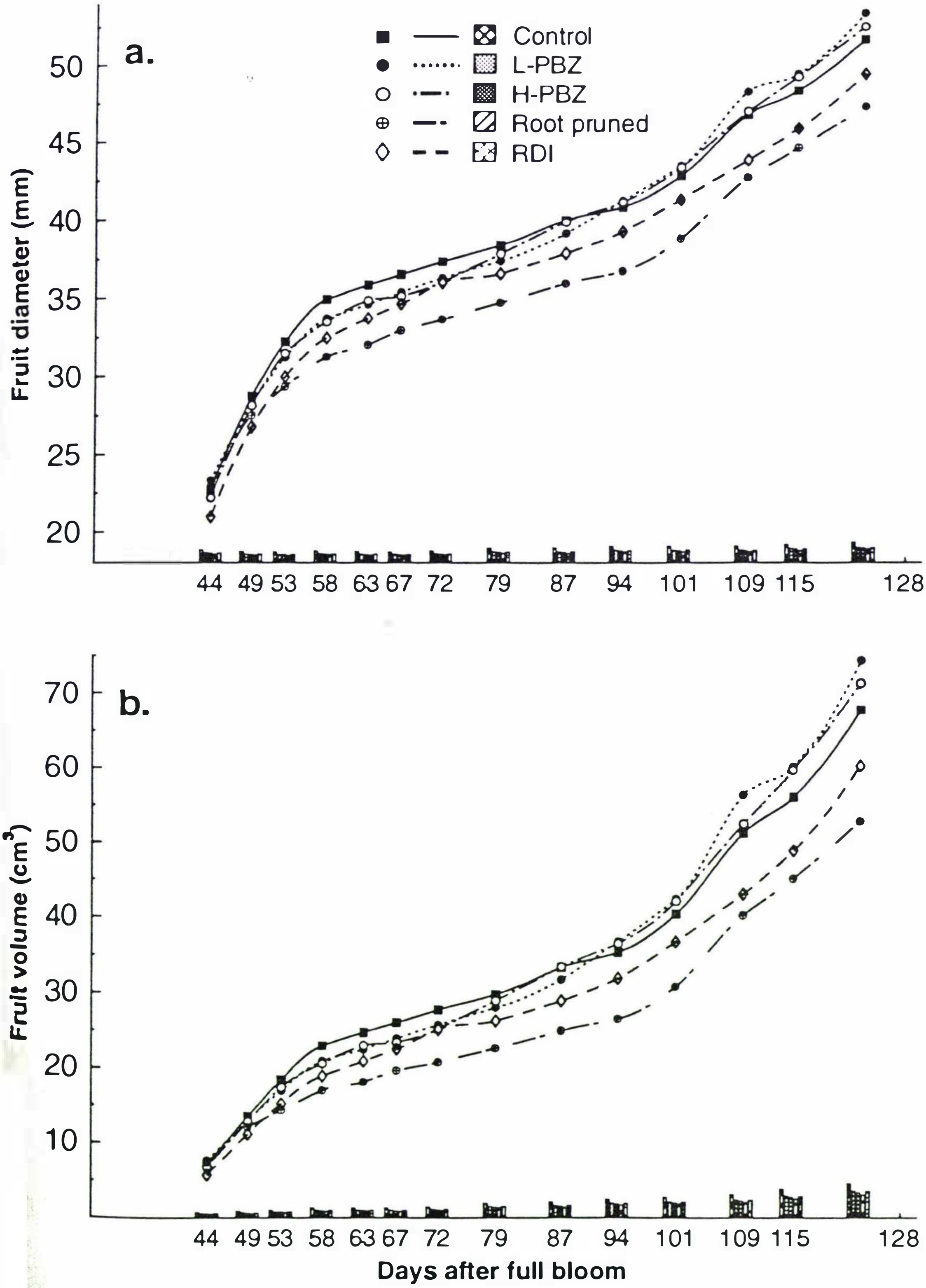


Figure 5.12 Seasonal changes in fruit size on wide planted 'Sundrop' apricot during 1990-1991 season. Bars show the standard errors of means. a). Fruit diameter (mm); b). Computed fruit volume (cm^3). Fruit number at the time of harvest was used as a covariate for data.

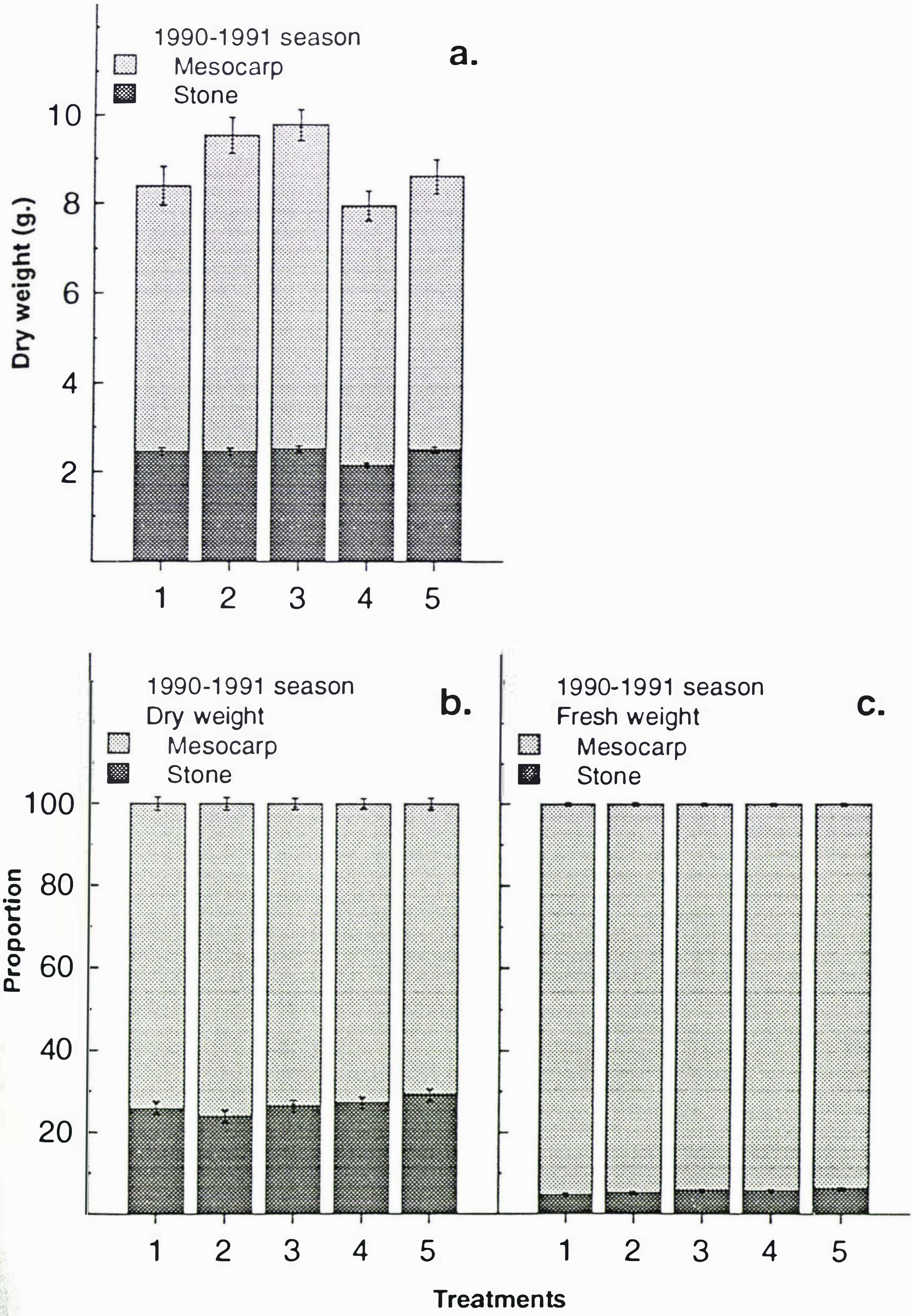


Figure 5.13 The influence of treatments on wide planted 'Sundrop' apricot fruit recorded at the time of fruit harvest from middle canopy zone. a). Mesocarp and stone dry matter of fruit; b). Proportion of fruit dry matter, and c). Proportion of fruit fresh weight; 1=Control; 2=L-PBZ; 3=H-PBZ; 4=Root pruned and 5=RDI. Vertical lines represent standard error of the mean.

Table 5.8. Fruit number, yield and adjusted fruit size of harvested fruit by treatment and canopy position of wide spaced 'Sundrop' apricot trees in January 1991.

Canopy zone[z]	Control	L-PBZ	H-PBZ	Root pruned	RDI	Significance of F values
			a). Fruit number			
Zone 1	27 ± 7[y]	14 ± 7	22 ± 6	10 ± 6	5 ± 6	*
Zone 2	121 ± 53	158 ± 53	89 ± 45	200 ± 45	62 ± 45	ns
Zone 3	18 ± 11	28 ± 11	21 ± 9	29 ± 9	21 ± 9	ns
Tree canopy	166 ± 62	200 ± 62	132 ± 53	239 ± 53	88 ± 53	ns
			b). Yield fresh weight (kg)			
Zone 1	1.71 ± 0.49	0.96 ± 0.49	1.49 ± 0.42	0.59 ± 0.42	0.55 ± 0.49	ns
Zone 2	8.53 ± 3.00	12.4 ± 3.00	8.02 ± 2.57	10.9 ± 2.57	5.54 ± 2.57	ns
Zone 3	1.99 ± 0.93	2.23 ± 0.76	1.64 ± 0.65	1.74 ± 0.65	1.41 ± 0.65	ns
Tree canopy	12.2 ± 3.71	15.6 ± 3.71	11.15 ± 3.18	13.2 ± 3.18	7.50 ± 3.18	ns
			c). Average fruit size (gram)			
Tree canopy	68 ± 4.22	79 ± 4.22	76 ± 3.62	57 ± 3.62	67 ± 3.62	**

[z] Lower, middle and upper regions of tree canopy for zone 1, zone 2 and zone 3 respectively.
[y] ± Standard error of the mean.
ns,*,**,*** Nonsignificant or significant at P = 0.05, 0.01 or 0.001 respectively.

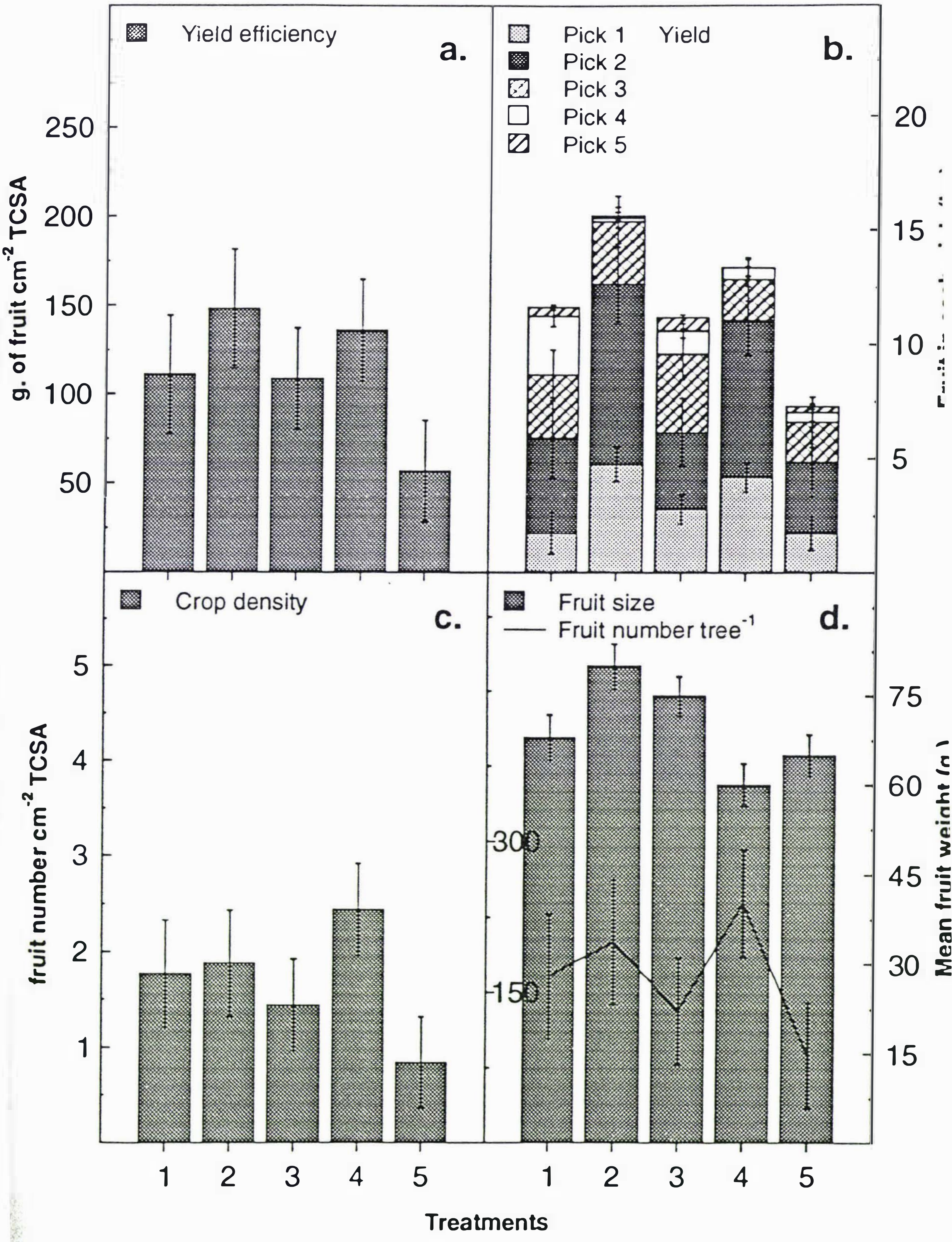


Figure 5.14 The influence of treatments on yield of wide planted 'Sundrop' apricot in 1990-1991 season. a). Yield efficiency; b). Early harvest and fruit maturity; c). Crop density and d). Mean fruit weight and mean total number of harvested fruits. 1= Control; 2= L-PBZ; 3= H-PBZ; 4= Root pruned and 5= RDI. Vertical lines represent standard error of the means.

Table 5.9. The influence of treatments on mesocarp colour (Hue angle and Lightness) of sample fruits from wide spaced 'Sundrop' apricot trees recorded at different harvest dates during January 1991.

dafb[z]	Control	L-PBZ	H-PBZ	Root pruned	RDI	Significance of F values
			a). Hue angle (degrees)			
127	100.3 ± 4.19[y]	94.65 ± 3.46	97.45 ± 2.96	93.86 ± 3.46	98.46 ± 3.46	ns
131	71.25 ± 2.44	68.96 ± 2.44	66.60 ± 2.44	66.81 ± 2.09	69.87 ± 2.44	*
142	66.72 ± 3.08	73.05 ± 3.08	72.04 ± 3.08	73.20 ± 2.64	72.73 ± 2.64	ns
146	81.06 ± 3.65	73.29 ± 3.65	73.85 ± 2.58	69.98 ± 3.01	72.88 ± 3.01	*
149	75.15 ± 1.90	-	68.22 ± 3.52	71.80 ± 2.70	76.27 ± 2.26	ns
			b). Lightness (%)			
127	49.73 ± 1.82	46.74 ± 1.50	49.57 ± 1.29	47.41 ± 1.50	49.29 ± 1.50	ns
131	55.86 ± 1.65	54.91 ± 1.65	51.23 ± 1.65	55.41 ± 1.41	55.50 ± 1.65	*
142	55.62 ± 1.83	57.41 ± 1.82	56.81 ± 1.82	56.08 ± 1.56	54.98 ± 1.56	ns
146	59.14 ± 3.07	52.46 ± 3.07	57.59 ± 2.17	51.84 ± 2.54	56.38 ± 2.54	ns
149	58.61 ± 1.04	-	57.78 ± 1.92	57.04 ± 1.47	57.23 ± 1.23	ns

[z] Fruits harvested at 5 different picking time in 1991 season.

[y] ± Standard error of the mean.

ns,*,**,*** Nonsignificant or significant at P = 0.05, 0.01 or 0.001 respectively.

Table 5.10. 'Sundrop' apricot fruit total soluble solids sampled from the wide planted experiment as influenced by different vigour control techniques, recorded at the time of fruit harvest during January 1991.

dafb^{z}	TSS (•Brix) for season 1990-1991					Significance of F values
	Control	L-PBZ	H-PBZ	Root pruned	RDI	
127	13.20 ± 1.18^{y}	12.06 ± 0.97	13.91 ± 0.83	14.35 ± 0.97	12.21 ± 0.97	ns
131	11.91 ± 0.75	12.79 ± 0.75	13.38 ± 0.64	13.86 ± 0.64	15.06 ± 0.64	**
142	12.94 ± 0.74	12.48 ± 0.74	12.08 ± 0.74	13.53 ± 0.63	12.15 ± 0.63	ns
146	11.45 ± 0.91	12.94 ± 1.00	12.65 ± 0.64	12.21 ± 0.81	11.68 ± 0.91	ns
149	9.55 ± 0.83	-	11.36 ± 1.48	13.14 ± 1.22	8.70 ± 0.95	*

^{z}Fruits harvested at 5 different picking dates in 1991 season.

^{y} ± Standard error of the mean.

^{ns},*,**,*** Nonsignificant or significant at P = 0.05, 0.01 or 0.001 respectively.

The crop density of the root-pruned treatment was highest (Figure 5.14 c.), although significantly different only from RDI ($P<0.05$). There were no significant differences in yield efficiency of any treatments (Figure 5.14 a.).

The hue angle and lightness of the surface of the harvested fruit at different picking dates are shown in Table 5.9 a. & b. Fruit were picked to colour standard and this is reflected in the absence of a marked effect of treatment on these parameters. Control fruit sometimes had a higher hue angle (less red) than H-PBZ ($P<0.05$ 131 dafb) and root-pruned ($P<0.05$ 146 dafb), and a higher L value (lighter colour) than H-PBZ ($P<0.05$ 131 dafb).

Table 5.10 shows that at the second picking date (131 dafb) RDI treatment had the highest value of TSS (15.06 °Brix) (control $P<0.01$, L-PBZ $P<0.05$) and with a trend towards a difference from H-PBZ ($P=0.07$). At the final pick (149 dafb) root-pruned TSS was higher than control and RDI ($P<0.05$).

5.5 DISCUSSION

One of the objectives underlying this experiment was to limit vegetative growth without having undesirable effects on yield, and to maintain or enhance fruit quality of 'Sundrop' apricot. Proebsting *et al.* (1989) suggested that successful fruit growing requires good management in maintaining sufficient vegetative and root growth to support maximum carbon fixation and assimilate partitioning for good fruit size, yield and quality. The study and understanding of the factors involved in fruit growth, maturity, yield and quality are important in the commercial application of any vigour control technique. This fruit growth study in both close and wide spaced trees provided information on the effects of the applied treatments in the orchard management system in which the experiment was carried out.

In both seasons fruit growth data and average fruit size were adjusted for crop load, which varied between trees. The highest number of fruits and also the highest percentage of harvested yield were produced in the middle canopy zone in both close (Tables 5.2 & 5.3) and wide spaced trees (Table 5.8). The middle canopy zone occupied a bigger surface area under the Tatura trellis training system (see chapter 3). Dann and Jerie (1988) reported the existence of gradients in maturity and sugar levels of peach fruit within a tree canopy. For example, they reported that flower opening started at the bottom of their two year old trees, but ripening of fruits commenced in the top canopy region. On the day of fruit harvest fruit from the tops of trees were over-ripe, whereas fruit from the bases of trees were still green. They discussed the possibilities of effects of light level, temperature and concentration gradients of endogenous factors changing the maturity between the canopy height regions. Under conditions of their experiment mesocarp dry weight from the upper canopy was consistently greater throughout the season than that fruits from the lower canopy region. In the present study, when fruit size within treatments in each canopy region was compared the results suggested that fruit in the top canopy region grew larger than those in the lower canopy regions. Variation in fruit quality within the tree canopy has also been reported by Heinicke (1966) and Jackson (1967) as cited by Palmer (1989). They reported larger and well-coloured fruits on the upper and outer sections of trees. Palmer (1989) concluded that high yields of good quality fruit can only be produced in systems having high light interception enabling high dry matter production, and that light well distributed within the canopy is needed to maintain fruiting and fruit quality.

Fruit growth in both close and wide planted trees followed a similar pattern of double sigmoid curve (Figures 5.4 to 5.7 and Figure 5.12). This result agrees with the previously reported growth pattern for apricot and for the other stone fruits. For example, Chalmers and van den Ende (1975a) cited the suggestion of Connors (1919) who divided the growth curve of peaches into three phases:

the initial phase of exponential growth (stage I), the lag phase (stage II) and stage III the second phase of exponential growth until fruit harvest. In the present study, treatments did not affect this pattern, the length of each stage was not changed but growth rates did change in some of the stages.

Lakso *et al.* (1989) mentioned the importance of maximizing early fruit development to increase carbohydrate partitioning into the fruit component over the whole season. The present study revealed that when vegetative growth was decreased by applied treatments the harvestable yield increased. For example, Figure 5.11 showed that the annual increase of TCSA in both seasons was significantly decreased by both PBZ treatments while the P-index was significantly increased by these treatments. Chapter 4 has reported the decrease in shoot growth by PBZ and other treatments. The reduction of activity of shoot tips as a sink for carbohydrate would allow more carbohydrate to be partitioned to fruits. Another possible reason would be that by reducing vigour the treatments had an indirect effect by improving light penetration into the canopy (see section 4.3.4). Jackson (1978) suggested that high yield, fruit bud formation, fruit set, fruit size and fruit quality, especially fruit colour, were affected by light interception. Lakso *et al.* (1989) reviewed the literature and suggested that variation in canopy light microclimate plays an important role in the carbohydrate support for fruit development.

5.5.1 Tree response to PBZ

PBZ is a powerful growth retardant. Anon (1984) suggested that the retardation of vegetative growth by PBZ results from diversion of assimilates to reproductive growth, giving increased yield potential and increasing fruit size. The results from both close and wide spaced trees revealed that the yield and average fruit size were increased (ie. ≈6 cm^3 in fruit volume at harvest) by both PBZ treatments (Tables 5.2 & 5.3, and Figures 5.4 to 5.7). The stimulation in fruit growth in close spaced trees occurred from early season and in wide spaced trees during stage III of fruit growth. This presumably reflects the reduced vegetative growth of trees caused by the PBZ treatments (see chapter 4) allowing more carbohydrates to be available for fruit growth. There was also some evidence of advanced fruit maturity at both spacings (Figures 5.9, 5.10 & 5.14). For example, in both close and wide spaced trees 46% of the crop of control was harvested in the first two picks, compared with ≈ 62% on the PBZ treatments. The increased proportion of harvested yield promoted by PBZ treatment during the early picks offers a great opportunity to growers seeking to advance harvest date when fruits may obtain a higher price and hence give a higher return. Increase in fruit yield and size of 'Flavorcrest' peach following PBZ application has been reported by Martin *et al.* (1987). Hillier and Rudge (1989) reported that PBZ had good potential for growth control and increased yield an average of 24% in stonefruits. Williams (1984) reported increased fruit yield and advanced maturity of apple following PBZ application.

The effect of PBZ treatments on fruit quality in terms of TSS and colour at both spacings was not constant (Tables 5.4 to 5.6, 5.9 & 5.10). It is likely that as commercial harvests removed fruits on the basis of colour, which would also reflect TSS, these colour and TSS results do not, except at the early harvests, well reflect differences between treatments. At early picking dates in both seasons on close spaced trees TSS was increased by PBZ treatment, but usually fruit colour from treated trees was not different from control. However, it was obvious in visual observation that PBZ treatments advanced fruit colour compared with control. Ogata *et al.* (1986) found that application of PBZ to nectarine and peach trees had no effect on fruit acidity and TSS, however flesh firmness was decreased. Also, the work on sweet cherry by Facteau and Chestnut (1991) showed that PBZ had no effect on fruit size, soluble solids concentration or firmness of fruits, however fruit density increased.

L-PBZ increased fruit set and fruit number in the second year (Figure 5.1 a. and Tables 5.1 b. & 5.3 a.), but flower density was not affected by PBZ treatments. Soil applied PBZ was reported to have either a negligible or slight beneficial effect on fruit set, yield and fruit size of European plum, with the increase in fruit size being noted for several years after soil application (Webster, 1990a). PBZ has been reported to have enhanced flowering and fruit set of sweet cherry, sour cherry and peach (Edgerton, 1986) and apple (Bubán, 1986; Miller and Swietlik, 1986; Tukey, 1989). In the present study, both PBZ treatments tended to advance flowering in the following season by 2-4 days relative to control (Figure 5.1 b.). PBZ advanced the flowering of sweet cherry by several days for 3 years (Webster, 1990b) and by 6-7 days without affecting the length of flowering period (Jacyna *et al.,* 1989).

In both seasons fruit dry weight at harvest (Figure 5.8) tended to be higher following PBZ treatment, although the differences relative to control were significant only in the first season of close spaced trees. The greater fruit dry weight was due to an increase in mesocarp dry weight. As discussed above the stimulation of fruit growth on close spaced trees started from early in the season when shoots and fruits were competing for assimilates. The reduction of vegetative growth by PBZ treatments early in the season which allowed more assimilate to be diverted to fruits contributed to the higher fruit growth, mainly by mesocarp growth. Lever (1986) suggested that the direct morphological evidence for a PBZ response is reduction in vegetative growth and the secondary effect is alteration of sink strength within the plant, allowing greater partitioning of assimilates to reproductive growth. Costa *et al.* (1986) emphasized that the reduction in vegetative growth changed the competition between vegetative and reproductive growth in favour of fruit growth and this possibly meant more assimilate was diverted to fruits.

5.5.2 Tree response to root-pruning

The results from close spaced trees showed that root-pruning had no significant effect on flowering period, flower density (Figure 5.1), yield efficiency (Figures 5.9 & 5.10), yield and mean fruit size (Tables 5.2 & 5.3). The annual increase of TCSA in the second season was reduced by root-pruning but it did not increase the P-index (Figure 5.11). In close spaced trees a negative effect of root-pruning on fruit growth occurred in the first season, when at the time of fruit harvest (128 dafb) the mean volume of fruits in the middle canopy zone was reduced by 5 cm^3 ($P<0.05$, Figure 5.5 b.). However, in the wide spaced trees root-pruning more severely reduced growth of tagged fruit from late stage I until harvest (Figure 5.12), and overall mean fruit size in the whole tree canopy was reduced ($P<0.01$, Table 5.8 c.). Root-pruning is reported to have had no effect on return bloom, fruit set or yield, but it did reduce fruit size of 'Golden Delicious' (Schupp *et al.,* 1992), and 'Melrose' apples (Schupp and Ferree 1987a). However, Elfving *et al.* (1991) reported that fruit size of 'McIntosh' apple was not affected by root-pruning. In the present study the fruit size response of root-pruned apricot was not accounted for by any difference in tree crop load either early (Tables 5.1 & 5.7) or at the time of fruit harvest (Tables 5.2, 5.3 & 5.8). The effect of fruit number on the response of apple fruit to root-pruning was considered by McArtney and Belton (1992) who reported that when the number of fruits was high, root-pruning had a negative effect on fruit size, otherwise no evidence of decreasing fruit size was found. Reduction of fruit size in apple was reported by Brunner (1990) who concluded that trees carrying high fruit numbers in the year of root-pruning may not be able to regenerate their roots as well as those trees with less crop load. Root growth and regeneration after root-pruning was suggested to depend on the inverse ratio of fruit load, and fruit bud formation might be affected, being dependent on root activity.

Root-pruning tended to advance maturity in the first season on both close spaced (35 and 68% of fruit in first two picks of control and root-pruned respectively) and wide spaced trees (51 and 84% respectively) (Figures 5.9 b. & 5.14 b.). There was a similar effect in the second season on close spaced trees (58 and 73% of fruit respectively, Figure 5.10 b.). In the first season, but not in the second, fruit from root-pruned trees tended to have higher TSS (for the same skin colour) at each pick (Tables 5.6 a. & 5.10). A reduction of fruit size and increased fruit colour and TSS have been reported for apple trees following root-pruning either during dormancy or at June drop (northern hemisphere) by Schupp and Ferree (1987a, 1988b and 1989).

5.5.3 Tree response to RDI

The results showed that RDI applied during stage I and II of fruit growth followed by full irrigation during stage III had no detrimental effect on fruiting performance in both seasons of study. In the second year on close spaced trees RDI increased percentage fruit set (Figure 5.1), and fruit number (Table 5.1 b.). The small increase in flower density was not significant, although Mitchell *et al.* (1984) reported increased flower density on pear trees in the following year. A drought may reduce apricot fruiting density (Marangoni *et al.*, 1988), flower number and yield in the subsequent year (Uriu, 1964). This suggests the severity of water stress as well as its timing have important roles affecting flowering and fruiting of fruit trees. In this study, a small reductions in soil water content and Ψ_{xylem} for 20 to 30 days in early summer had no negative effects on flowering and fruiting, and improved some cropping characteristics. For example, the result from close spaced trees revealed that RDI significantly improved yield, yield efficiency, crop density (Figure 5.10), fruit number and fruit set in the second season. In addition, RDI had no negative effect on fruit development through the season and on final fruit size on close spaced trees (Figures 5.4 to 5.6). On wide spaced trees generally fruit growth (Figure 5.12 a. &b.) was less than on control through the season, although the only significant differences occurred during late stage I and early stage II (49-67 dafb), with final fruit size not significantly affected. Mitchell and Chalmers (1982) found peach fruit growth was faster on RDI trees in stage III when trees were fully irrigated. RDI of pears before the period of final rapid fruit size increase, followed by full irrigation during rapid fruit expansion either did not affect fruit size or yield or increased them (Chalmers *et al.,* 1986; Mitchell, *et al.,* 1984, 1986 & 1989). Reduced early season irrigation has been used to control shoot growth of pear and peach in the spring without detrimental effects on yield (Chalmers *et al.,* 1981, 1986; Mitchell and Chalmers, 1982; Mitchell *et al.,* 1984, 1986 & 1989).

There was some evidence RDI advanced maturity on both close and wide spaced trees and also improved TSS and colour on some occasions. In the first season on close spaced trees yield in the first two picks was 74% of total harvested yield, which was 14% higher than control. Reduction in fruit size and increasing TSS following RDI applied to apple trees has been reported by Ebel *et al.* (1993), but fruit colour and firmness were not affected. In contrast, fruit size was not affected by RDI applied to 'Cox's Orange Pippin' apple (Irving and Drost, 1987), pear (Mitchell *et al.* 1984) and peach fruit size was increased (Li *et el.*, 1989).

SUMMARY

Strong relationships were observed between fruit diameter, fruit volume and fruit fresh weight in both seasons (r^2=0.98), with no effect of treatments on these relationships.

PBZ advanced flowering (by 2-4 days) in the year following application. PBZ treatments increased fruit set and final fruit number and enhanced crop density and yield efficiency. PBZ treatments reduced annual increase of TCSA and gave more yield per unit increase in TCSA (P-index) in both seasons of the close spaced experiment. Fruit growth and final fruit size were increased without any detrimental effect on fruit quality. Fruit maturity was advanced on both spacings. The results suggested that trees at close spacing showed better performance in terms of reproductive criteria with the low but annual dosage of PBZ.

Flowering period, flower density, yield efficiency, P-index and fruit dry weight were not affected by root-pruning. However, fruit size at harvest was reduced by root-pruning in the first season. The effect of root-pruning on fruit growth and final fruit size reflected its effect on vegetative growth. Only when root-pruning increased shoot growth was fruit size decreased. There was some evidence of a trend to advanced maturity and increased TSS after root-pruning.

RDI applied in the first season generally enhanced flowering, flower and fruit density, and especially fruit set and fruit number in the subsequent year. In general, RDI had no negative effect on flowering and fruiting, yield and final fruit size. There was evidence of advanced maturity and improved TSS with RDI.

Both RDI and L-PBZ had greater effects on fruit set and fruit number in the second year of treatment. The L-PBZ treatment gave more uniform responses than the H-PBZ treatment. Generally, reproductive growth performed better with PBZ treatment than with root-pruning or RDI.

CHAPTER SIX

EFFECTS OF TREATMENTS ON WATER RELATIONS OF 'SUNDROP' APRICOT

6.1 INTRODUCTION

The response of vegetative and reproductive growth of 'Sundrop' apricot trees to different vigour control techniques was discussed in chapters 4 and 5. This chapter reports the water relations responses of trees to the treatments during the orchard experiment.

The difficulties faced in imposing the RDI treatment were described in chapter 3. The objective was to induce a mild water stress during stage I and II of fruit growth, and resume irrigation during stage III. This was achieved by isolating the root zone of the experimental trees from rainfall and irrigation early in fruit growth. In non-stress treatments normal orchard irrigation was applied all season based on tensiometer readings. In general, during the growing season (except for the RDI in stages I and II) when soil water suction reached -50 kPa, trees were irrigated to close to field capacity i.e. -25 to -30 kPa (FC ≈ -20 to -25 kPa).

In stage III of fruit growth normal irrigation was resumed in the RDI treatment (see section 3.5.1.4). However, in the second season (on 12th., and 17th., December 1991 in early stage III) supplemental irrigations of 30-60 litre m^{-2} were applied to raise soil moisture of the RDI plots close to field capacity. Supplemental irrigation was based on soil moisture recorded during the experiment. The aim was to supply water to maintain the soil moisture content at this level in the third stage of fruit growth. Jerie *et al.* (1989a) suggested that irrigation management oriented to optimize the proper balance between vegetative and reproductive growth was a major determinant in orchard productivity. Irrigation scheduling suggested by Elfving (1982) was based on pan evaporation (E_{pan}). The crop water requirement was expressed as ET_{crop}

(mm day^{-1}). RDI is usually scheduled based on the principle that predictable plant water deficit will be obtained if a reduced proportion of potential evapotranspiration is replaced at close intervals. So an assessment of ET is required. The methods of estimating evapotranspiration were described in detail by Sharma (1985). According to that report, numerous methods have been applied to estimate water use of plants, eg. based on the water balance method, micro-meteorological and plant physiological methods. For scheduling irrigation, using pan evaporation data and calculating the crop factor was suggested by Jerie (pers. comm., 1994). In the literature irrigation scheduling reported by many authors has been applied using evaporation from a U.S. class A pan and calculating percentage of water evaporated from a free water surface equivalent in size to the area of the tree planting square (E_{ps}). Water has been supplied as a percentage of replacement of evapotranspiration from the E_{ps} or litre m^{-2} of row. Examples may be found in Chalmers *et al.* (1984); Chalmers *et al.* (1986); Ebel *et al.* (1993); Huslig *et al.* (1993); Jerie *et al.* (1989b); Mitchell and Chalmers (1982); Mitchell *et al.* (1984) and Mitchell *et al.* (1989).

Elfving (1982) in his review of the response of plants to trickle irrigation suggested that soil-water status monitoring is one of the major techniques for irrigation scheduling. This method has advantages in humid regions because it eliminates the need to budget rainfall. Any water entering the root zone, regardless of its source, becomes available to plants and also influences the operation of soil-based irrigation-scheduling equipment. Campbell and Campbell (1982) pointed out that proper irrigation scheduling needs to answer two questions, when should irrigation be performed and when should it be turned off. They concluded that as soil is the primary recipient of irrigation water, so it is reasonable to conclude that these questions are answered by monitoring soil moisture. However, exceptions might occur when the monitoring of soil moisture is too expensive or of insufficient accuracy to satisfy irrigation-scheduling needs. They stated that the neutron probe is widely used for monitoring soil moisture, but its use for irrigation scheduling is relatively recent. Based on their experience Campbell and Campbell (1982) suggested that compared with other methods used for monitoring soil moisture, the neutron probe offered the best combination of features for irrigation scheduling. They suggested that even when a climatological scheduling approach, such as pan evaporation, is employed for irrigation scheduling, it should be checked by occasional measurements of soil moisture to ensure that cumulative errors in the climatological scheduling method did not lead to either crop stress or over irrigation.

Xylem water potential (Ψ_{xylem}) measurement, as an indicator of plant water status, was carried out predawn and at midday during both experimental seasons, and also diurnally in the second season simultaneously with measurement of photosynthesis and stomatal conductance.

Osmotic adjustment has been suggested as a mechanism of drought tolerance by plants under shortage of water supply. Lowered osmotic potential in response to water deficit stress is proposed to play a significant role in turgor maintenance and regulating plant growth processes (Morgan, 1984). The possible existence of osmotic adjustment was investigated in the second season by measuring leaf and fruit osmotic potentials. Also, some simple carbohydrates in fruit were extracted from sample fruits to investigate the role of simple carbohydrates in any possible osmotic adjustment of fruits.

This chapter will establish the degree of internal stress, through both seasons of study, to indicate its relationship to some physiological responses to applied treatments on 'Sundrop' apricot trees grown under the humid climate. For example, to see if root-pruning affected performance by inducing internal water stress, and examine whether osmotic adjustment was an adaptive mechanism in either fruit or leaves under the conditions of this experiment. Data on the wide planted trees was restricted to the first season as previously described.

6.2 MATERIALS AND METHODS

6.2.1 Measurements

6.2.1.1 Climatic conditions

The overall climatic conditions of the experimental site (mean of 30 years) were presented in chapter three (Section 3.4.1). The daily and monthly rainfall and evaporation, temperature, relative humidity and sunshine hours during this study were monitored based on data from the nearest weather station, AgResearch Grasslands, Crown Research Institute (CRI), Palmerston North (≈ 1 Km away from experimental site).

6.2.1.2 Soil moisture measurements

Aluminium tubes of 50.8 mm external diameter (Ullrich Aluminium Company Ltd., Palmerston North, NZ) were cut into 1.20 m lengths and the lower end of each tube was sealed using twin pack Plasti-Cast cable joint sealer (Fraser Brown and Stratmore Ltd., Lower Hutt, Wellington, NZ). Access tubes were driven into the soil to a depth of 110 cm after using a 50 mm diameter auger to open a hole (Plate 6.1 a.) at distances of 50, 80 and 110 cm from tree trunk of control and RDI treatments, and 50 and 80 cm from trunk of the root-pruned trees (Plate 6.1). After installation, the upper end of each tube was protected from rainfall and irrigation using a rubber bung and an inverted tin can.

In close spaced trees a total of 32 access tubes (4 replicates on control, root-pruned and RDI with 12, 8 and 12 tubes respectively) were used in the first season (1990-1991). In the second season (1991-1992) the number of access tubes was increased to 44 to include all the RDI plots. However, in the wide planted experiment two tubes were installed at a distance of 50 cm and 1 tube each at distances of 80 and 110cm from the tree trunk, so a total of 16 access tubes were installed on the control and RDI treatment in the two replicates.

Soil moisture in 0-1.0 m soil depth was determined using a neutron hydroprobe (Plate 6.2 a.)(Campbell Hydroprobe Model 503-DR, from Campbell Pacific Nuclear Corporation, Martinez, CA, USA) which had previously been calibrated for the site by the method of Campbell and Campbell (1982). Soil moisture was determined weekly at 10 cm increments from 20 cm to 100 cm depth in the soil throughout the first season (.2, .3, .4, .5, .6, .7, .8, .9 and 1.0 m) and at 20 cm increments in the 1991-1992 season (.2, .4, .6, .8 and 1.0 m). The uppermost reading (.2 m depth) was multiplied by two (year 1) or three (year 2) to estimate the amount of soil moisture (mm) in the uppermost 20 or 30 cm layer of soil. Similarly, in the second season measurements at soil depths of .4, .6 and .8 m were multiplied by two to calculate the content of soil water in the 20 cm increments of soil measured in that season. Measurement at 1.0 m depth represented the amount of water in the 90-100 cm soil depth. Field capacity for the 0-1.0 m depth of soil was recorded (316 mm) with the hydroprobe during the winter and used for scheduling irrigation.

6.2.1.3 Xylem water potential (Ψ_{xylem})

Xylem water potential (Ψ'_{xylem}) was monitored using a pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, CA, USA). Measurements were made before sunrise (pre-dawn Ψ_{xylem}) and in the hour following solar noon (midday Ψ'_{xylem}) on the same trees throughout the first season on close and wide spaced trees and in the second season on closely spaced trees, on all treatments except L-PBZ.

In addition, diurnal changes of xylem water potential (Ψ_{xylem}) from 0600 to 1900 h were monitored on control, root-pruned and RDI treatments of close spaced trees on 4 occasions in the second season during stage II and III of fruit growth (69, 83, 101 and 110 dafb).

In both seasons measurement was made on two mature leaves shaded at the time of measurement in the middle canopy zone, taken from 8 replications of each treatment on close spaced trees and 4 leaves from 2 replications of wide spaced trees. Each leaf was enclosed in a small plastic bag prior to excision to avoid rapid water loss. The reading was taken within 30 seconds of excision.

6.2.1.4 Leaf photosynthesis and stomatal conductance

Net CO_2 assimilation rate (A) and leaf conductance (g_s) were determined using a Li-Cor Li-6200 Portable Photosynthesis System. Measurements were made during stage III of fruit growth, on two mature sun exposed leaves on each of the eight replications of control, H-PBZ, root-pruned and RDI treatments in close spaced trees and 4 exposed leaves of each of two replications of wide spaced trees during 1990-1991. In the second season these measurements were carried out on all treatments of close spaced trees from late stage I until the time of fruit harvest. All measurements were made at solar noon.

In addition, in the second season (1991-1992) on close spaced trees diurnal records of photosynthesis and stomatal conductance were made on control, root-pruned and RDI treatments during stage II (69 and 83 dafb), and III (101 and 110 dafb) of fruit growth from 0900 to 1900 hours at two hourly intervals. Diurnal measurements were made on 2 sun exposed leaves of the middle canopy zone from all 8 replicates of control, root-pruned and RDI treatments.

6.2.1.5 Leaf and fruit water potential, pressure potential, osmotic potential and leaf relative water content

In the second season of the close spaced experiment predawn and midday seasonal changes of water status of leaves and fruits were monitored using a dew point depression method (Flower and Ludlow, 1986; George and

Nissen, 1992a). Leaf and fruit disc samples were loaded into the Sample Chamber Model C-52 connected to a Wescor HR-33T Microvoltmeter (Wescor, INC, Utah, USA) and the microvolt output recorded (Plate 6.4).

Leaf (Ψ_l) and fruit (Ψ_f) water potential and osmotic potential (Ψ_s) were measured weekly from 46 dafb until harvest on control, root-pruned and RDI treatments. However, the measurements on root-pruned treatment were interrupted after the first record in late stage I until early stage III of fruit growth. This was caused by a shortage of correctly operating sample chambers during this period. Leaves and fruits were each measured on separate days each week, samples being collected predawn and midday on the day of measurement. At each measurement 1 leaf or fruit was sampled from each of 5 replicates of each of control, root-pruned and RDI treatments. Samples were put in labelled black plastic bags and loaded into the sample chamber within 5 minutes. In all cases delay was minimized between sampling and measurement to avoid any water loss from leaf or fruit samples.

Disc samples of 5 mm diameter were used of both leaf and fruit. Leaf discs were sampled from the centre of the lamina of a fully expanded and mature leaf and loaded into the 7 mm diameter X 1.25 mm deep sample holder. However, fruit samples 5 mm X 2 mm height were taken from the mesocarp of the cheek in the equatorial region and loaded into the 7 mm X 2.5 mm deep sample holder. All precautions were observed according to the equipment manual. All sample holders were washed and cleaned with de-ionized water and acetone after each measurement. In addition, all sample chambers were tested for contamination prior to each measurement, and if needed washed with de-ionized water and dried by a cool air blast and properly tested prior to measurement.

All sample chambers were calibrated weekly to allow conversion of the microvolt output readings to either actual leaf (Ψ_l) or fruit (Ψ_f) water and osmotic (Ψ_s) potential. Calibration was carried out with a series of concentrations of NaCl solution (range 0.05, 0.2, 0.4, 0.6, 0.8 and 1 molar) prior to each set of measurements. The correlation between readings of known NaCl solutions and the standards (recorded with a range of NaCl concentrations at different temperatures and provided in the Wescor manual) were computed using Regression Procedure (SAS Institute Inc. 1991). For the 20 chambers available this relationship varied and those with higher correlation (r^2 = 0.97 to 0.99) were selected for leaf and fruit sample assessments. If the correlation coefficient was low (r^2 < 0.97) chambers were re-cleaned or replaced. Water or osmotic potential of fruit or leaf samples were determined from the microvolt readings using the regression equation of the form Y = a+bX established from the standards. In this equation Y is the water or osmotic potential of leaf or fruit in MPa, a is constant, b the slope and X the microvolt reading.

Before starting the first measurement early in the season (46 dafb) a series of preliminary measurements and conversions were performed to determine the thermal and vapour equilibrium time needed for apricot leaf (2.5 h) and fruit (2.0 h) tissue. Thereafter all readings for leaf and fruit samples were taken after allowing the necessary equilibrium time (Plate 6.4 b.).

After the first reading on fresh tissue, leaf and fruit sample discs were removed from the sample chamber and wrapped with 'Gladwrap' plastic cling film and then with aluminium foil for protection. After labelling, the samples were inserted into liquid air for 30 seconds. After unwrapping, frozen samples were loaded back into the appropriate sample chamber, sealed and after the equilibrium time (2-2.5 h) a second reading was made. This allowed recording of osmotic potential by rupturing cell walls, so removing pressure potential from the tissue. Pressure potential (Ψ_p) was calculated according to the following formula (Kramer, 1983):

$$\Psi_l \text{ or } \Psi_f = \Psi_p + \Psi_s$$

where Ψ_l and Ψ_f is leaf or fruit water potential respectively, Ψ_p is pressure potential (turgor potential) of leaf or fruit samples and Ψ_s is osmotic potential (solute potential) of leaf or fruit samples.

Relative water content of leaves was also recorded at the same time as leaf osmotic potential measurement to calculate the amount of adjusted osmotic potential and remove the effect of tissue dehydration. Osmotic potential of leaves at full turgor was calculated based on recorded Ψ_s and relative water content (RWC) data according to the method described by Wang and Stutte (1992) with the following formula:

$$\Psi_s \text{ at full turgor} = \frac{\text{measured } \Psi_s \times RWC}{100}$$

This method allowed elimination of the effect of passive tissue dehydration and adjustment of recorded Ψ_s to 100% RWC.

Discs from the same leaves used for Ψ_l and Ψ_s determination at solar noon were used to record leaf RWC. Precautions were taken to minimize the time between sampling and RWC measurement, to avoid any water loss (Bennett, 1990). Between 10-15 discs of 15 mm diameter were sampled from each leaf (with 5 replications per treatment) avoiding main veins of leaves. Initial fresh weights of leaf discs were recorded prior to floating on distilled water in petri dishes for 4 hours at room temperature to constant turgid weight. In the dishes leaf discs were separated from each other using tweezers, so that all floated freely in the water. Dishes were then covered with paper towels to keep samples under day light intensity approximating the photosynthetic compensation point. After 4 hours the turgid weight (TW) of surface dried leaf discs was recorded. Dry weight (DW) was recorded after oven drying at 70°C for 24 h. RWC was determined according to the method of Barrs and Weatherley (1962):

$$RWC = \frac{(FW - DW)}{(TW - DW)} \times 100$$

where RWC is relative water content in percent, FW is initial fresh weight (g), TW is turgid weight (g) and DW is dry weight (g) of sample discs.

6.2.1.6 Fruit carbohydrates

Samples for measurement of simple carbohydrates of fruit (from 52 to 137 dafb) were taken at the same time as the midday fruit water status samples. Two fruit were randomly sampled from the middle canopy zone from each of 5 replications of control, H-PBZ, root-pruned and RDI treatments. Fruit were kept in labelled plastic bags and within 0.5 h after sampling 5-10 g of mesocarp from each cheek was sampled and fresh weight recorded.

Samples were stored in 20 ml vials with screw lids. Spare samples were also taken for preliminary work to establish the levels of carbohydrates to be determined. Samples were kept in a freezer (-20°C) prior to freeze drying. Samples were freeze dried for a week and then kept in a sealed air tight plastic bag with silica gel in the freezer (-20°C) until the time of extraction.

At the time of extraction the dry weight of samples was recorded and samples were crushed and ground manually using a glass bar. Ground samples were homogenized with 20 ml boiling ethanol (95%). According to Paull *et al.* (1984) this method is effective in inactivating invertase activity and failure to follow these precautions leads to significant increases in glucose and fructose of up to 30%. Homogenized samples were shaken at 100 rpm for 24 h at room temperature. Samples were kept in the cool room (4°C) for a week, then centrifuged at 5000 rpm for 10 minutes. Five ml of clear supernatant was taken and put into labelled vials. Samples were stored for 8 weeks in the freezer (-20°C) to allow various components to precipitate before analysis

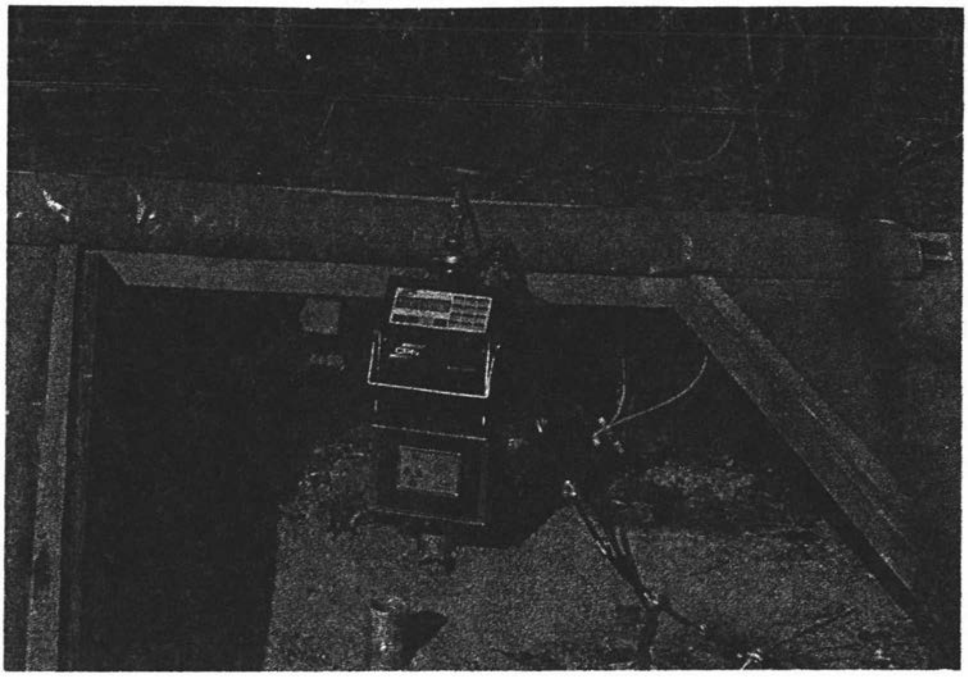
(Paull *et al.*, 1984).

After this time, an aliquot of 1 ml cleared supernatant was removed from the samples and put into 1.5 ml Eppendorf tubes. A stream of oxygen free nitrogen was passed over the samples in the Eppendorf tubes for 2.5 h to remove the ethanol (Plate 6.2 b.). The samples were completely dried by placing them in a vacuum oven (60°C) overnight. The residue was redissolved in 1 ml of de-ionized water and filtered (Plate 6.3 a.) through 0.2 µm Nylon Membrane Filters followed with rinsing the Eppendorf tubes with another 1 ml. For determination of simple carbohydrates 20 µl of each sample was injected into a high performance liquid chromatography (HPLC) system (Waters, Milford, MA, USA) and eluted with water (Plate 6.3 b.). Samples were analysed for glucose, fructose, sucrose and sorbitol. After 10 samples 20 µl of a standard was injected to check the retention time for each simple carbohydrate. The standard was a mixture of glucose, fructose, sucrose and sorbitol (600 µg ml^{-1} of each). The carbohydrate column (Bio-Rad Aminex, HPX87C) was maintained at 85°C, and the detector (Optilab 5922 RI Chromatography Module, Tekator AB, Högnäs, Sweden) at 25°C. Injected samples were eluted with water at a flow rate of 0.6 ml min^{-1}. The areas under the curves were computed by a β-RAM package.

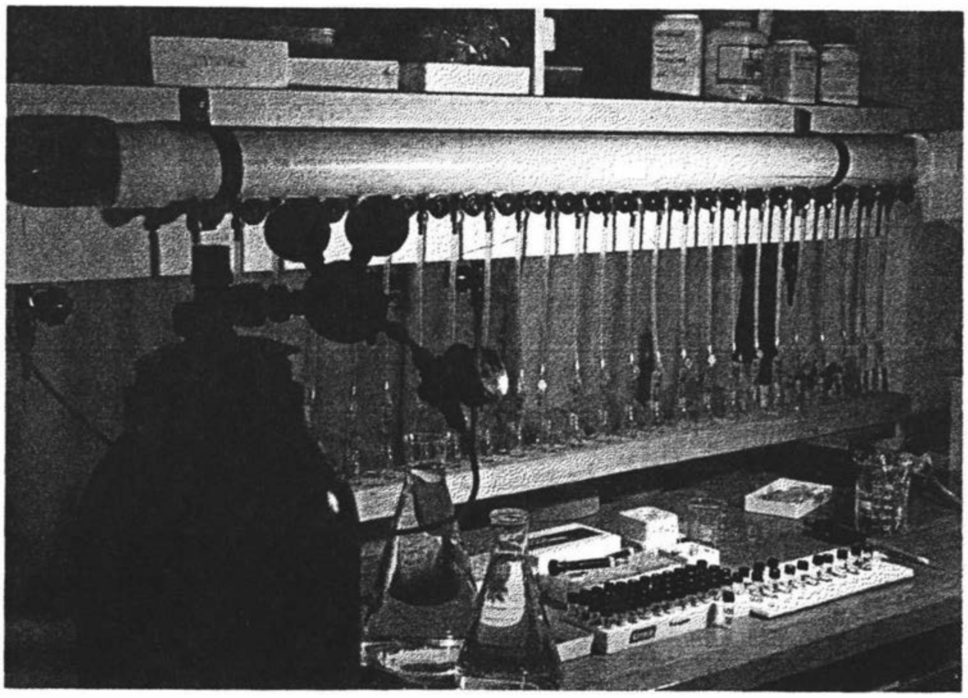
The influence of each simple carbohydrate on osmotic adjustment of fruit was computed using Van't Hoff's equation (Kramer, 1983):

$$\Psi_{cho} = \frac{n\ mol_{cho} \times RT}{V}$$

where Ψ_{cho} is osmotic pressure (MPa) of each individual carbohydrate (sucrose, glucose, fructose or sorbitol), $n\ mol_{cho}$ is moles of solute, R is the universal gas constant (0.00832 litre MPa/degree mol at 273°K), T is fruit temperature in °K, and V is the volume of solvent in litres.

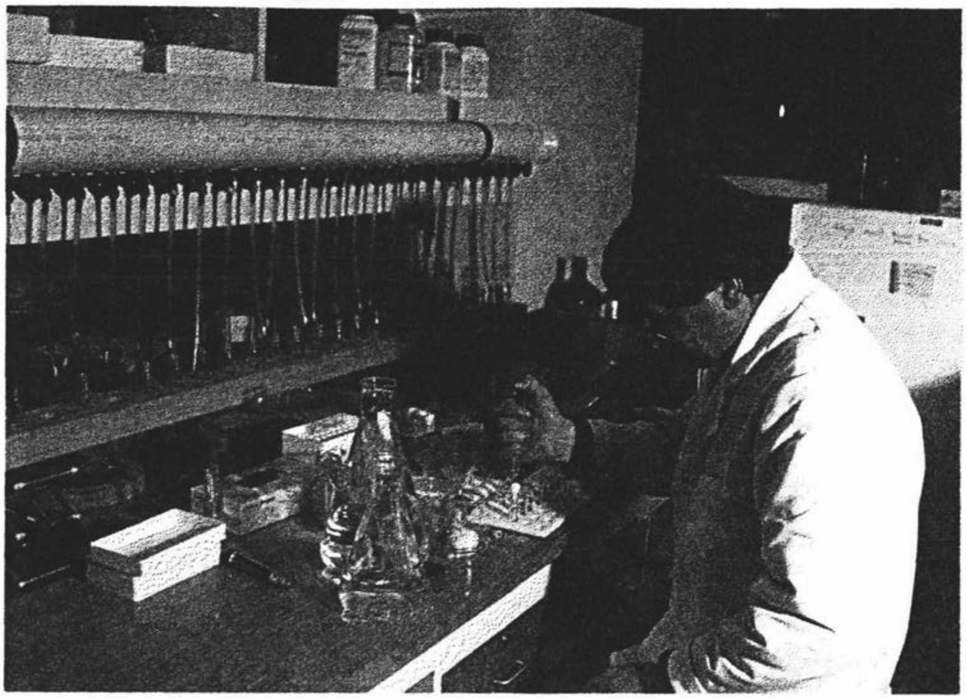


a.



b.

Plate 6.2 a). Recording soil moisture using a neutron hydroprobe; **b).** Using a stream of nitrogen gas to remove ethanol from fruit extracts for carbohydrate determination.



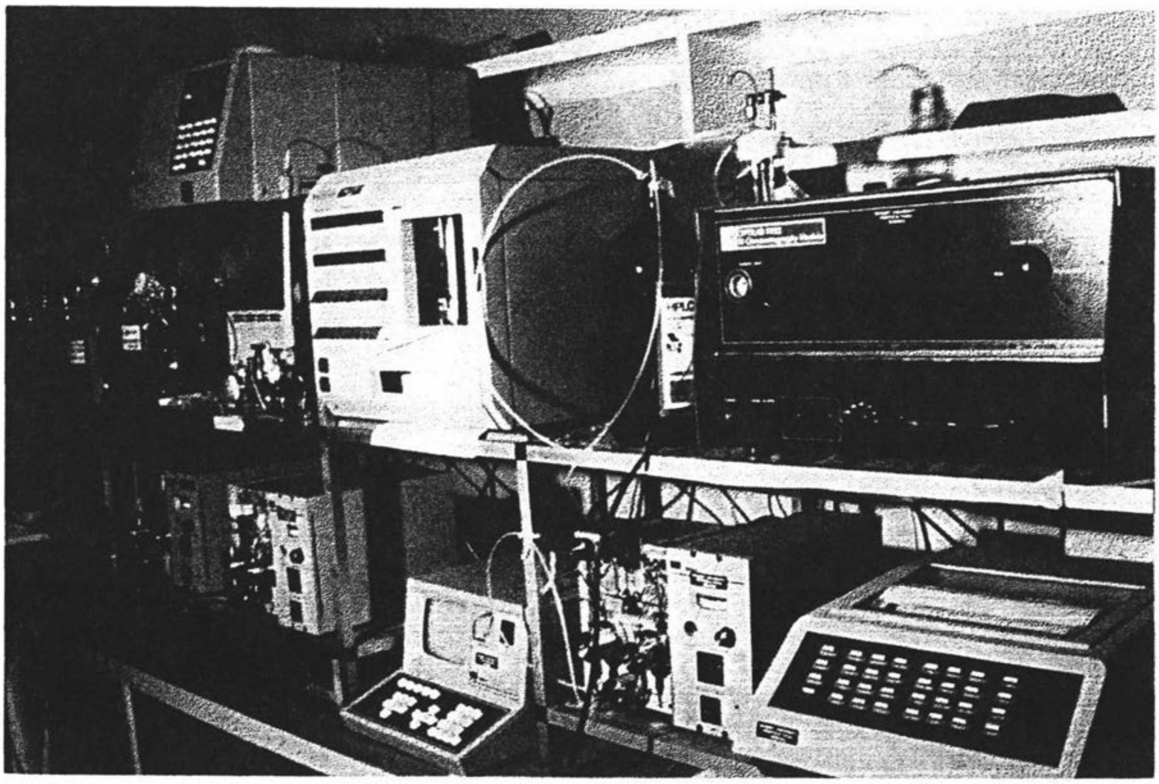
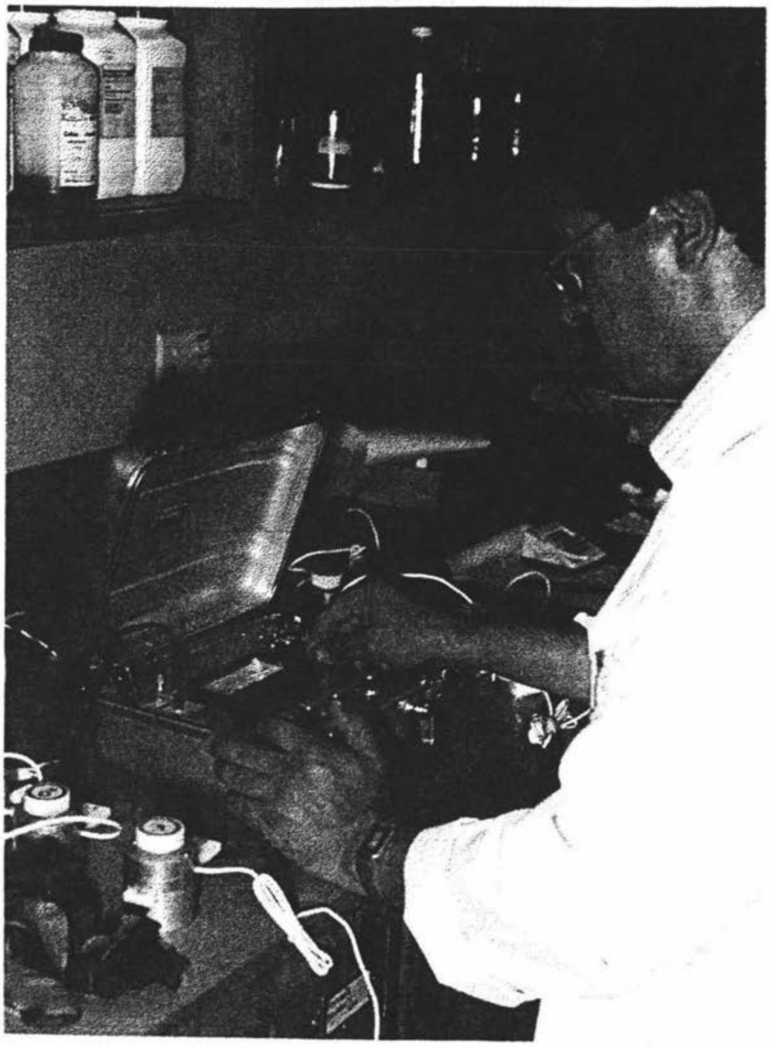


Plate 6.3 a). Filtering fruit carbohydrate samples using Nylon Membrane Filter prior to injection into high pressure liquid chromatography system; **b).** HPLC.



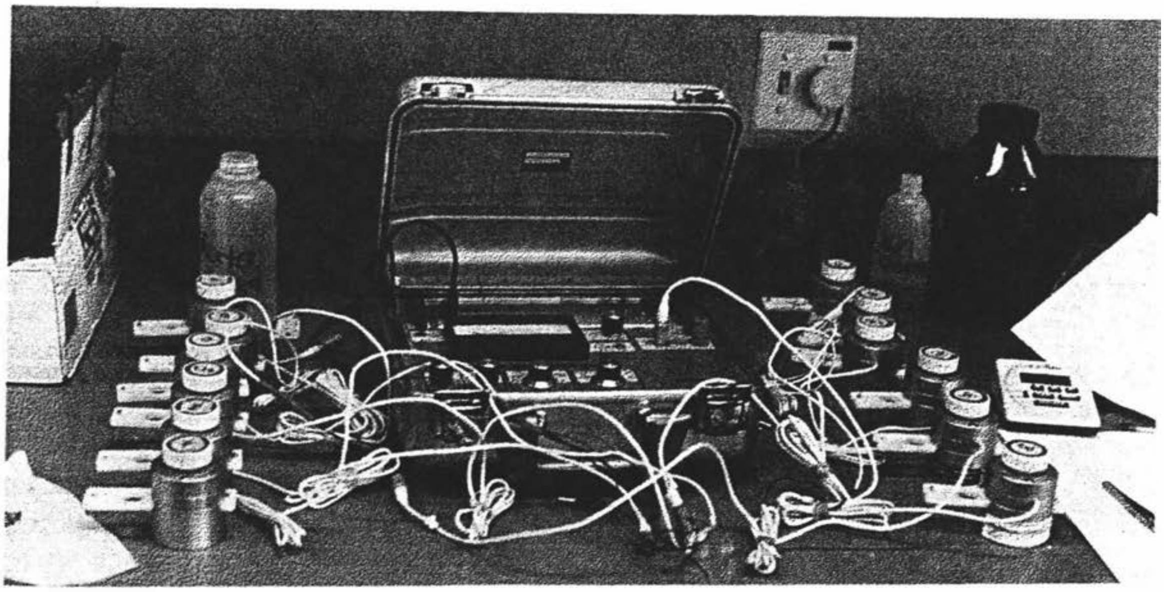


Plate 6.4 a). Recording Ψ_l and Ψ_s of samples using Wescor HR-33T; **b).** Samples loaded in the Sample Chamber Model C-52 during equilibration time.

6.2.2 STATISTICAL ANALYSIS

Data were analysed as described in chapter four (Section 4.2.2).

Soil moisture data were analysed in different categorised soil regions according to depth of soil and distance from tree trunk (Table 6.1).

Table 6.1. The categorisation of soil volume in monitoring soil moisture throughout the orchard experiment during the 1990-1991 and 1991-1992 seasons.

Soil region[z]	Depth (cm)	Distance from tree trunk (cm)
"a."	0 - 50	0 - 50
"b."	0 - 50	50 - 150
"c."	50 - 100	0 - 50
"d."	50 - 100	50 - 150
"e."	0 - 100	0 - 150

[z] "a." and "c." regions include 1 access tube per replication of each treatment.
"b." and "d." regions include 2 access tubes per replication of control and RDI and 1 access tube per replication of root-pruned treatment.
"e." region is pooled data from all above regions, and includes 2-3 access tubes per replication.

6.3 RESULTS: CLOSE SPACED TREES

6.3.1 Climatic data

The long term pattern of climatic conditions at the experimental site was presented in chapter 3 (Section 3.4.1). Long term average annual precipitation (mean of 30 years) is 966 mm, of which 541 mm fall during the main growing period 1 September to 31 March. The average annual pan evaporation is 995 mm, the highest occurring in January with 157 mm. Mean minimum and maximum temperatures are 4.5 and 22.6°C respectively.

Flowering time of 'Sundrop' in this orchard is usually in late August. The fruit grows until mid January which is the normal harvest time. Approximate timing for stage I (in September and October) and II (November) is 3 months and stage III normally starts in December (see chapter 5). Irrigation was withheld from RDI treatments during stages I and II then resumed in both seasons in early stage III of fruit growth (from 92 dafb).

6.3.1.1 Season 1990-1991

The climatic record of the first season of experiment is shown in Figure 6.1. The annual precipitation in this year was 948 mm, of which 369 mm fell between September and the end of January. However, rainfall was not well distributed during this period, as shown by the daily rainfall and evaporation (Figure 6.2). The monthly rainfalls in this period were 16.9, 83.7, 98.3, 50.7 and 120.2 mm in September, October, November, December and January respectively.

The maximum temperature (22.2°C) was registered during the harvest period in January with high evaporation (163 mm). The sunshine hours and relative humidity (%RH) were also recorded and are shown in Figure 6.1 c. and d.

6.3.1.2 Season 1991-1992

Figure 6.3 shows the climatic record for the second season of experiment. The annual rainfall in this season was 1028 mm, of which 385.4 mm fell between September and the end of January (65.1, 80.9, 81, 81.2 and 77.2 mm monthly means respectively). The daily rainfall and evaporation (Figure 6.4) showed that rainfall in this season was more evenly distributed through the 3 stages of fruit growth than in the first season. However, in each stage of fruit growth

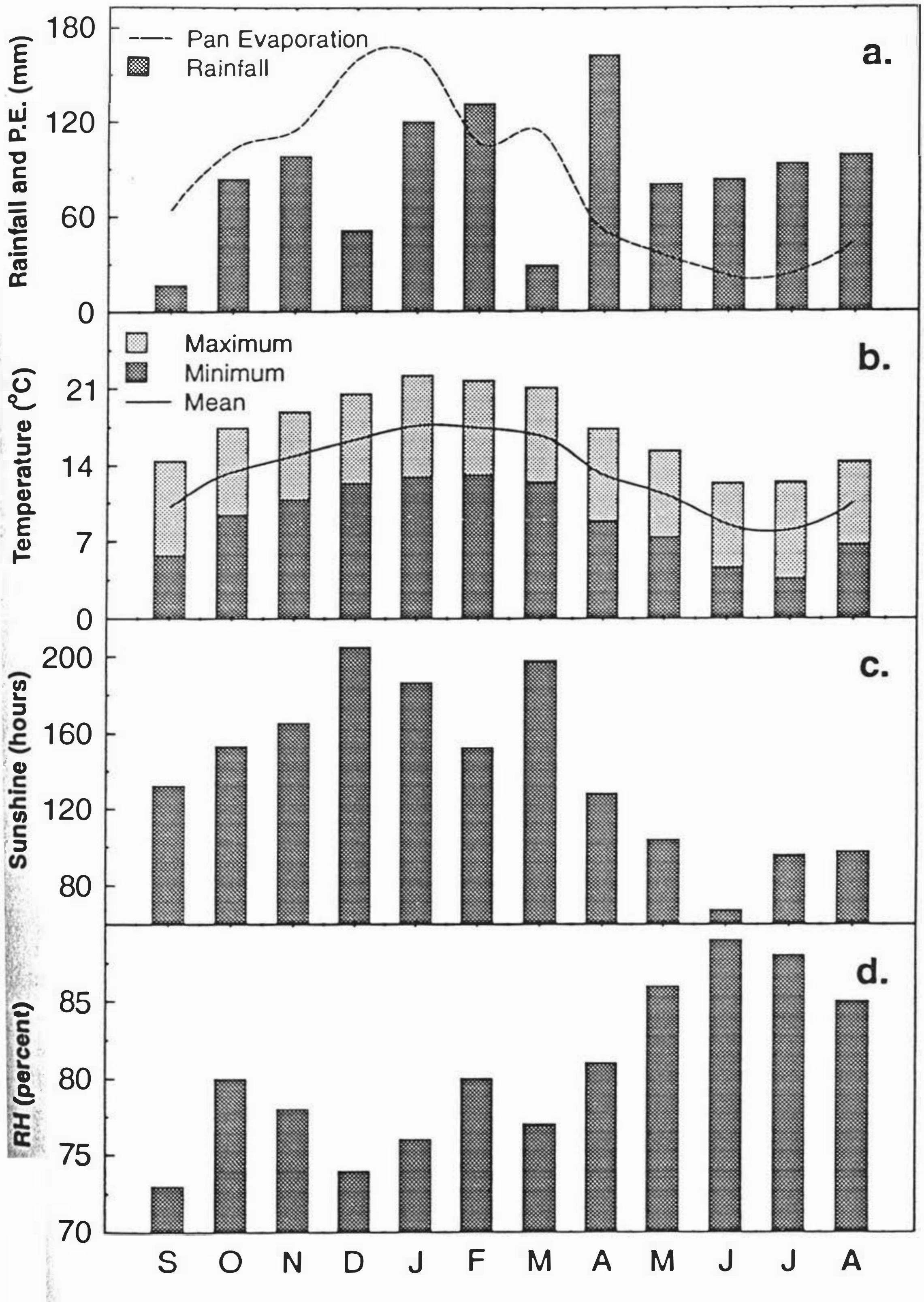


Figure 6.1 The monthly climatic data for the 1990-1991 season (1 Km away from the experimental site) at Palmerston North, New Zealand. a). Rainfall and pan evaporation (P.E.); b). Temperature; c.). Sunshine hours; d). Relative humidity (RH).

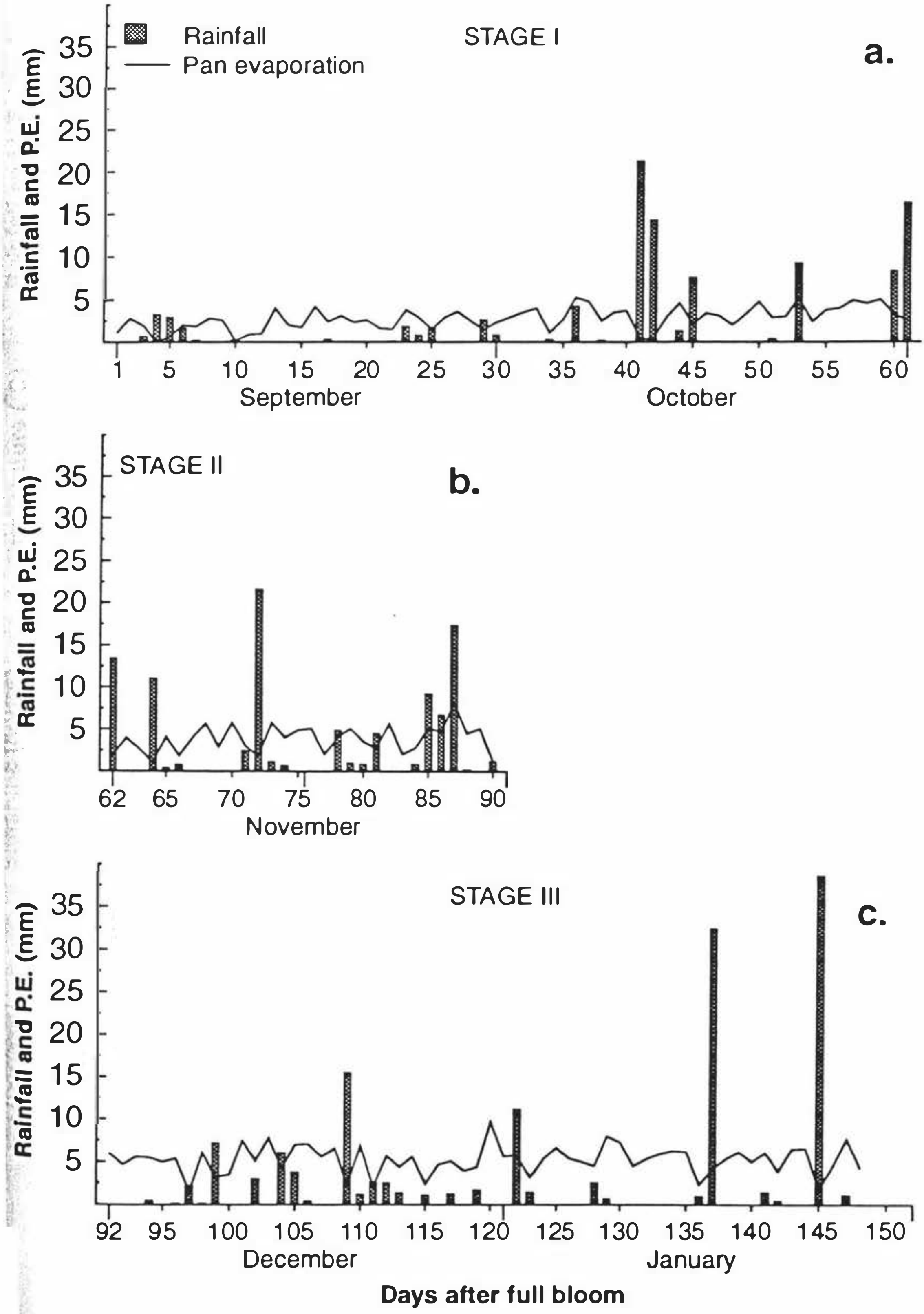


Figure 6.2 Daily rainfall and pan evaporation (P.E.) during 1990-1991 season (1 Km away from the experimental site) at Palmerston North, New Zealand. a). During stage I; b). During stage II; and c). During stage III of fruit growth

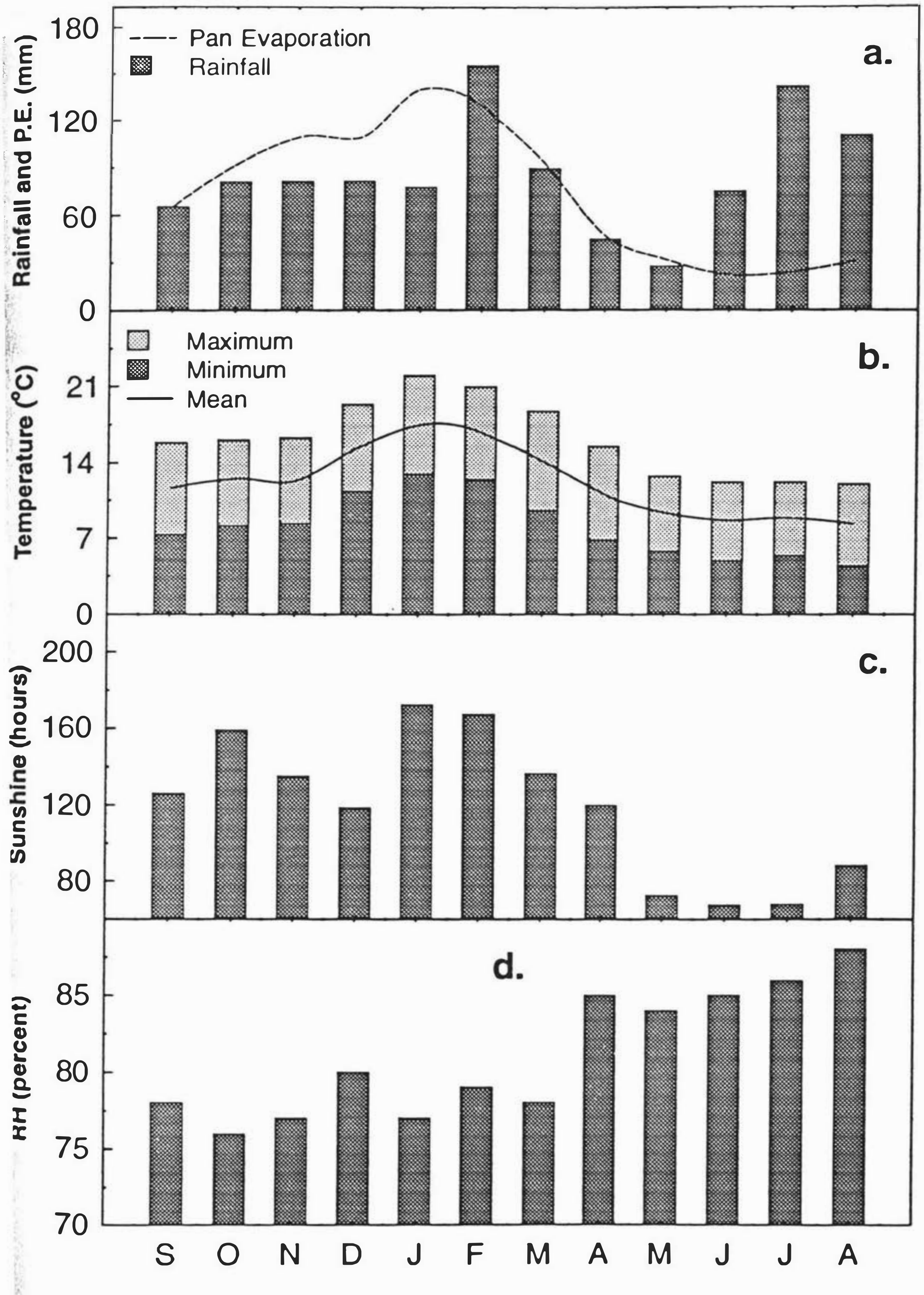


Figure 6.3 The monthly climatic data for the 1991-1992 season (1 Km away from the experimental site) at Palmerston North, New Zealand. a). Rainfall and pan evaporation (P.E.); b). Temperature; c.). Sunshine hours; d). Relative humidity (RH).

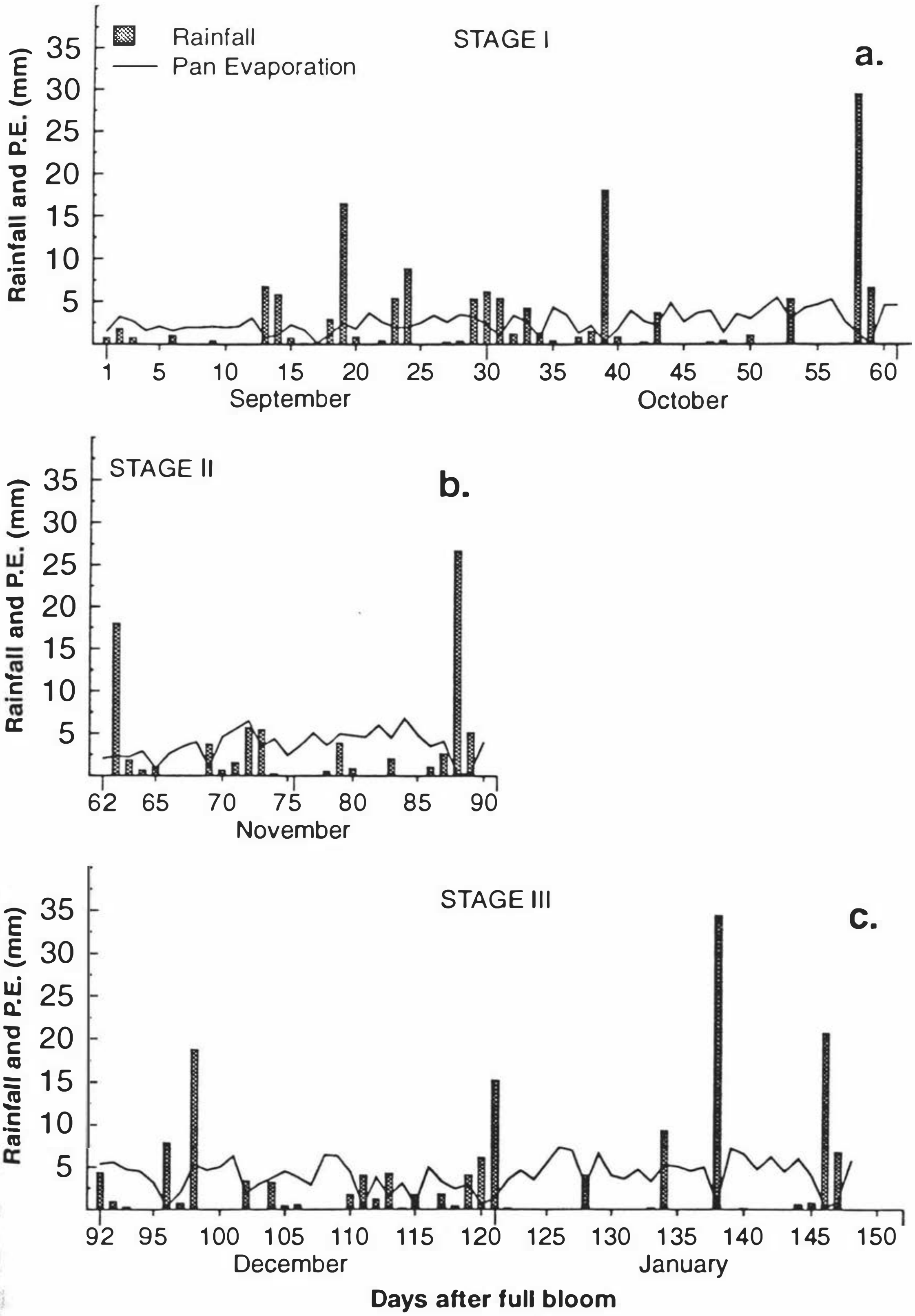


Figure 6.4 Daily rainfall and pan evaporation (P.E.) during 1991-1992 season (1 Km away from the experimental site) at Palmerston North, New Zealand. a). During stage I; b). During stage II; and c). During stage III of fruit growth

torrential rainfall occurred. This season was also cooler than the first season with higher rainfall and lower evaporation in January (140.3 mm), the maximum temperature 21.9 °C in this month. The sunshine hours were lower and RH generally greater than during the first season throughout the fruit growth period (Figure 6.3).

6.3.2 Soil moisture

The soil type of the experimental site was described in chapter 3 (Section 3.4.2). Field capacity (FC) in the 0-100 cm soil depth was 316 mm water, of which 182 mm existed in the top 50 cm layer of soil and 134 mm in the 50-100 cm soil depth.

6.3.2.1 Season 1990-1991

The record of total soil moisture in this season is shown in Figure 6.5. In general at all depths and distances the root-pruned treatment showed higher soil moisture content throughout the season. However, the differences between this treatment and control were significant in the region beyond the pruning cut only during stage III of fruit growth ($P<0.05$ at 104, 111 dafb; $P<0.01$ at 120 and $P<0.001$ at 130 and 137 dafb, Figure 6.5 b.). Differences between root-pruned and control in the "a." , "c." and "d." regions were not significant. When data was pooled for all regions the differences between root-pruned and control disappeared (Figure 6.5 e.).

The RDI treatment showed lower levels of soil moisture than control and root-pruned throughout the season ($P<0.01$) when analysis was performed on the pooled data (Figure 6.5 e.). However, in the top 50 cm layer of the soil and closer to the tree trunk (Figure 6.5 a.) RDI showed lower soil moisture ($P<0.05$) than root-pruned and control during stages I and II of fruit growth. Deeper in the soil (50-100 cm soil depth) and closer to the trunk (Figure 6.5

c.) no significant differences were observed among the treatments until the end of the season when the root-pruned treatment showed a higher soil moisture than RDI ($P<0.05$ at 130 and 137 dafb). In the "b." soil region (Figure 6.5 b.) both control and root-pruned showed more soil moisture than RDI throughout the season ($P<0.05$ for 48, 61, and 89 dafb and $P<0.001$ at all other dates). In the "d." region (Figure 6.5 d.) RDI showed significantly lower soil moisture than control and root-pruned until 54 dafb ($P<0.05$), and than root-pruned again late in stage III ($P<0.05$ at after 111 dafb).

6.3.2.2 Season 1991-1992

The soil moisture for this season is shown in Figure 6.6. RDI soil moisture was significantly lower than control and root-pruned until early stage III, 102 dafb ($P<0.001$, Figure 6.6 e.). Soil moisture content for root-pruned trees was significantly higher than control only during stage I, until 60 dafb ($P<0.05$).

The greater soil drying for RDI trees was mainly accounted for by the increased water loss from the top 50 cm of soil (Figure 6.6 a.). Close to the trunk RDI was drier ($P<0.05$) than root-pruned and control only until 38 dafb (Figure 6.6 a.), but in the more distant "b." region it was drier than both until early stage III (Figure 6.6 b., $P<0.01$ until 102 dafb). At greater depth, in the inner region (Figure 6.6 c.) moisture differences between treatments did not attain significant levels, and further out (Figure 6.6 d.) the only significant differences ($P<0.05$) occurred until 53 dafb, root-pruned being more moist than RDI.

6.3.3 Xylem water potential

6.3.3.1 Season 1990-1991

Seasonal changes in predawn and midday xylem water potential (Ψ_{xylem}) during

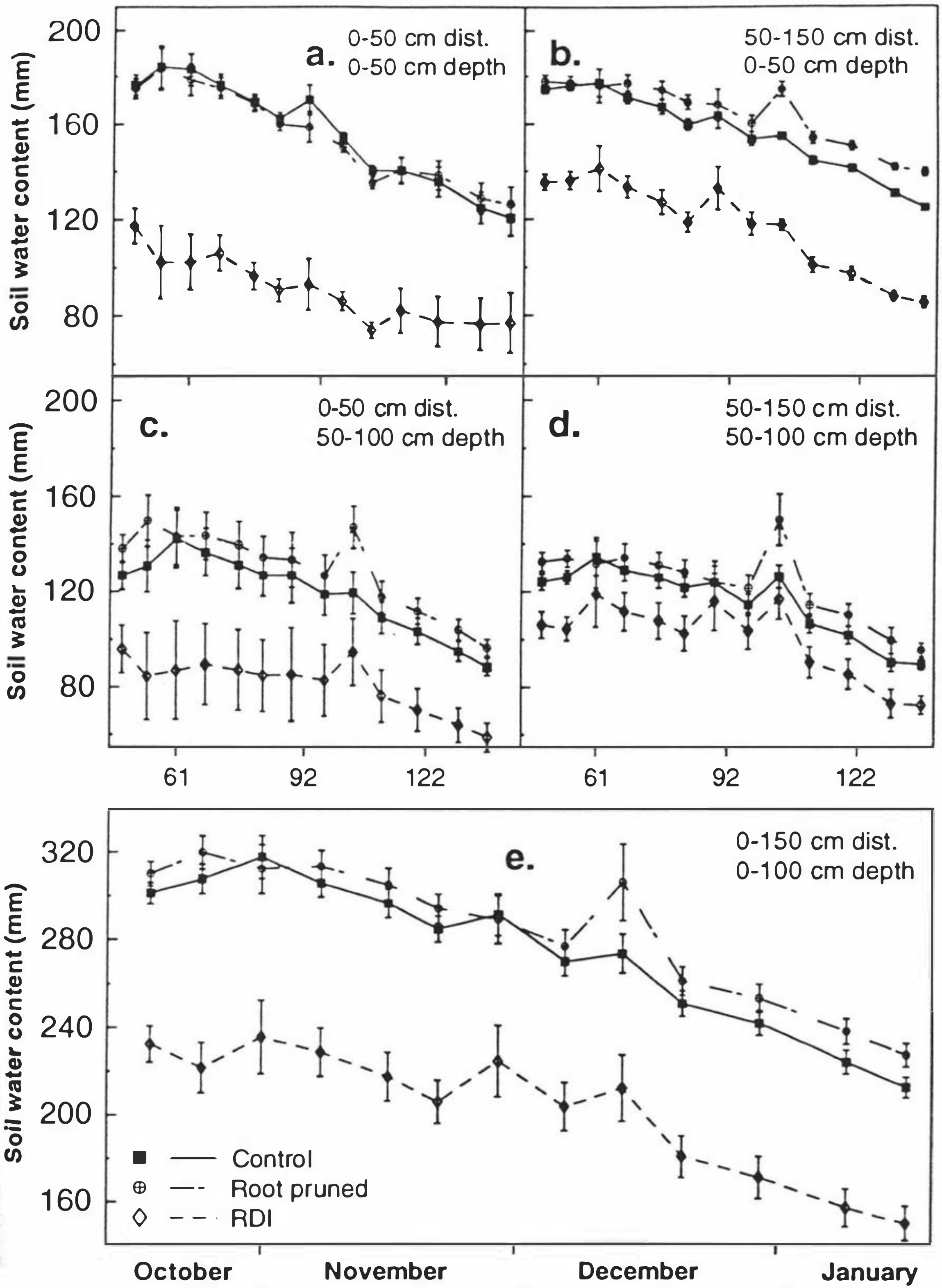


Figure 6.5 The influence of treatments on soil water content (mm) of close spaced 'Sundrop' during 1990-1991 season. a). and b). Top 50 cm layer of soil. c). and d). Lower 50 cm layer of soil and e). Top 100 cm of soil (Dist. is distance from tree trunk). Bars show the standard error of the mean.

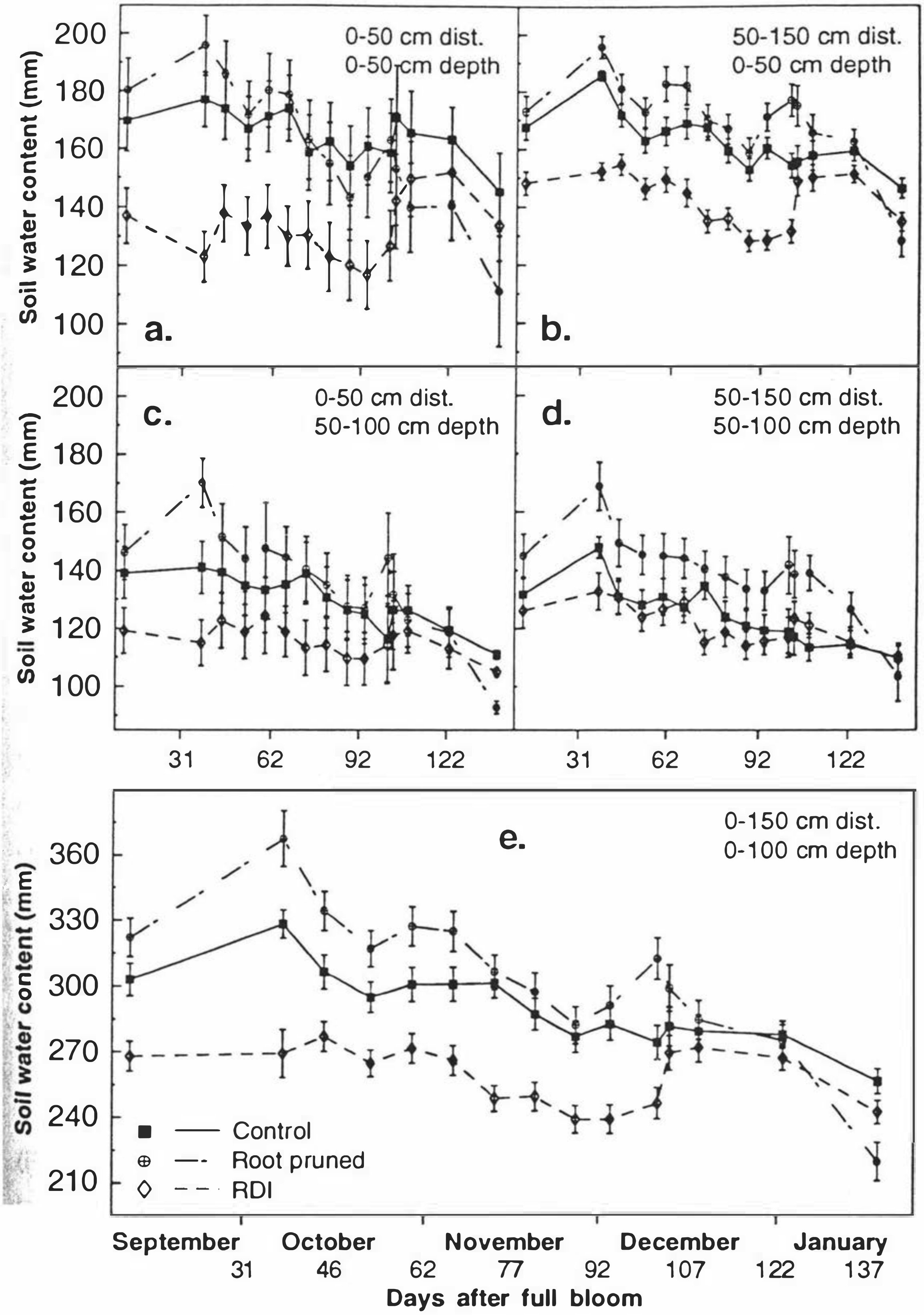


Figure 6.6 The influence of treatments on soil water content (mm) of close spaced 'Sundrop' during 1991-1992 season. a). and b). Top 50 cm layer of soil. c). and d). Lower 50 cm layer of soil and e). Top 100 cm of soil. (Dist. is distance from tree trunk). Bars show the standard error of the mean.

the first season are shown in Figure 6.7. In general no differences were detected either at predawn or midday between control and H-PBZ treatments. Root-pruning had no significant effect on predawn Ψ_{xylem}, but midday Ψ_{xylem} was lower than control ($P<0.05$) throughout the season. RDI had lower predawn Ψ_{xylem} than control during early stage II (72 and 76 dafb) and early stage III ($P<0.05$). RDI midday Ψ_{xylem} was significantly lower than control during later stage II ($P<0.05$ from 79 to 90 dafb) and in mid-stage III of fruit growth ($P<0.05$ from 105 to 119 dafb except day 112).

6.3.3.2 Season 1991-1992

The pattern of predawn and midday xylem water potential (Ψ_{xylem}) in the second season is shown in Figure 6.8. Both root-pruned and RDI showed lower predawn Ψ_{xylem} than control ($P<0.01$) at all dates of measurement (except for day 101 when root-pruned and control were similar), and RDI was similarly significantly lower ($P<0.01$) than H-PBZ. Predawn root-pruned showed lower Ψ_{xylem} than H-PBZ at 83, 104 and 110 dafb ($P<0.01$). At midday (Figure 6.8 b.) root-pruned trees Ψ_{xylem} was always significantly lower ($P<0.01$) than control and H-PBZ (except on 62 and 143 dafb). RDI treatment did not significantly reduce noon Ψ_{xylem} except during stage II (69 and 83 dafb) and early stage III (96 and 101 dafb, at $P<0.05$).

6.3.3.2.1 Diurnal pattern of xylem water potential

During the 1991-1992 season, Ψ_{xylem} was recorded diurnally on 4 occasions (Figure 6.9). In all treatments and dates Ψ_{xylem} reached its most negative value by 3 pm and then started to recover. On all occasions root-pruned treatment had lower Ψ_{xylem} than control by 3 pm ($P<0.01$), and RDI was also significantly lower ($P<0.05$) than control except at 83 dafb. During early morning control had higher Ψ_{xylem} than root-pruned and RDI treatments ($P<0.05$), except on day 101 when root-pruned and control were similar.

6.3.4 Net CO_2 assimilation (A) and stomatal conductance (g_s)

6.3.4.1 Season 1990-1991

Figure 6.10 shows the stomatal conductance and CO_2 assimilation rate during stage III of fruit growth. There were no significant differences among the treatments for g_s (Figure 6.10 a.). RDI showed lower A (Figure 6.10 b.) than H-PBZ at 112 dafb, and at 140 dafb than control treatment (both $P<0.05$).

6.3.4.2 Season 1991-1992

The influence of treatments on g_s and A during stage III in this season are shown in Figure 6.11. L-PBZ treatment increased both g_s and A values during stage II and early stage III of fruit growth relative to control ($P<0.05$ on day 55 and $P<0.01$ at 76, 83, 101 and 110 dafb). In early stage II (69 and 76 dafb) both RDI and root-pruned showed greater g_s than control ($P<0.05$), but this did not increase A during these periods.

6.3.4.2.1 Diurnal pattern of net CO_2 assimilation rate and stomatal conductance

The diurnal records of A and g_s determined on four occasions during 1991-1992 are shown in Figures 6.12 and 6.13 respectively. Generally, g_s had lowest value for all treatments during day 83 with no major changes during the course of the day for any treatment. The review of diurnal climatic data (from the nearest weather station) showed that RH (57%) was lower on 83 dafb than on 69, 101 and 110 dafb (78%), 4.5 h sunshine were registered on day 69 and 9 h for the other occasions. During stage II of fruit growth usually no significant treatment differences occurred in A and g_s. During stage III a stronger diurnal pattern occurred in A and g_s with maxima at 1300 - 1500 h which were generally higher than in stage II, but treatment differences usually were not significant (Figures 6.12 and 6.13).

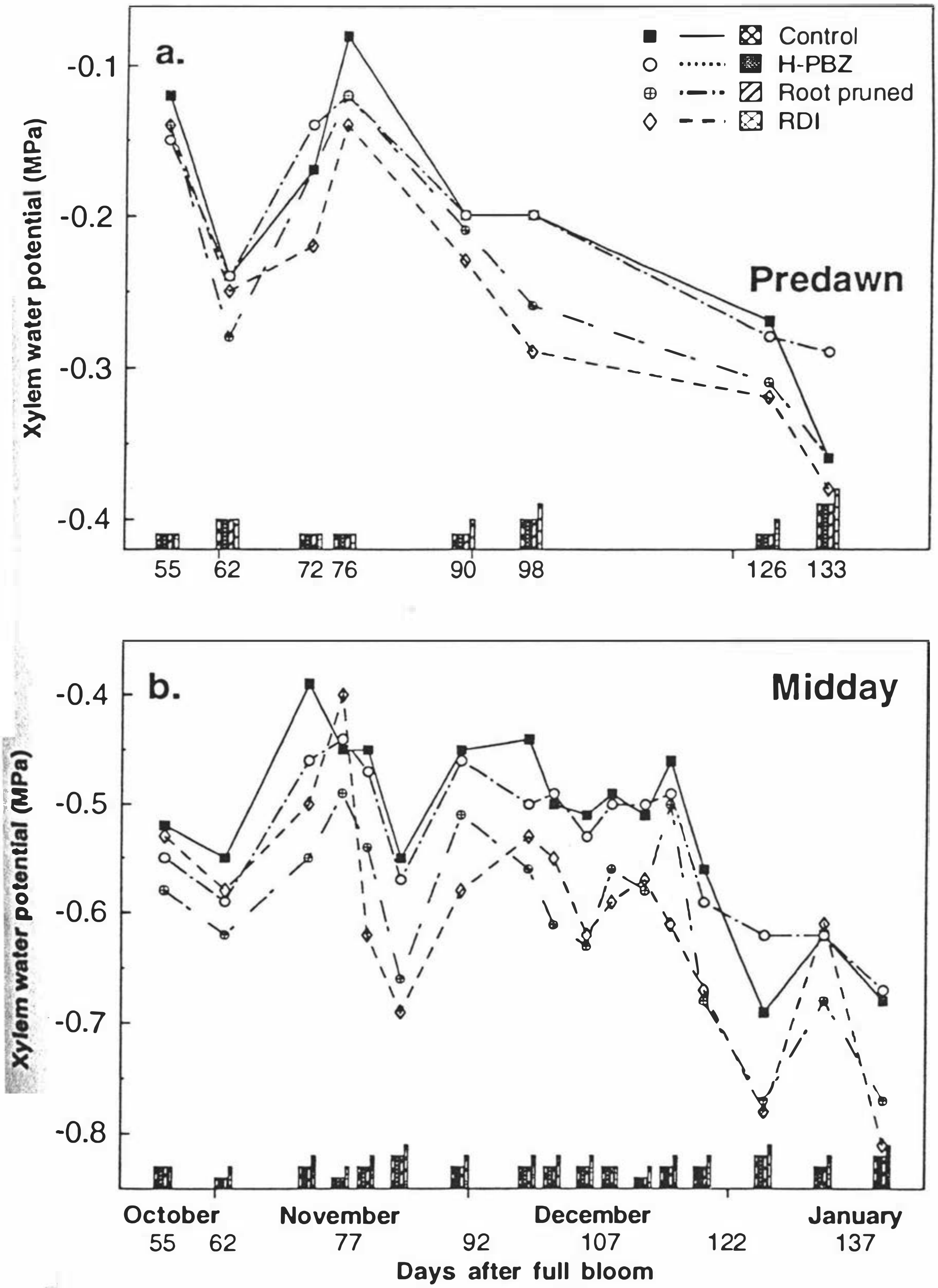


Figure 6.7 The influence of treatments on xylem water potential (ψ_{xylem}) in close spaced trees in 1990-1991 season. a). Predawn xylem water potential; b). Midday xylem water potential. Bars show the standard error of the mean. Each point represents the mean of two leaves on each replicate (n=16 for Control, H-PBZ and Root pruned; and n=10 for RDI treatment).

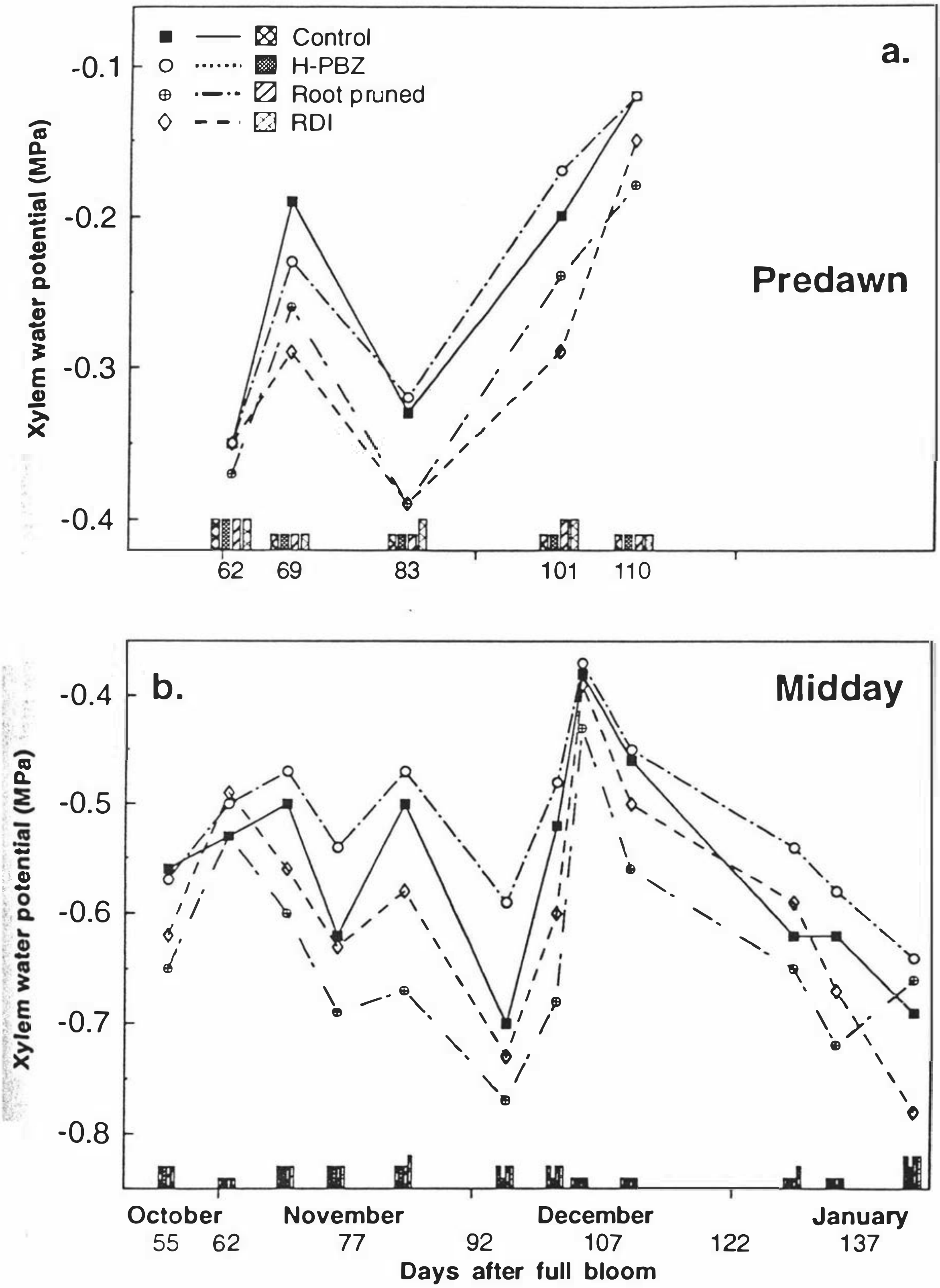


Figure 6.8 The influence of treatments on xylem water potential (ψ_{xylem}) in close spaced trees in 1991-1992 season. a). Predawn xylem water potential; b). Midday xylem water potential. Bars show the standard error of the mean. Each point represents the mean of two leaves on each replicate (n=16 for Control, H-PBZ and Root pruned; and n=10 for RDI treatment).

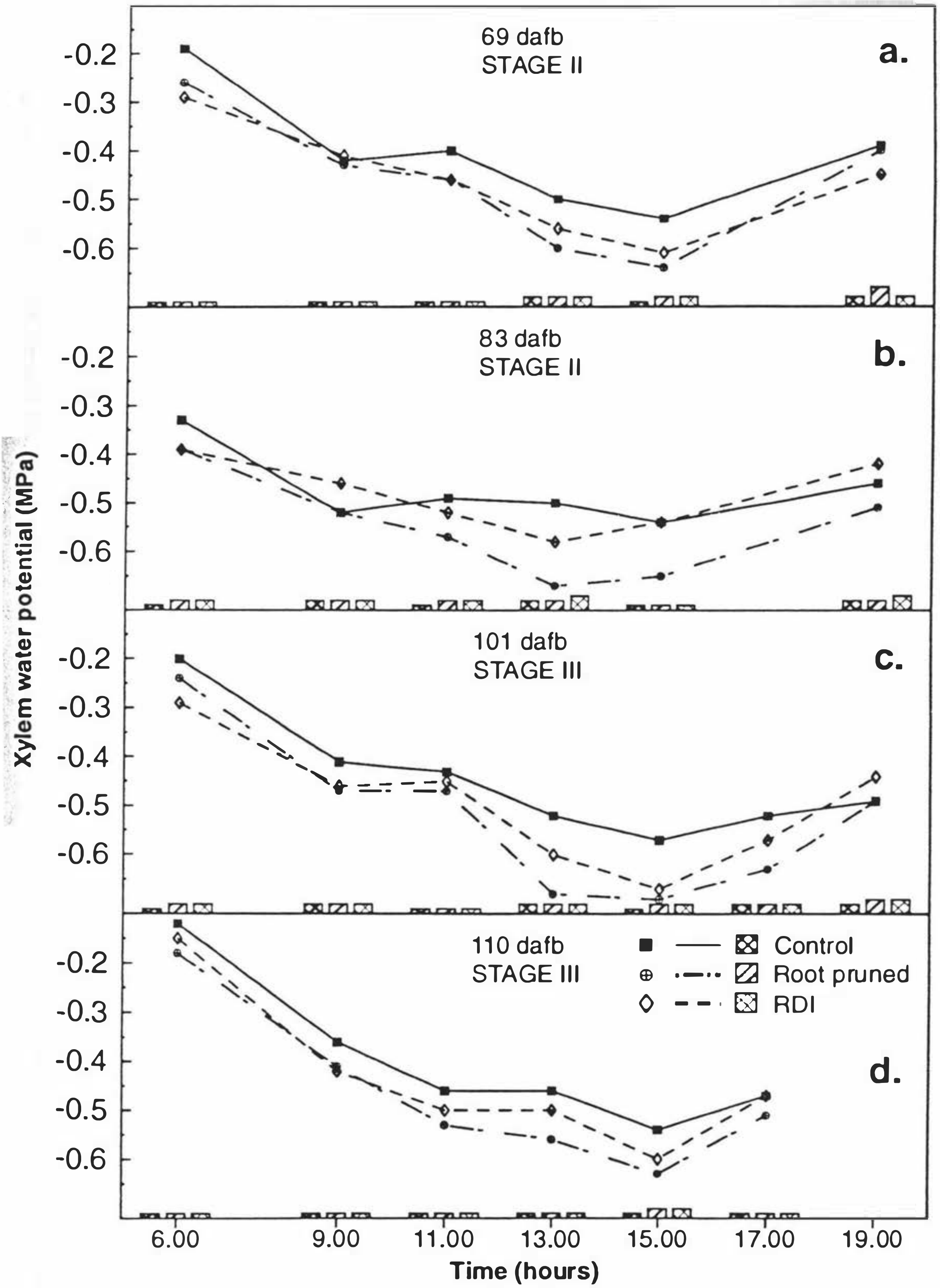


Figure 6.9 The influence of treatments on diurnal patterns of xylem water potential (ψ_{xylem}) in close spaced trees in 1991-1992 season. a). 69 dafb; b). 83 dafb; c). 101 dafb; d). 110 dafb. Bars show the standard error of the mean. Each point represents the mean of two leaves on each replicate per treatment (n=16 for Control and Root pruned; and n=10 for RDI treatment).

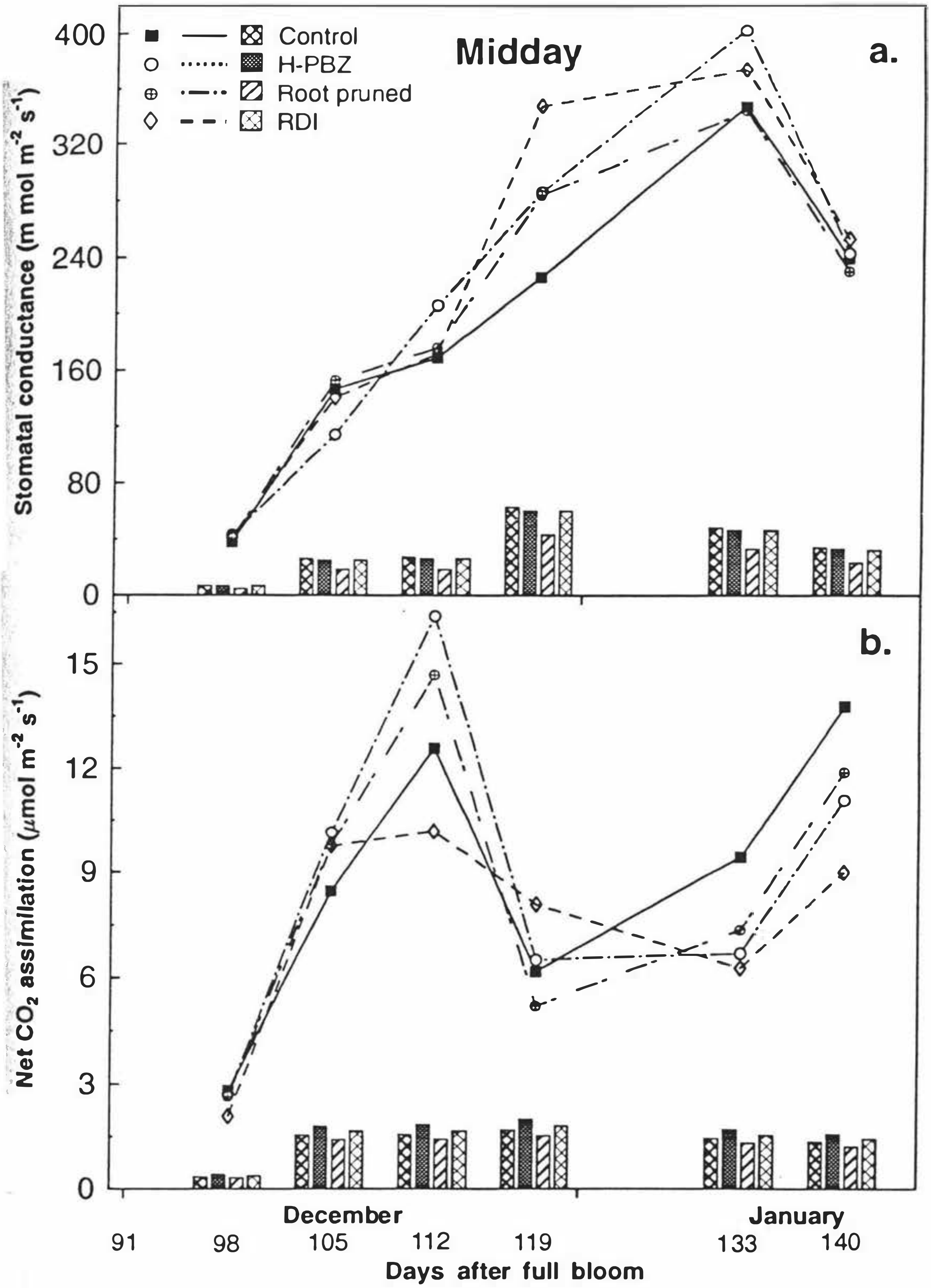


Figure 6.10 The influence of treatments on a). Stomatal conductance (g_s) and b). Net CO_2 assimilation (A) of close planted 'Sundrop' apricot trees during stage III of fruit growth in 1990-1991 season. Bars show the standard error of the mean. Each point represents the mean of two leaves on each replicate per treatment (n=16 for Control, H-PBZ and Root pruned; and n=10 for RDI treatment).

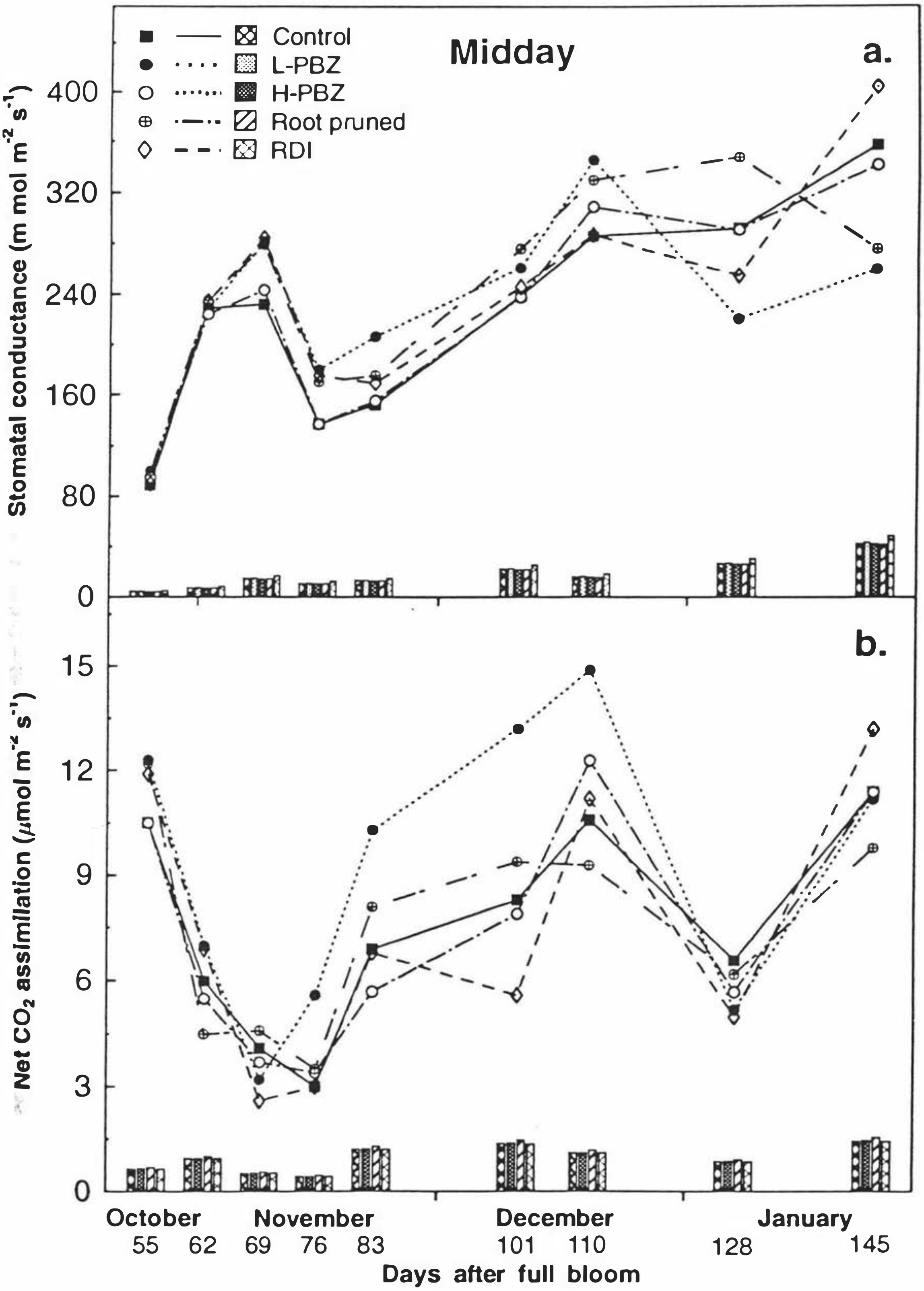


Figure 6.11 The influence of treatments on a). Stomatal conductance (g_s) and b). Net CO_2 assimilation (A) of close planted 'Sundrop' apricot trees in 1991-1992 season. Bars show the standard error of the mean. Each point represents the mean of two leaves on each replicate per treatment (n=16 for Control, L-PBZ, H-PBZ and Root pruned; and n=10 for RDI treatment).

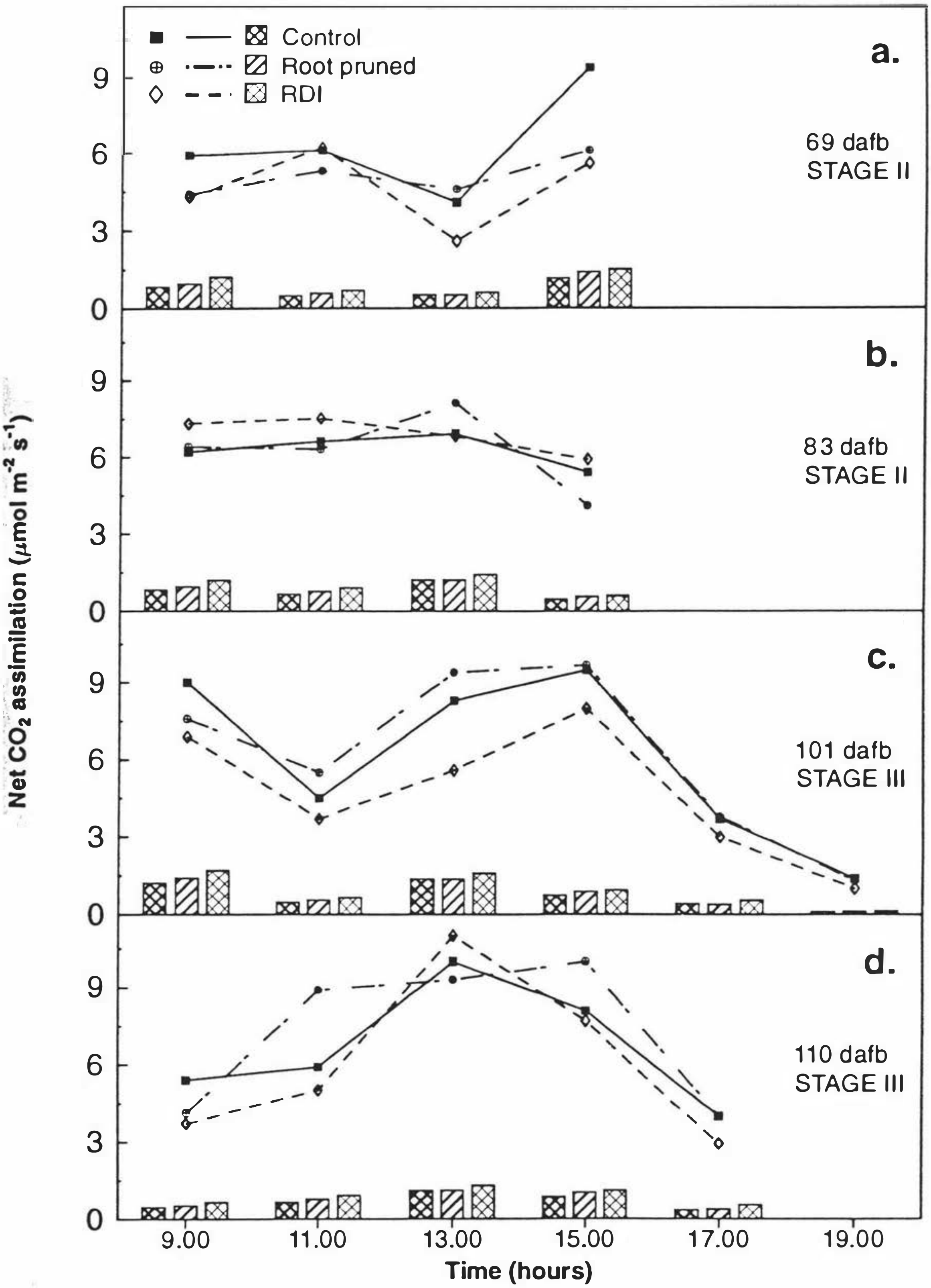


Figure 6.12 The influence of treatments on diurnal patterns of net CO_2 assimilation (A) of close spaced trees in 1991-1992 season. a). 69 dafb; b). 83 dafb; c). 101 dafb; d). 110 dafb. Bars show the standard error of the mean. Each point represents the mean of two leaves on each replicate per treatment (n=16 for Control and Root pruned; and n=10 for RDI treatment).

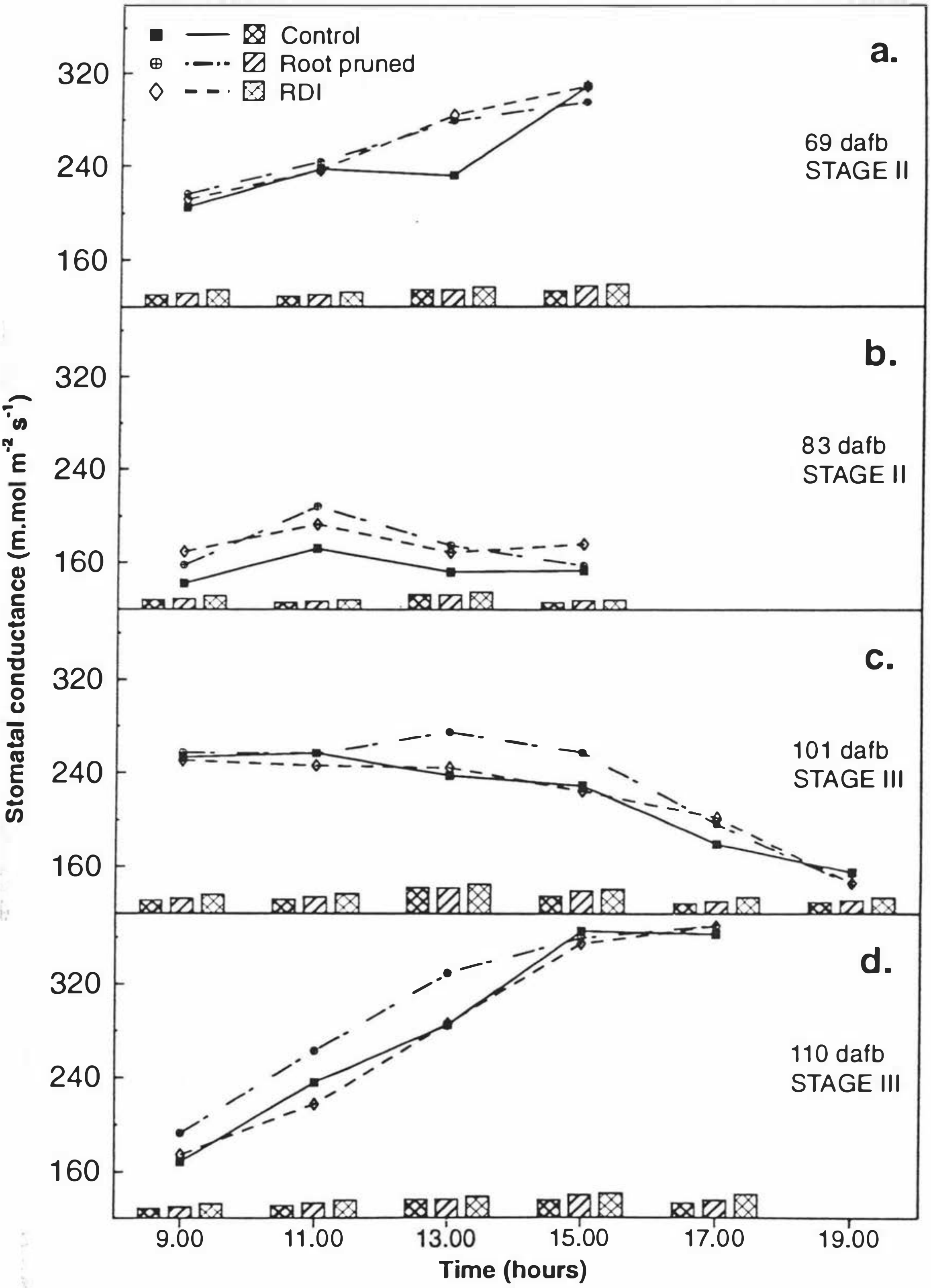


Figure 6.13 The influence of treatments on diurnal patterns of stomatal conductance (g_s) of close spaced trees in 1991-1992 season. a). 69 dafb; b). 83 dafb; c). 101 dafb; d). 110 dafb. Bars show the standard error of the mean. Each point represents the mean of two leaves on each replicate per treatment (n=16 for Control and Root pruned; and n=10 for RDI treatment).

6.3.5 Osmotic adjustment (Season 1991-1992 only)

6.3.5.1 Osmotic adjustment of leaf

The seasonal records of leaf predawn and midday water status (Ψ_l, Ψ_p and Ψ_s) are shown in Figures 6.14 and 6.15. Treatment differences in Ψ_l, Ψ_p and Ψ_s either at predawn or midday during stage I were not significant. During stage II of fruit growth RDI showed a lower predawn Ψ_l on 68 dafb (P=0.077) however its Ψ_p was also lower than control at this time ($P<0.05$) with no difference in Ψ_s (Figure 6.14). In stage II at midday all leaf water status components were similar (Figure 6.15). When midday leaf Ψ_s was adjusted (Figure 6.16 b.) for relative water content (RWC), Ψ_s of RDI (-2.05 MPa) showed a significant difference from control (-1.83 MPa) on 75 dafb ($P<0.05$).

In stage III of fruit growth predawn Ψ_l of all treatments were not significantly different, but RDI showed higher Ψ_p than control and root-pruned treatments ($P<0.05$) on 124 dafb (Figure 6.14). Midday RDI Ψ_l and Ψ_s during stage III was higher than control ($P<0.05$) on some occasions (108, 115 and 131 dafb). However, midday Ψ_p of control was lower than RDI on 108 dafb and was higher than root-pruned treatments on 96 and 108 dafb. Both RDI and root-pruned showed significantly lower adjusted osmotic potential than control ($P<0.05$) on 124 dafb (Figure 6.16 b.).

6.3.5.2 Osmotic adjustment of fruit

Fruit Ψ_f, Ψ_p and Ψ_s at predawn and midday during the second season are shown in Figures 6.17 and 6.18. During stage I and stage II, generally both predawn and midday Ψ_f and Ψ_s of RDI fruit were lower than control, although the only significant differences were on day 59 ($P<0.05$). However, at this date both predawn and midday Ψ_p of these two treatments were not significantly different (59 dafb). No significant differences occurred in midday

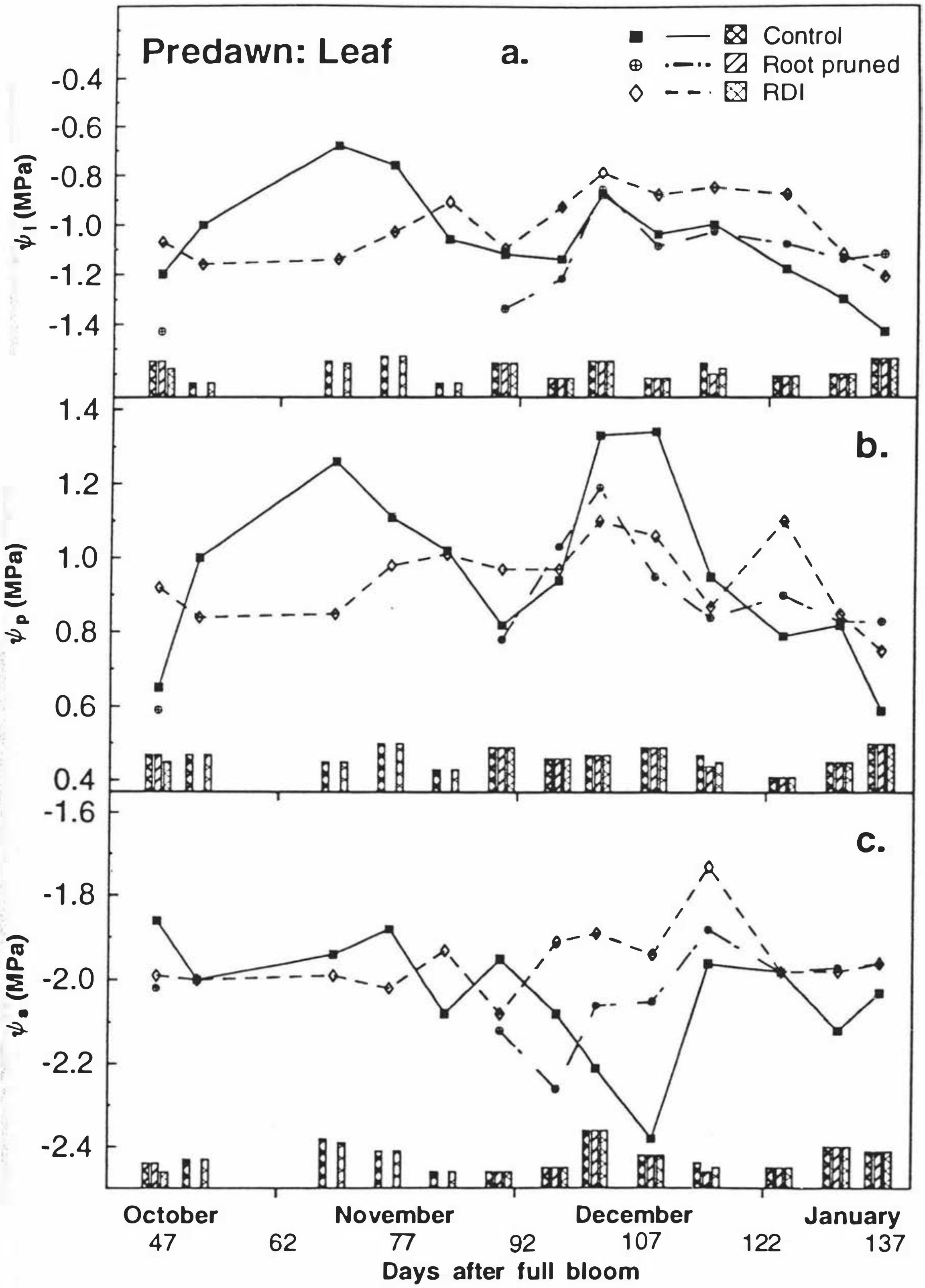


Figure 6.14 The influence of treatments on predawn leaf water relations of close spaced trees in 1991-1992 season. a). Leaf water potential (ψ_l); b). Turgor potential (ψ_p); c). Osmotic potential (ψ_s); Bars show the standard error of the mean. Each point represents the mean of one leaf on each replicate treatment^{-1} (n=5 for all treatments at each date).

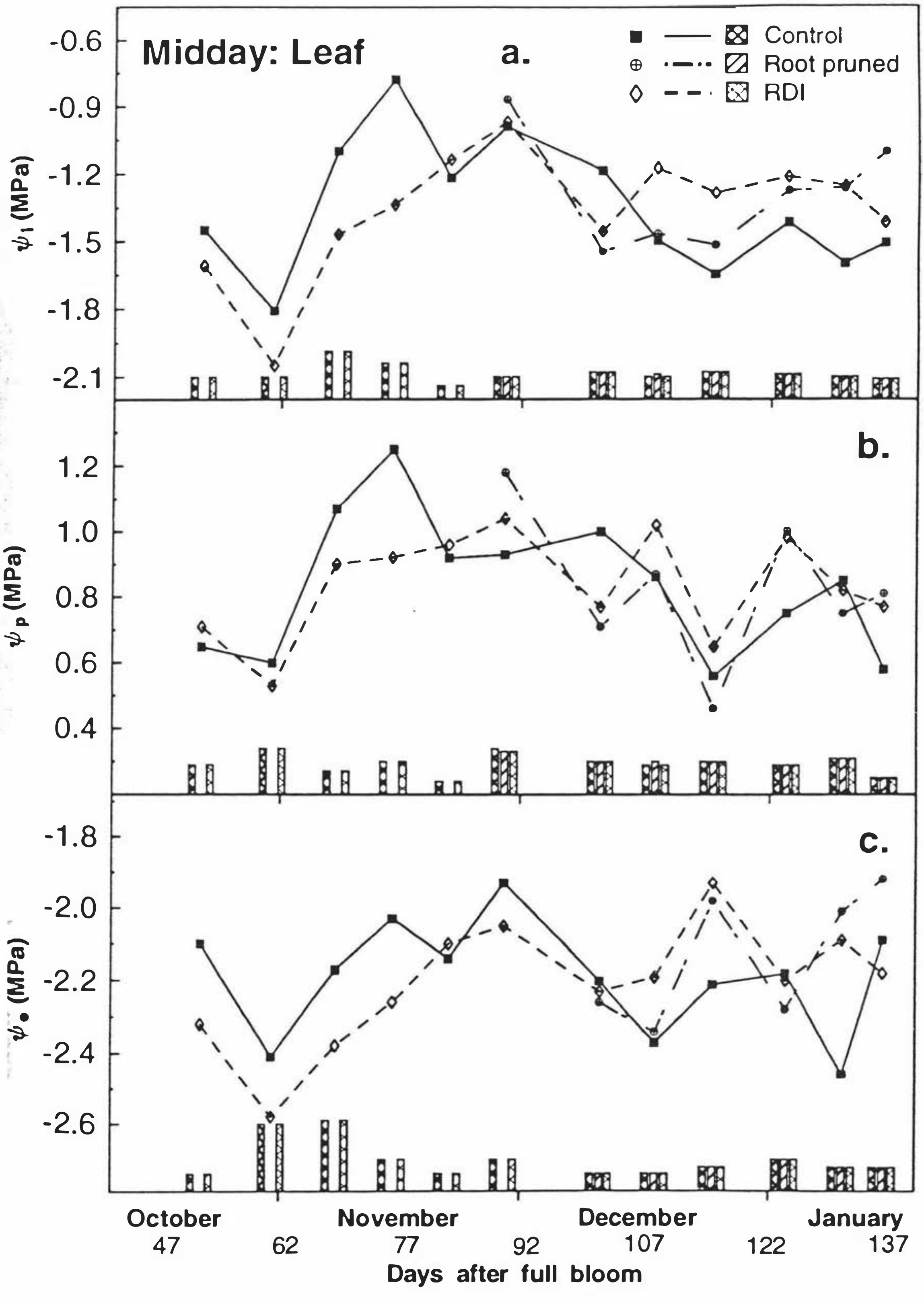


Figure 6.15 The influence of treatments on midday leaf water relations of close spaced trees in 1991-1992 season. a). Leaf water potential (ψ_l); b). Turgor potential (ψ_p); c). Osmotic potential (ψ_s); Bars show the standard error of the mean. Each point represents the mean of one leaf on each replicate per treatment (n=5 for all treatments at each date).

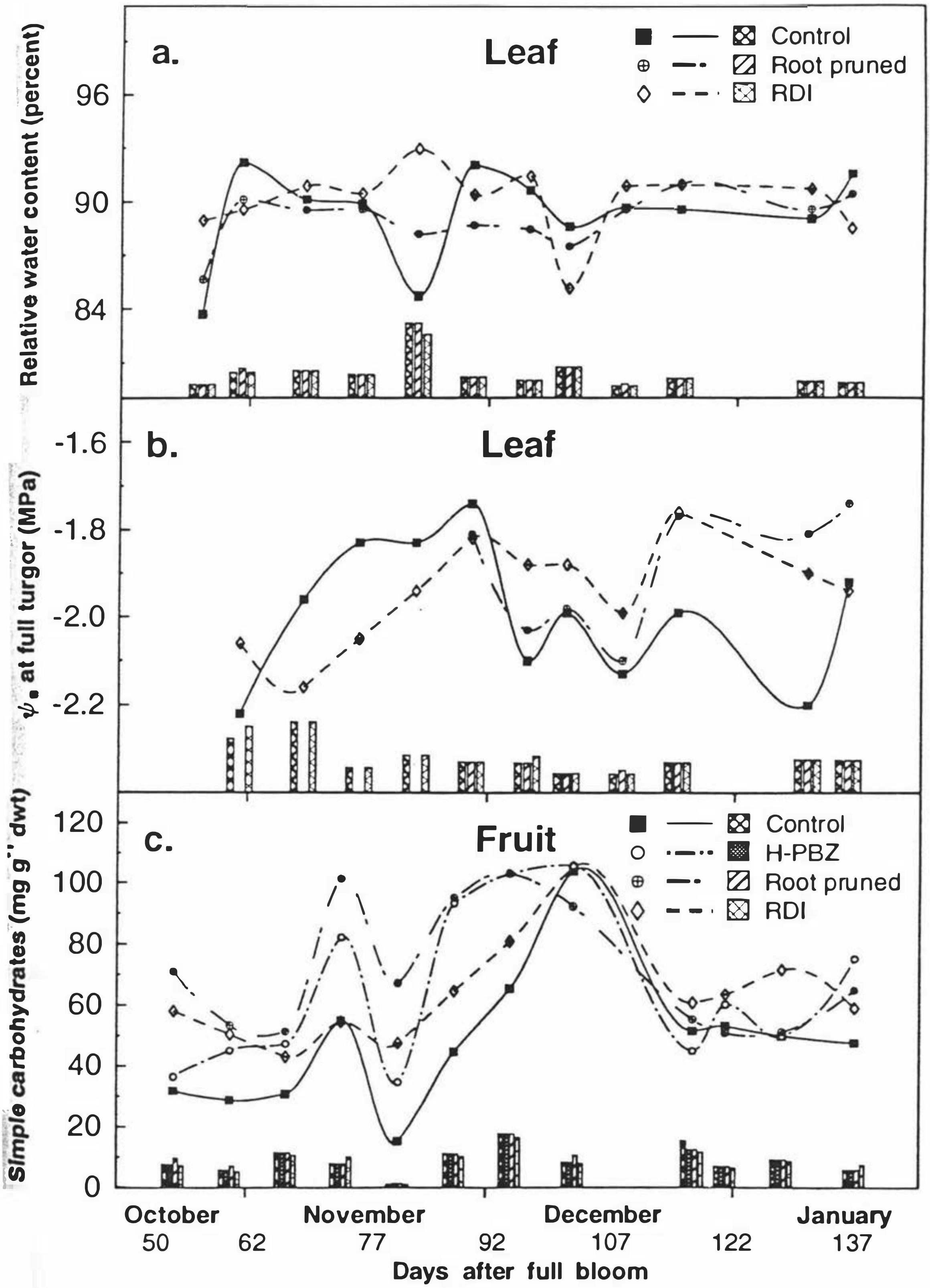


Figure 6.16 The changes of a). Relative water content (RWC); b). Osmotic potential at full turgor (ψ_s); and c). Simple carbohydrates (sucrose + glucose + fructose + sorbitol). Midday on close spaced 'Sundrop' apricot during 1991-1992 season. Bars show the standard error of the mean. Each point represents the mean of five samples treatment^{-1}.

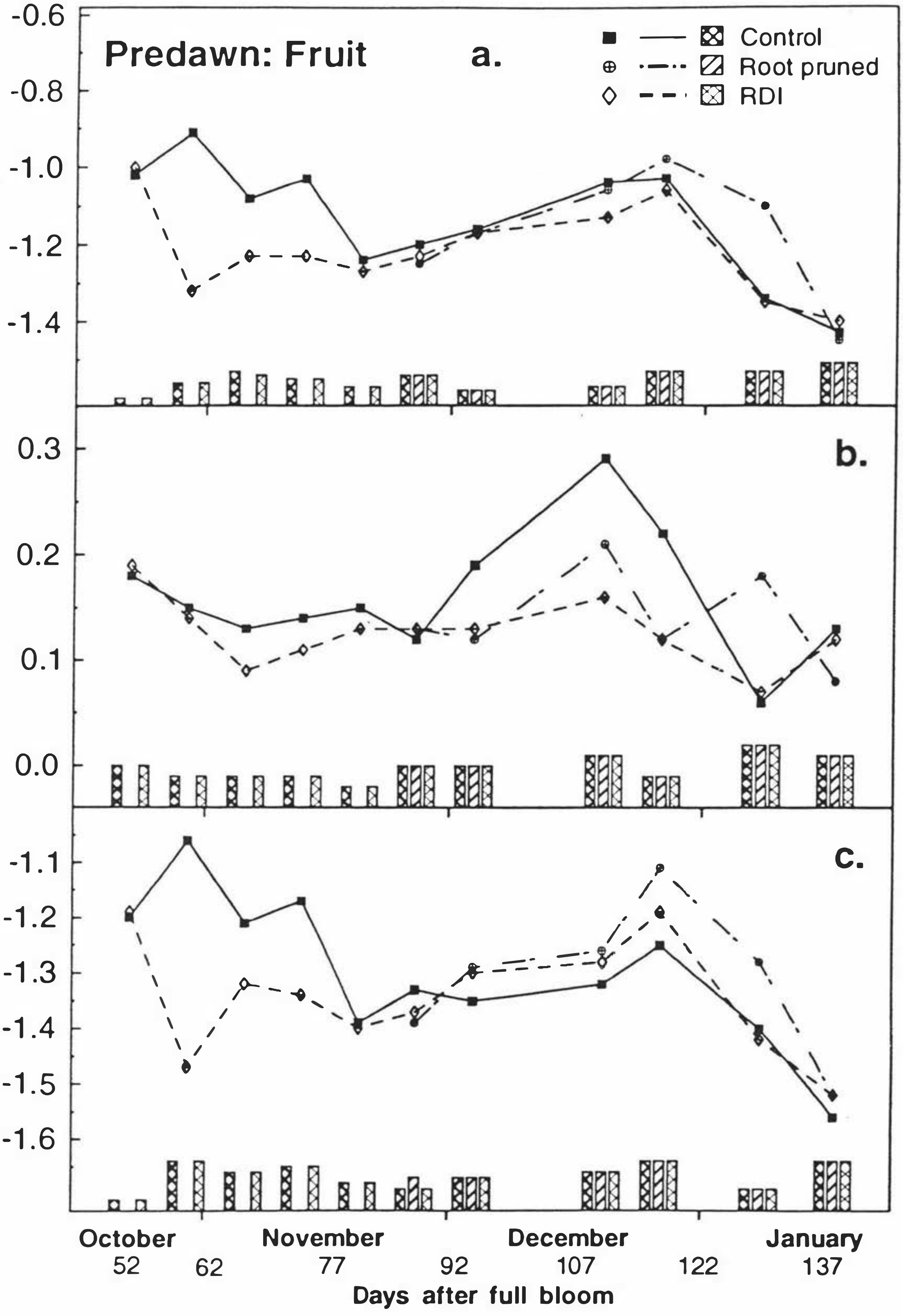


Figure 6.17 The influence of treatments on predawn fruit water relations of close spaced trees in 1991-1992 season. a). Fruit water potential (ψ_f); b). Turgor potential (ψ_p); c). Osmotic potential (ψ_s); Bars show the standard error of the mean. Each point represents the mean of one leaf on each replicate per treatment (n=5 treatment^{-1}).

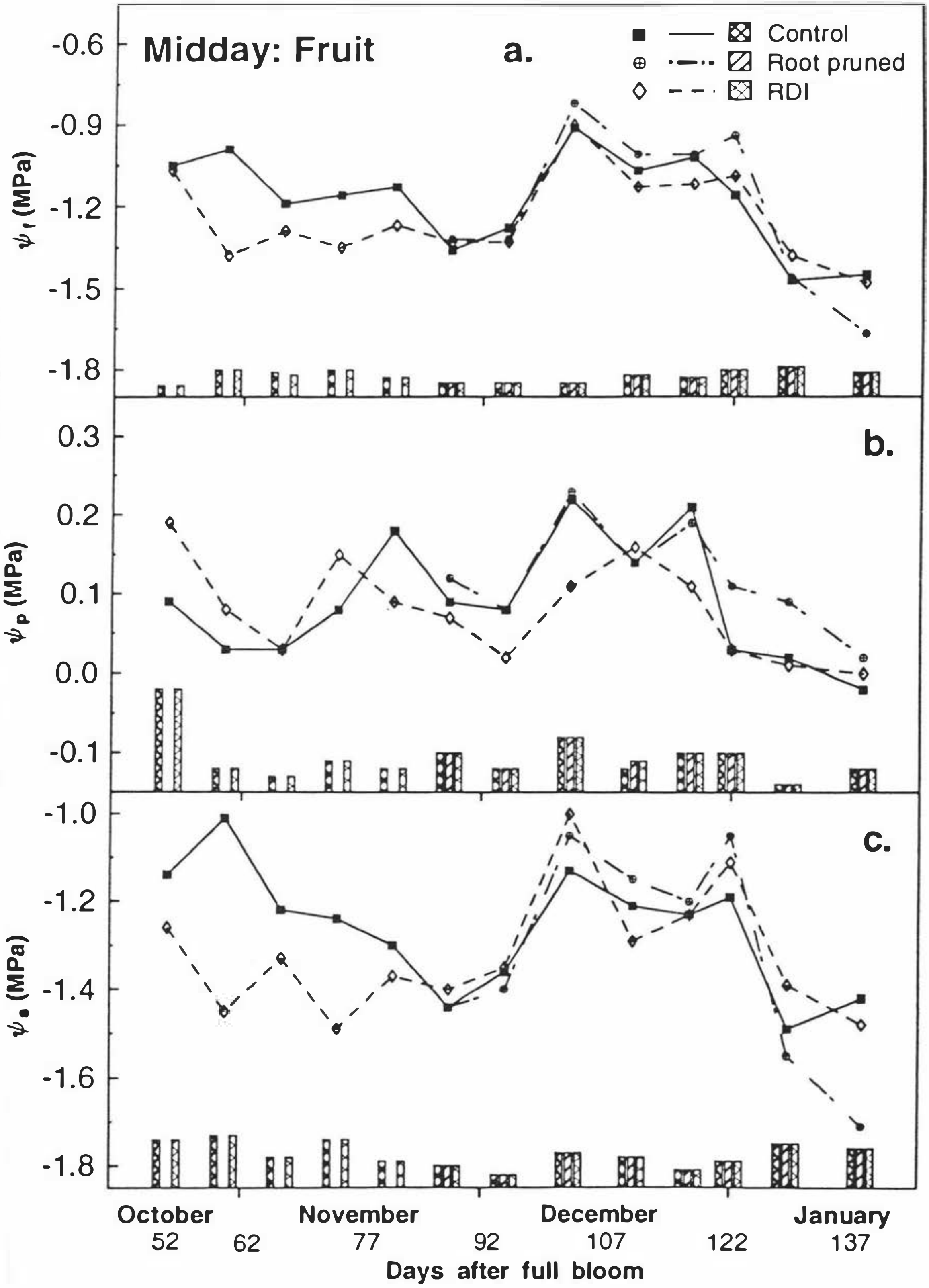


Figure 6.18 The influence of treatments on midday fruit water relations of close spaced trees in 1991-1992 season. a). Fruit water potential (ψ_f); b). Turgor potential (ψ_p); c). Osmotic potential (ψ_s); Bars show the standard error of the mean. Each point represent the mean of one leaf on each replicates per treatment (n=5 treatment^{-1}).

fruit Ψ_s among treatments during stage II of fruit growth. At the time of fruit harvest (137 dafb) root-pruned trees showed lower Ψ_t and Ψ_s than control and RDI ($P<0.05$). At the same time Ψ_p of root-pruned fruit remained at the level of control and RDI and differences between these treatments were not significant (Figure 6.18 b.).

6.3.6 Fruit simple carbohydrates (CHO)

The partial contribution of each simple carbohydrate to osmotic adjustment in fruits during stage I (59 dafb) and stage III (137 dafb) as calculated by Van't Hoff's equation in relation to the magnitude of osmotic adjustment was very small for sucrose, fructose and sorbitol. The result showed that on both dates and in all treatments (control, RDI and root-pruned) glucose had a greater influence in decreasing Ψ_s of fruits than the other recorded simple carbohydrates ($\Psi_{glucose}$ was 0.0340 and 0.0606 for control and RDI respectively on 59 dafb, and 0.0408 and 0.0560 for control and root-pruned respectively on 137 dafb).

The seasonal concentration of the fruit simple carbohydrates (sucrose, glucose, fructose and sorbitol) are shown in Figure 6.19 and 6.20. Treatments had no significant effect on sucrose concentration throughout the season (Figure 6.19 a.). Figure 6.19 b. shows that both RDI and root-pruned treatments contained more glucose than control in later stage I and II of fruit growth. The differences between root-pruned and control treatments were significant at 52, 59, 73, 80 and 87 dafb ($P<0.05$), and between RDI and control at 52, 59 and 80 dafb ($P<0.05$). In addition, root-pruned trees tended to increase fruit glucose concentration above control at the time of fruit harvest ($P=0.09$ at 137 dafb). H-PBZ increased glucose at 80, 87 and 137 dafb relative to control, but showed lower glucose at 52 and 80 dafb relative to root-pruned and RDI treatments ($P<0.05$).

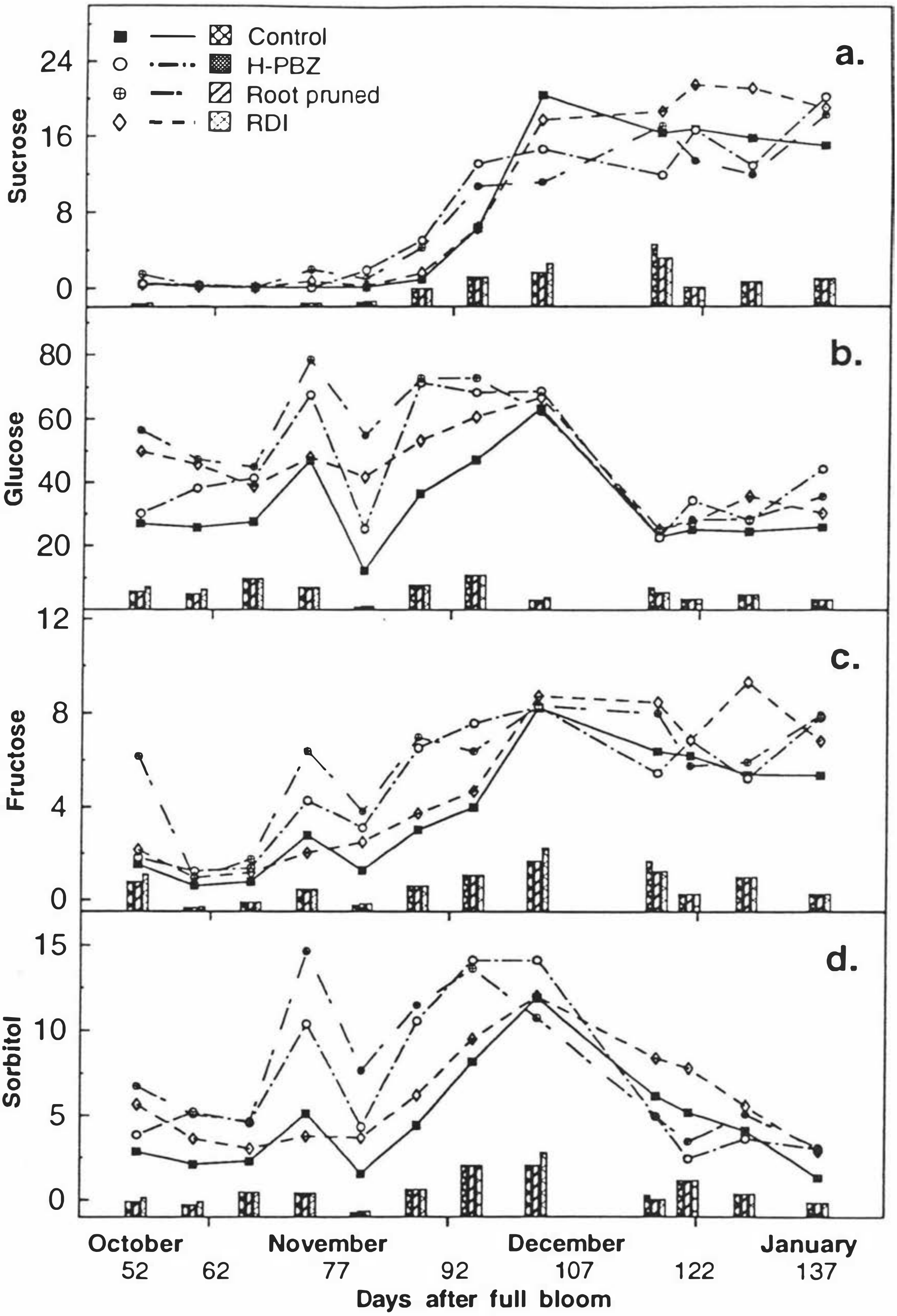


Figure 6.19 The influence of treatments on seasonal concentration of simple carbohydrates (mg g^{-1} dry weight) of close planted 'Sundrop' apricot fruit during 1991-1992 season. Bars show the standard error of the mean. Each point represents the mean of five samples.

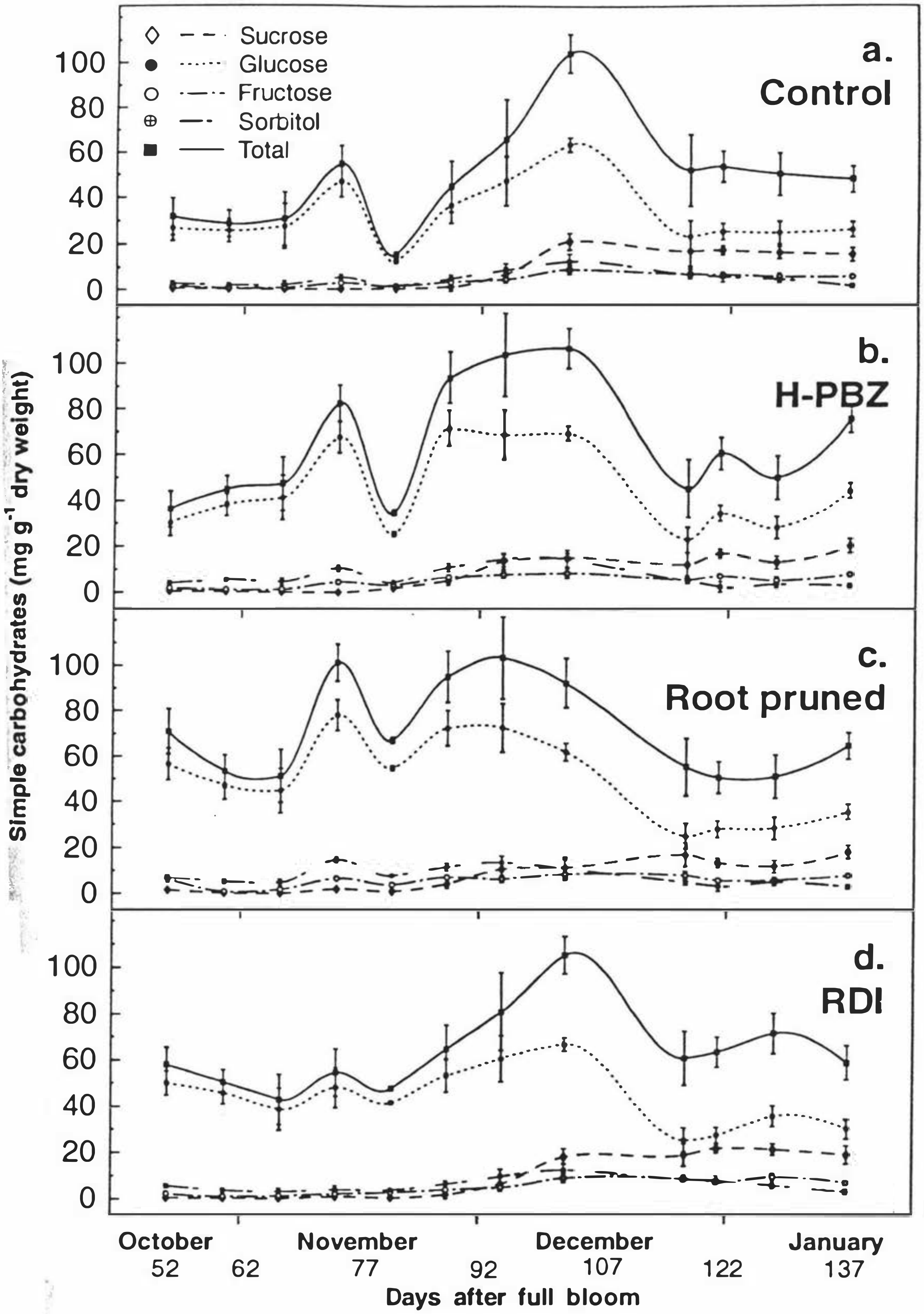


Figure 6.20 The influence of treatments on seasonal patterns of concentration of simple carbohydrates of close planted 'Sundrop' apricot fruit during 1991-1992 season. Total simple carbohydrates (CHO) is the sum of (sucrose + glucose + fructose + sorbitol). Bars show the standard error of the mean. Each point represents the mean of five samples.

During later stage II the amount of fructose (Figure 6.19 c.) was increased by all treatments relative to control. At this time significant increases relative to control occurred for the root-pruned treatment at 73, 80 and 87 dafb ($P<0.01$), for RDI at 80 dafb ($P<0.05$) and for H-PBZ at 59, 80 and 87 dafb ($P<0.01$). During later stage I and stage II of fruit growth (59, 73, 80 and 87 dafb) H-PBZ and root-pruned treatments raised the amount of sorbitol (Figure 6.19 d.) relative to control ($P<0.05$). The sorbitol in root-pruned fruit was also greater than in RDI fruit at later stage II (73 and 80 dafb, $P<0.05$). However, in stage III no significant differences were observed in sorbitol among the treatments.

Figure 6.19 and 6.20 show that the major fruit CHO throughout the season was glucose in all treatments, although there was a marked decline in stage III when sucrose increased. On day 80 late in stage II of fruit growth there was a sharp drop in total CHO of all treatments except RDI (Figure 6.16 c.). At that time fruit CHO was highest in root-pruned trees (67 mg g^{-1} dry weight, $P<0.001$), and lowest in control and H-PBZ (15 and 35 mg g^{-1} dry weight respectively). RDI total simple carbohydrates (48 mg g^{-1} dry weight) at this time was greater than that of control ($P<0.001$) and H-PBZ ($P<0.01$), but was lower than in the root-pruned treatment ($P<0.01$). The total CHO in all treatments declined in stage III (110-115 dafb), but with no significant differences among the treatments. At 137 dafb H-PBZ and root-pruned showed higher CHO than control ($P<0.05$ and $P=0.09$ for H-PBZ and root-pruned respectively).

6.4 RESULTS: WIDE SPACED TREES (SEASON 1990-1991 ONLY)

6.4.1 Soil moisture

The soil moisture content recorded in the first season of the experiment is shown in Figure 6.21. Irrigation was resumed on RDI plots at the start of stage III of fruit growth (from 92 dafb). In the RDI treatment soil moisture was lower than control in late stage I and during stage II ($P<0.01$) in the top 50 cm layer of soil (Figure 6.21 a. and b.). No differences from control were significant during stage III in the upper part of the root zone. At lower depth (50-100 cm, Figure 6.21 c. and d.) RDI and control were essentially similar, although until 54 dafb RDI had higher soil moisture content ($P<0.05$). When data for all soil depths and regions were pooled (Figure 6.21 e.) this difference disappeared, and generally the control contained greater soil moisture during stages I and II of fruit growth, this being significant between 61 and 82 dafb ($P<0.05$).

6.4.2 Xylem water potential

The only significant difference in predawn Ψ_{xylem} (Figure 6.22 a.) was that RDI was lower ($P<0.05$) than control at the end of stage II of fruit growth (90 dafb) immediately before resuming irrigation. Midday Ψ_{xylem} was lower for the RDI treatment ($P<0.05$) late in stage I and early stage II (55 to 76 dafb) and again in early stage III (94, 101 and 105 dafb) of fruit growth (Figure 6.22 b.).

6.4.3 Net CO_2 assimilation rate (A) and stomatal conductance (g_s)

Figure 6.23 a and b shows g_s and A during stage III of fruit growth. No significance differences occurred between the control and RDI in g_s values (Figure 6.23 a.). However, the net CO_2 assimilation rate (A) of RDI trees was higher ($P<0.05$) than of control, H-PBZ and root-pruned (Figure 6.23 b.) in early stage III (98 dafb), and RDI continued this trend relative to control on 105 dafb ($P=0.055$). Also, at this date (105 dafb) root-pruned and H-PBZ showed higher A than control ($P<0.01$ and $P<0.05$ respectively).

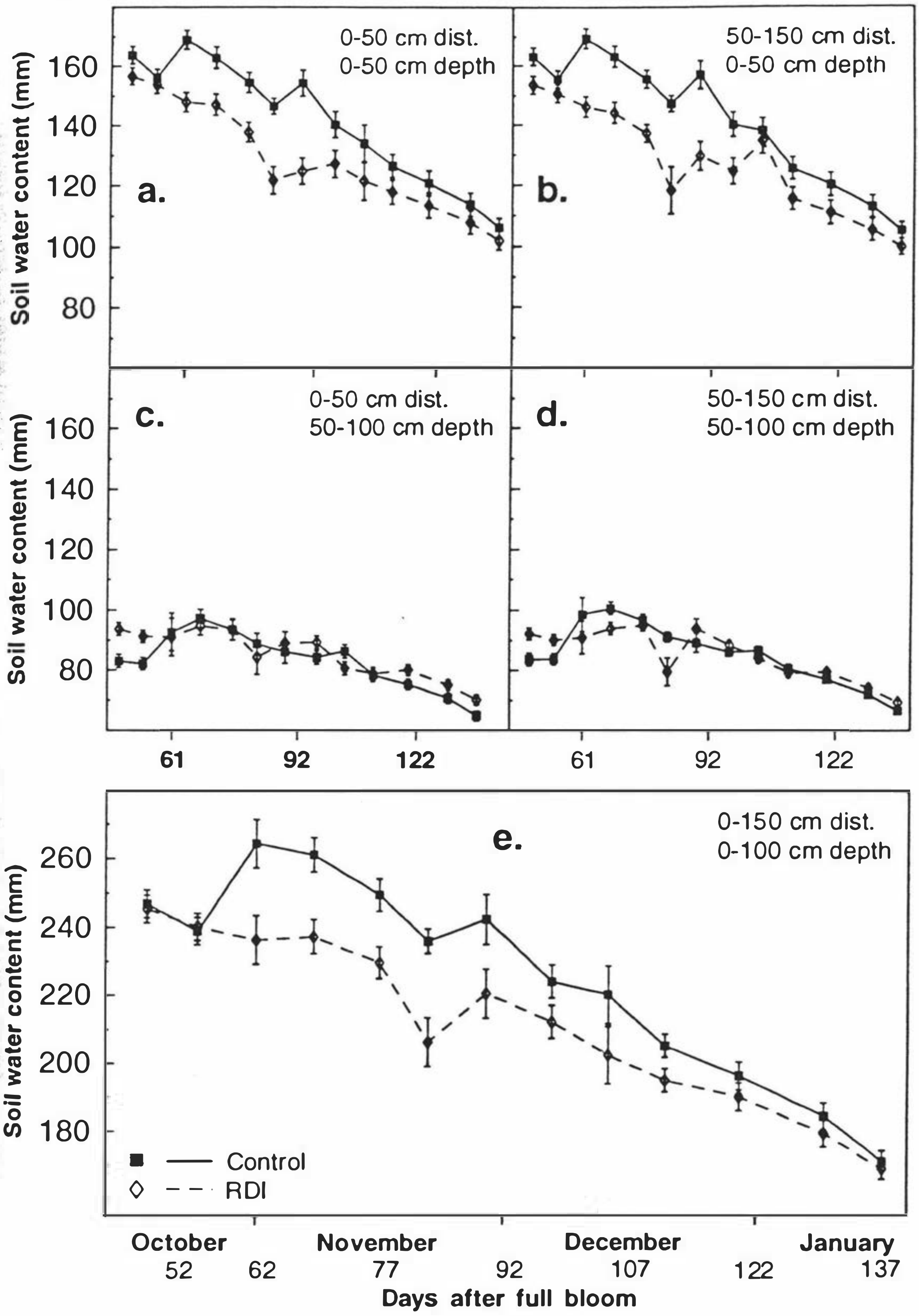


Figure 6.21 The influence of treatments on soil water content (mm) of wide spaced 'Sundrop' during 1990-1991 season. a). and b). Top 50 cm layer of soil. c). and d). Lower 50 cm layer of soil and e). Top 100 cm of soil (Dist. is distance from tree trunk). Bars show the standard error of the mean.

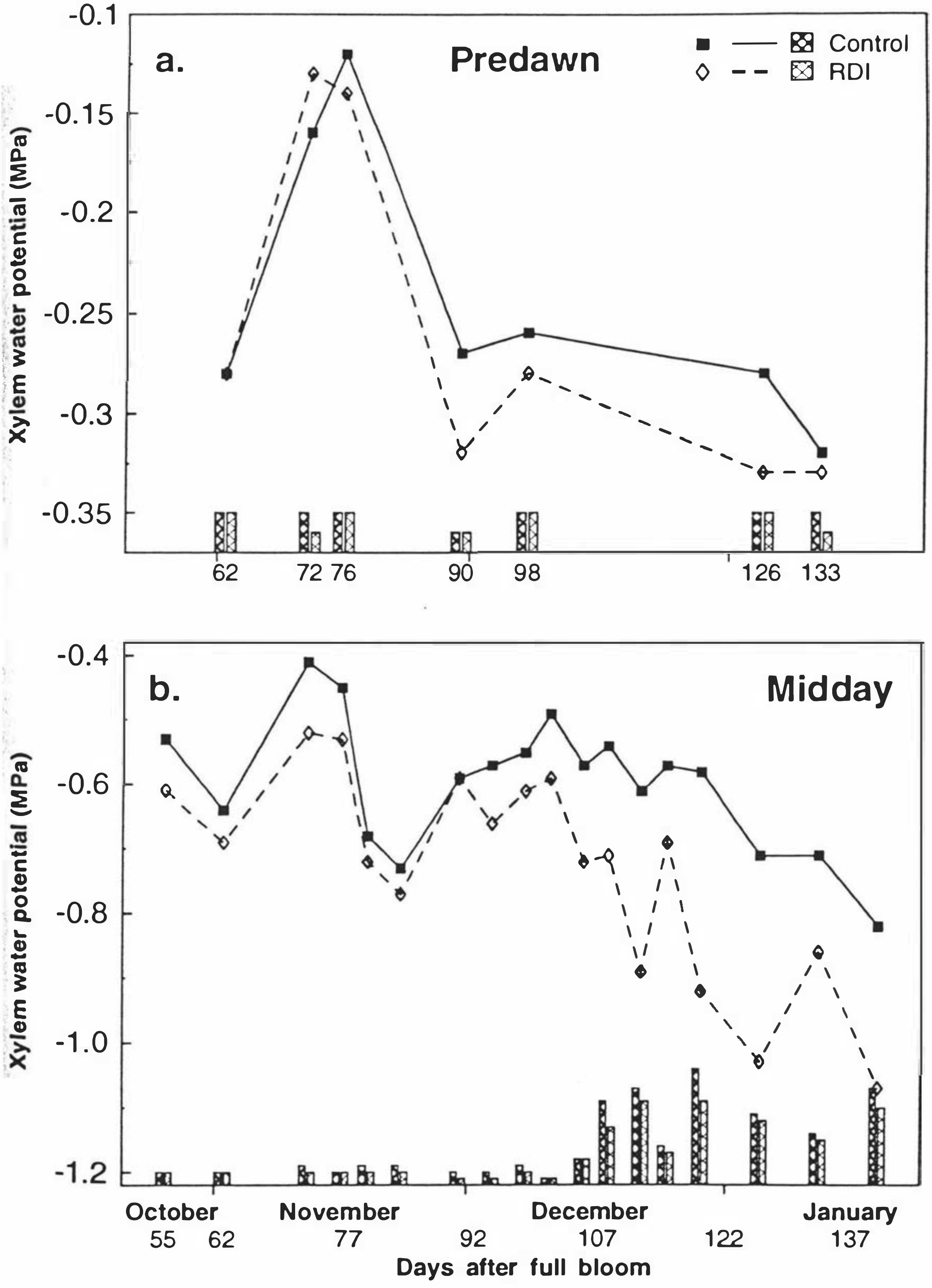


Figure 6.22 The influence of treatments on xylem water potential (ψ_{xylem}) in wide spaced trees in 1990-1991 season. a). Predawn xylem water potential; b). Midday xylem water potential. Bars show the standard error of the mean. Each point represents the mean of four leaves on each replicate (n=8 for each treatment).

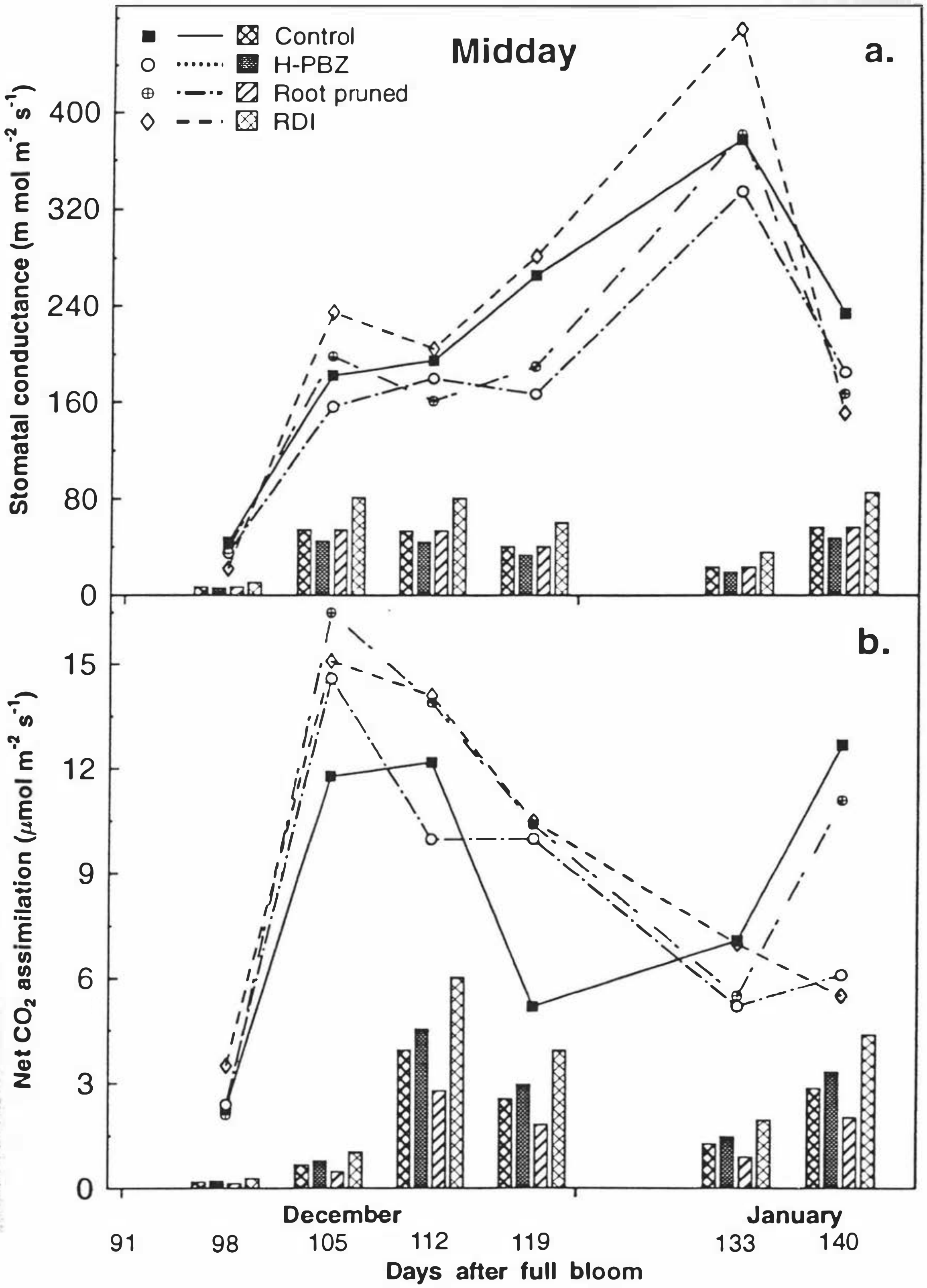


Figure 6.23 The influence of treatments on a). Stomatal conductance (g_s) and b). Net CO_2 assimilation (A) of wide planted 'Sundrop' apricot trees during stage III of fruit growth in 1990-1991 season. Bars show the standard error of the mean. Each point represents the mean of four leaves on each replicate per treatment (n=8 for all treatments).

6.5 DISCUSSION

The experimental trees were grown in a humid climate with relatively high rainfall and high relative humidity. The long term study of climatic data (Figure 3.1) revealed that usually there is a deficit between evaporation and rainfall between September and March and this reaches a maximum at the time of fruit harvest (January for 'Sundrop' at this experimental site, Figures 3.1, 6.1 and 6.3). Climatic data recorded during this experiment (Figure 6.1 to 6.4) revealed that the rainfall was more uniformly distributed throughout the main growing season in the second year (season 1991-1992) than during the first year (season 1990-1991). When daily rainfall and evaporation during the first growth stage was studied, it was found that in both seasons the variation in rainfall was very high (ie. more than 30 mm rainfall fell in one day). Presumably, if irrigation were established based on the climatological approach described earlier in this chapter (section 6.1), torrential rain could upset irrigation scheduling in this climate, and in the present study this usually occurred during each stage of fruit growth in both seasons (Figure 6.2 and 6.4).

6.5.1 Tree response to PBZ

In close spaced trees in both seasons Ψ_{xylem} for PBZ and control were the same. Although soil water content of PBZ treatments was not evaluated in this study, it is assumed that soil water fell to ≈ 80 %FC during stage III of fruit growth. For example, soil water content was recorded on control treatment as having declined to 77 and 87 %FC by harvest in the first and second seasons. Over this time (ie. stage III) Ψ_{xylem} of H-PBZ treatment fell to -0.2 (MPa) at predawn and -0.5 at solar noon. Under the conditions of this study, on some occasions PBZ treatments showed an increase in g_s and A and significant differences appeared in stage II and early stage III during the second season (Figure 6.11). This might suggest that in the non-water stressed conditions of this study PBZ increased g_s on some occasions, and allowed higher net CO_2 assimilation rate, which could contribute to improved productivity (see chapter 5). Biasi *et al.* (1989) found that PBZ under water stress conditions reduced stomatal conductance of peach seedlings, although at 100% FC it increased stomatal conductance. The increase in A by L-PBZ treatment in the second season is in agreement with the report of Elfving and Proctor (1986), who found that generally A was stimulated in apple trees in the year of PBZ treatment.

The fruit CHO study (Figure 6.16 c.) showed that total CHO content was significantly increased in later stage II and stage III in the second season. The TSS increase in PBZ treated fruits at harvest reported in chapter 5 (Table 5.6 b.) supports this finding. A small increase in soluble solids in apple fruits following PBZ application has been reported by Miliou and Sfakiotakis (1986). In the present study, the amount of glucose (later stage II and III), and fructose and sorbitol (late stage I and II) in the fruit mesocarp tissue was significantly increased by H-PBZ treatments (Figures 6.19 and 6.20). These results are in agreement with Lever (1986) who suggested that a secondary effect of PBZ is alteration of sink strength within the plant, allowing greater partitioning of assimilate to reproductive growth. Hodairi and Canham (1990a) found that the decrease in shoot growth of young apple trees by PBZ was usually associated with a redistribution of assimilates within the vegetative and reproductive growth. The diversion of increased assimilate to the fruits as a result of a reduction of vegetative growth was also reported by Costa *et al.* (1986).

6.5.2 Tree response to root-pruning

In general the amount of soil moisture around the root-pruned trees was similar to that around the control (Figures 6.5 and 6.6). However, on some occasions in both seasons the root-pruned soil moisture increased significantly relative to control. Such a difference appeared during stage III of fruit growth in the "b." region in the first season of experiment (Figure 6.5 b., from 104 dafb). At the same time, Ψ_{xylem} of this treatment at midday was lower than control. Ψ_{xylem} was also lower than control during stage I and II of fruit growth. This suggested that root-pruned trees, due to reduced root volume, gave a water stress type response. This presumably was caused by the reduction of absorbing roots by this treatment in the "b." region. After root-pruning some regeneration of roots probably occurred in the "a." and possibly "b." regions. Apparently the overall reduction in absorbing root surface meant it could only partly supply the water requirements of the trees, which resulted in the decline in Ψ_{xylem}. Midday Ψ_{xylem} throughout the second season was significantly lower than that of control. Reduction in water absorption and induction of water stress as a result of root-pruning have been reported previously by Randolph and Wiest (1981). They also suggested reduction in assimilate production, nutrient uptake and effects on the source of growth regulating hormones as other possibilities.

When all soil moisture data were pooled in the first season the significant difference between control and root-pruned trees disappeared (Figure 6.5 e.). This suggested that roots of the root-pruned treatment closer to the tree trunk in the "a." region increased water uptake, although no significant difference in soil moisture was observed between control and root-pruned trees in the "a." region (Figure 6.5 a.). Similarly, in the second season no significant differences occurred in soil moisture in the "a." region between control and root-pruned treatment (from 12 dafb, Figure 6.6 a.). However, when data for this season from all soil regions were pooled, root-pruned treatment showed higher soil moisture during stage I of fruit growth (Figure 6.6 e.). The root sampling study one year later (see chapter 4, section 4.3.2) revealed higher RLD in the "a." region of root-pruned plots. The presence of a larger volume of roots close to the trunk, within the area bounded by the pruning cut, is consistent with the idea of greater water uptake occurring in this region.

Geisler and Ferree (1984a) suggested that immediately after root-pruning the supply of water, mineral nutrients and hormones from the roots to the shoots declined because the root:shoot ratio had decreased. They also proposed that a greater proportion of assimilate was directed to root growth so that the plant started to recover its root:shoot ratio by increased root growth. In the present study, while in the first season root-pruning tended to increase soil moisture in mid summer, in the second season it increased soil moisture content only until the end of stage I (60 dafb, Figure 6.6 e.). It is concluded that root-pruned trees started to recover their root:shoot ratio following the first season root-pruning, by producing more roots within and immediately beyond the pruning cut (chapter 4, section 4.3.2). However, root-pruning in the second season was only able to remove regenerated roots in the "b." region, as pruning was applied in the same position as in the first season. So, it is possible to conclude that the increased roots within the "a." region resulting from the first season pruning added to the ability of root-pruned trees for increased water uptake from late October in the second season within the "a." region. Thus in the second season root-pruning was less effective in controlling vigour of root-pruned trees, and more shoot growth occurred than in the first season.

Assimilation and g_s of root-pruned trees were not affected in either season of study on dates between October and late January (Figures 6.10 and 6.11). This was different from the finding of Poni *et al.* (1992), who reported reductions in A, g_s and transpiration two days after root-pruning potted apple, grape, peach and pear trees, whilst in the present study A and g_s were first measured 55 dafb (1991-1992 season). The diurnal study of Ψ_{xylem} revealed that the root-pruned treatment reached its minimum Ψ_{xylem} at 3 pm and was lower than control at this time of the day (Figure 6.9), with no negative effect on A and g_s (Figures 6.12 and 6.13). A and g_s were evaluated during stage III (from 98 dafb) in the first season and from late stage I (from 55 dafb) in the second season. As has been described earlier, soil moisture data revealed that root-pruned trees had similar or higher soil moisture than control. It is likely that when root-pruning is applied in drought condition it may have negative effects on A, g_s and transpiration. Work on 'Jonathan' apple trees by Ferree (1992) showed that trees subjected to root-pruning under severe drought conditions had reduced A and transpiration, while root-pruned trees given supplemental water showed increased transpiration and also greater fruit size at harvest.

Neither predawn or midday turgor of leaves and fruits (Figures 6.14 ,6.15, 6.17 and 6.18) was reduced compared to the control by root-pruning (except midday turgor of leaves at 96 and 108 dafb during stage III). At the time of fruit harvest (137 dafb) Ψ_p of fruits of root-pruned trees at midday was similar to that of control fruits. This possibly occurred as a result of osmotic adjustment (0.29 MPa) at this time (Figure 6.18). The fruit mesocarp carbohydrates recorded at the same time revealed that the concentration of all simple carbohydrates were generally higher on root-pruned trees than on control at this time, with a trend toward significant differences in glucose, fructose and total CHO. Calculation of the influence of the simple carbohydrates on osmotic adjustment revealed that glucose had the major effect on osmotic adjustment of fruit (0.04 and 0.06 MPa on control and root-pruned trees respectively).

Root-pruning generally increased total CHO concentration during stages I and II of fruit growth (Figure 6.16 c.). Glucose and sorbitol increased in both late stage I and II, and fructose was higher in stage II (Figures 6.19 and 6.20). The possible occurrence of osmotic adjustment was not investigated in the first two growth stages either at predawn or midday. However, the changes in simple carbohydrates during these periods might be explained by osmotic adjustment, or possibly by a reduction of root demand for assimilates and increased carbohydrates diversion to active young fruits. There do not appear to be any studies of fruit CHO of root-pruned apricot trees in the literature. Ferree (1989) reported that root-pruning of young greenhouse-grown MM.111 apple trees had no influence on the carbohydrate fractions in leaves and shoots, but increased soluble and insoluble fractions in the roots.

6.5.3 Tree response to RDI

Experimental trees did not experience severe water stress. During fruit stage II, the lowest midday Ψ_{xylem} was about -0.7 MPa (Figures 6.7 and 6.8), and at the time of greatest soil drying (late January 1991) it was -0.81 MPa. As described in chapter 4 (section 4.3.2.1), shoot growth was significantly reduced relative to control only during late stage I of fruit growth in the first season. This suggests that shoot growth is more sensitive to water stress during stage I. Jerie *et al.* (1989 a) suggested that deficit irrigation during the early stage of fruit growth was able to reduce shoot growth, while fruits grow slowly and are insensitive to water stress at this time. At later stages of fruit development fruits are sensitive to water deficit, and trees should be fully irrigated to maximize fruit size.

Soil moisture data obtained by neutron probe revealed that under close spaced trees the top 50 cm layer of soil was significantly drier with RDI during the first two stages of fruit growth (to day 92). However the amount of soil moisture in the deeper layer of the RDI plots was not significantly different from control (Figures 6.5 and 6.6). Under wide spaced trees the soil dried slowly at the beginning of season (Figure 6.21 e.), and in the top 50 cm layer of the soil (Figure 6.21 a.) the amount of soil moisture was close to that of control during stage I of fruit growth. Time was needed to reduce the amount of soil moisture in the RDI plots, and it became lower than control during stage II of fruit growth. Presumably, the design of the RDI plots covers and low evapotranspiration reduced the drying out of the top 50 cm layer of soil at the beginning of the season. The area covered by plastic sheeting to isolate the root zone of two wide spaced trees was bigger than the area covered to isolate three close spaced trees. So it may be assumed that more water was extracted from the smaller covered area of close spaced trees than from the bigger area of wide spaced trees. Mitchell *et al.* (1989) suggested that RDI is more efficient in close spaced trees than in wider spacings or on deep soils, where there is more space for growing the root system and a larger volume of water is available to reduce the potential effects of RDI. They suggested that in wider spaced trees or in deeper soil a more severe level of RDI may be required to control vegetative growth.

In both years the lowest predawn and midday Ψ_{xylem} of RDI trees was as low as -0.4 and -0.8 MPa, which indicates the trees developed a moderate degree of internal water deficit. RDI Ψ_{xylem} was often significantly lower than control from November to January (1990-1991 season) and to early stage III (1991-1992 season), at times of high evaporative demand and/or following a prolonged period of reduced soil moisture (Figures 6.7, 6.8 and 6.22). The limited degree of soil drying and internal plant water deficit during stage I and II indicates there was only a limited achievement of RDI conditions as described by Chalmers *et al.* (1981). This, and the deep soil and climatic conditions discussed previously, reduced the potential RDI effect. Jerie *et al.* (1989a) reported that there is good potential for RDI in high density plantings with shallow but well drained soil and/or in a dry climate, during periods of rapid shoot growth. Under similar environmental conditions to those in this study, Durand (1990) used lucerne as a cover crop in conjunction with RDI to reduce soil water content around 'Royal Gala' apple trees early in the season. Similarly, Caspari *et al.* (1994) have reported using annual cover crops to increase the potential effect of RDI on grapevines.

RDI reduced Ψ_{xylem} each year by about 0.1 MPa during fruit stage II and early stage III (70-100 dafb), and a little later in the drier first season (Figures 6.7, 6.8 and 6.9). At these times in close planted trees, and also with the more marked depression of Ψ_{xylem} during stage III of wide spaced trees (Figure 6.22), there was no reduction in A or g_s. Kramer (1983) suggested that water stress refers to conditions in which plant water potential and turgor is decreased enough to interfere with normal functioning. The level of water stress at which this occurs depends on the type of plant and the stage of development. He suggested that the degree of water deficit which causes wilting is a plant related characteristic. Farquhar and Sharkey (1982) reported that water stress caused stomatal closure restricting uptake of CO_2, and g_s and A were reduced under mild water stress of apple and cherry (Flore *et al.,* 1985). Salisbury and Ross (1992) stated that water is often the most limiting factor for A. However, water, light, ambient CO_2 and O_2 concentrations, mineral nutrients and leaf temperature are all factors affecting A (Lawlor, 1987). In the present study on some occasions g_s was significantly higher in RDI trees than control (ie. early stage II in the second season) while net CO_2 assimilation rate was similar to control. Presumably g_s responded to ambient RH which is normally high in this climate. Schulze *et al.* (1972) and DeJong (1983) reported g_s in apricot ranges from 2.7 to 11 mm s^{-1}. Apricot stomata were sensitive to relative humidity and environment (Schulze *et al.,* 1972). They found that at high RH stomata remained open in spite of a decrease in Ψ_{xylem}.

While leaves responded to drying soil, it is important to be aware of the effects on fruit. Leaves are often the first plant tissue to respond to a water deficit, and changes in their water status may affect fruit quality. Ψ_p of RDI fruits was not different from control fruit at midday. During late stage I (59 dafb) both predawn and midday Ψ_t and Ψ_s of RDI fruit was lower than control, but Ψ_p was similar to control at both times (Figures 6.17 and 6.18). At this time and until the end of fruit stage II, total soil moisture content of RDI plots was lower than control (Figure 6.6 e.) and Ψ_t was reduced while fruit turgor was maintained (Figures 6.17 and 6.18). This latter effect appears due to osmotic adjustment (0.44 MPa) in RDI fruit. The fruit glucose concentration dramatically increased in RDI fruits during stages I and II, with significant differences from control on 52, 59 and 80 dafb (Figures 6.19 and 6.20). This suggests that of the recorded simple carbohydrates glucose was the solute responsible for osmotic adjustment at the end of stage I. Analysis of the influence of simple carbohydrates on the osmotic adjustment revealed that glucose made the greatest contribution to it at this time (0.03 and 0.06 MPa in control and RDI treatments respectively). The work on Asian pear under glasshouse conditions by Behboudian and Lawes (1994) showed 0.68 MPa osmotic adjustment occurred in fruits, with partial contributions of 0.05, 0.12 and 0.13 MPa estimated for glucose, fructose and sorbitol respectively when compared with control.

Generally, the midday Ψ_s of RDI leaves was lower than control during stages I and II. This possibly demonstrates the normal adaptation of trees to the low water deficit, although the only significant difference detected was on day 68 at the midday measurement (Figure 6.15 c.). When the effect of tissue dehydration was eliminated, active osmotic adjustment at this time was calculated to be 0.23 MPa (Figure 6.16 b.). Kramer (1983) reported that in a situation of lowered water potential, osmotic adjustment enables cell enlargement and continuing growth. Maintenance of turgor by osmotic adjustment could therefore be an important adaptational response in some species during drought periods (Kramer, 1983 and Morgan, 1984). The work by Ruggiero *et al.* (1988) showed the ability of apricot to tolerate drought periods and to adjust its leaf Ψ_s, with a noticeable process of long term adaptation to increasing water stress. They reported the accumulation of soluble sugars in the leaves with decreasing Ψ_s.

During fruit growth stages I and II both the predawn and midday records of Ψ_{xylem} (Figure 6.8) showed lower values on RDI trees than on control, although the differences were not significant. During stage III both predawn and midday Ψ_f and Ψ_s were usually the same in re-watered RDI and control. Generally Ψ_s of RDI fruits in stage III was similar to higher than control. This was consistent with leaves Ψ_s, for example predawn Ψ_s (108 dafb) and midday Ψ_l and Ψ_s

(108, 115 and 131 dafb) of leaves were higher than control. These results suggested that irrigation did maintain turgor during stage III of fruit growth. Behboudian *et al.* (1994) found that midday turgor potential of leaves of Asian pear grown under greenhouse conditions became progressively lower with continuation of early-season water stress. They suggested early season water stress which resulted in a leaf water potential of less than -2.5 MPa had negative effects on photosynthesis and fruit growth, and should be avoided in Asian pear.

SUMMARY

From this study of the effect of treatments on the water relations of 'Sundrop' apricot it is concluded that:

PBZ treated trees had the same water status as control. In addition net CO_2 assimilation rate and stomatal conductance of PBZ treated trees were increased on some occasions through the fruit growth period and fruit carbohydrates were increased.

Root-pruned trees, in spite of having a similar or higher soil moisture content than control, showed a water stress type response in the leaves and fruits. The response was greatest at the time of highest evaporation and water demand during fruit stage III. It appeared that root-pruning increased internal water deficit by reducing the absorbing sites of the root system, and on some occasions trees could not fully recover their water status during the night and early morning. As time elapsed after root-pruning more water was extracted from the top 50 cm layer of soil within the area of the cut roots. This increased water uptake close to the tree and the greater RLD in this region after 2 years suggested there was root regeneration and recovery of the root:shoot ratio during the first growing season after root-pruning. The lower Ψ_{xylem} during both seasons suggests the root system was still not able to fully support the tree's water consumption and water loss through the transpiration stream. However, under the conditions of this experiment net CO_2 assimilation rate and stomatal conductance were not affected by root-pruning. The fruit mesocarp of root-pruned trees contained more simple carbohydrates. The increase in glucose, fructose and sorbitol tended to increase total CHO in late stage I and II of fruit growth and increased further during stage III of fruit growth. In the second season it was recorded that fruit turgor potential was maintained at the time of fruit harvest in conditions of rapidly drying soil through the mechanism of osmotic adjustment in fruits.

Soil moisture measurements in the both seasons showed it was very difficult to apply a sufficient level of water deficit in the RDI plots in the deeper layer of the soil in close spaced trees and also in the top layer of the soil in the wide spaced trees. This was caused by low temperature, high rainfall (especially during winter) and deep soil. This suggested that the horticultural application of RDI as a vegetative growth control technique under the conditions of this study is questionable, a situation that has also been recognised in a number of other regions. The greater soil drying below the RDI trees was mainly accounted for by the increased water loss from the top 50 cm layer of soil, but generally soil moisture in the lower 50 cm layer of soil was also less than control. The RDI treatment had no significant negative effect on net CO_2 assimilation rate and stomatal conductance, and increased these values on some occasions. The study of water status of fruits and leaves revealed that both predawn and at midday the level of stress during RDI application was not enough to produce lower Ψ_l and Ψ_s throughout the season. However at the soil water level which existed under these experimental conditions there was some evidence to suggest that Ψ_p of both fruit and leaves were maintained through the possible occurrence of osmotic adjustment in apricot fruit (at 59 dafb) and in leaves (at 68 dafb). The partial contributions of the recorded simple carbohydrates calculated by Van't Hoff's equation revealed that their contribution to osmotic adjustment in fruits was small, but with glucose making

the greatest contribution amongst the carbohydrates recorded.
In general, the results from RDI treatment and climatic data suggested that during times of high evaporation, adequate irrigation during fruit stage III (December, November and January) is essential to produce good quality fruit with large fruit size with this variety of apricot.

In general, the internal water stress of root-pruned trees was more severe than of RDI trees, however net CO_2 assimilation rate and stomatal conductance were not affected by root-pruning and were higher than RDI trees on some occasions. Root-pruned trees fruit showed higher amounts of some simple carbohydrates and higher total CHO than the RDI fruit on some occasions.

CHAPTER SEVEN

THE EFFECTS OF WATER STRESS AND CYTOKININ ON VEGETATIVE GROWTH, WATER RELATIONS AND ENDOGENOUS ABSCISIC ACID LEVEL OF 'TREVATT' APRICOT

7.1 INTRODUCTION

The effects of different vigour control techniques on the vegetative, reproductive and water relations responses of mature 'Sundrop' apricot trees grown under orchard conditions have been discussed in the last four chapters. This chapter will discuss the physiological responses of two-years-old 'Trevatt' apricot to water stress and the cytokinin 6-benzylaminopurine (BAP) grown under controlled environment conditions in an aeroponic system during 1992-1993.

The relatively cool temperate and humid climate under which the orchard study was conducted has been discussed. It was decided to carry out this experiment under conditions similar to the natural environment which exists for apricot culture in arid and semi arid areas. The growing system used for this study allowed a greater degree of water stress to be achieved, close control of the stress level, and use of an improved BAP application technique. Meblenbacher *et al.* (1991) noted that apricots are grown in many parts of the world, and their natural habitat includes extremes in temperature and humidity. However, apricots are grown most successfully in two very different climate regions. In central Asia, winters are long, cold, and dry and summers are hot, dry and have little atmospheric humidity. The second set of conditions is very short, dry and mild winters with a hot and dry summer, as occurs in the Mediterranean climate.

One of the objectives of this experiment was to examine the effects of water stress and of root applied BAP on the root:shoot ratio and dry matter partitioning of apricot plants. Wilson (1988) discussed some models which may explain the mechanisms controlling shoot:root ratio. A hormone model was one he discussed and he concluded hormones have an effect on shoot:root ratio. According to him, one type of evidence is the effect of exogenously applied hormones and, he considered it likely exogenous hormone application would modify shoot:root ratio. Chibnall (1954), as cited by Vaadia and Itai (1969), suggested that cytokinin may play a role in chemical signals between roots and shoots. Went (1938), as cited by Vaadia and Itai (1969), suggested the existence of hormones in the roots controls shoot growth. The influence of root applied cytokinin on the shoot:root ratio has been reported in the literature by Richards and Rowe (1977 a & b); Richards (1980) and Kuiper *et al.* (1989). Richards (1980) found that the result of exogenous BAP application varied with site of application.

Kramer (1983) suggested that water deficit caused reduction of cytokinin production in roots and reduced the supply to the shoots. If water stress reduces the level of endogenous cytokinins, and leads to a change in the root:shoot ratio, it is natural to ask whether exogenous cytokinin applied to water stressed trees would be able to restore this ratio. In the present study this was examined by adding BAP to water stress treatment. It was desirable to have access to clean intact root systems for direct observation, measurement and experimental purposes such as the application of precise levels of exogenous BAP and water stress. For this purpose an aeroponic growing system was employed to provide a selected level of BAP (in the nutrient solution) and of water stress (using an intermittent misting system). Plant nutrition and water requirements were provided by high pressure, low flow nozzles, which produced a fog within a chamber which enclosed the plant root zone. The aeroponic system allowed good control over the level of water stress by adjustment of misting off- and on-time. Work on tomato and Asian pear by Saunders (1991) showed intermittent misting in an aeroponic system was successful in generating mild to moderate water stress under glasshouse conditions.

It was planned to carry out the experiment using 'Sundrop' apricot seedlings, however a preliminary study showed the seedlings did not adapt well during the acclimation time in either a hydroponic or aeroponic system. Budded 'Trevatt' trees which replaced the seedlings were the only apricot trees available.

Loveys *et al.*, (1987) reported a low concentration of ABA in xylem sap of apricot showed no influence on stomatal conductance. They also suggested a possibility of osmotic adjustment occurring in apricot leaves under the conditions of their experiments in South Australia. In the present experiment the endogenous abscisic acid level in shoot tips and two adjacent partially expanded leaves was evaluated to examine whether ABA played a role in controlling shoot growth under water stress conditions. This evaluation was performed using an indirect enzyme-linked immunosorbent assay (ELISA) method, which could be applied to the small quantity of plant sample available at the beginning of the experiment. In addition the possible occurrence of osmotic adjustment was examined both in partially expanded leaves adjacent to shoot tips and in fully expanded leaves. Simple carbohydrates and some major ions in fully expanded leaves were analysed to investigate the mechanism of any osmotic adjustment.

7.2 MATERIALS AND METHODS

7.2.1 Preliminary study

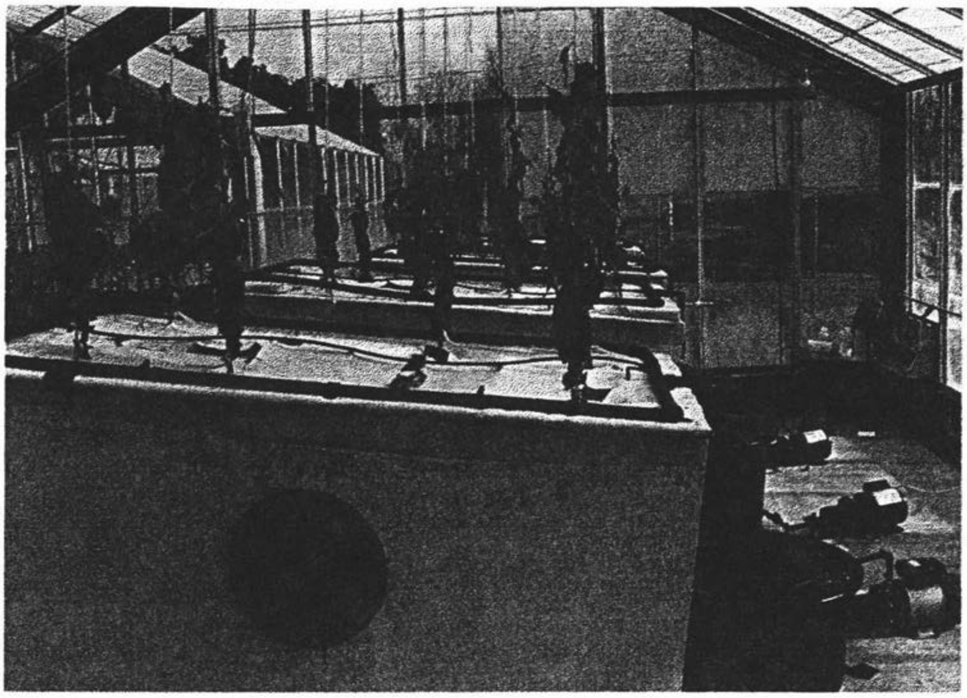
A series of preliminary studies were conducted (at the Plant Growth Unit, Massey University) under glasshouse conditions to study the adaptation of 'Sundrop' apricot seedlings to aeroponic and hydroponic growing systems

(Plates 7.1 & 7.2), and to estimate what level of BAP would be appropriate for the main experiment (Plates 7.2 b. & 7.3 b.).

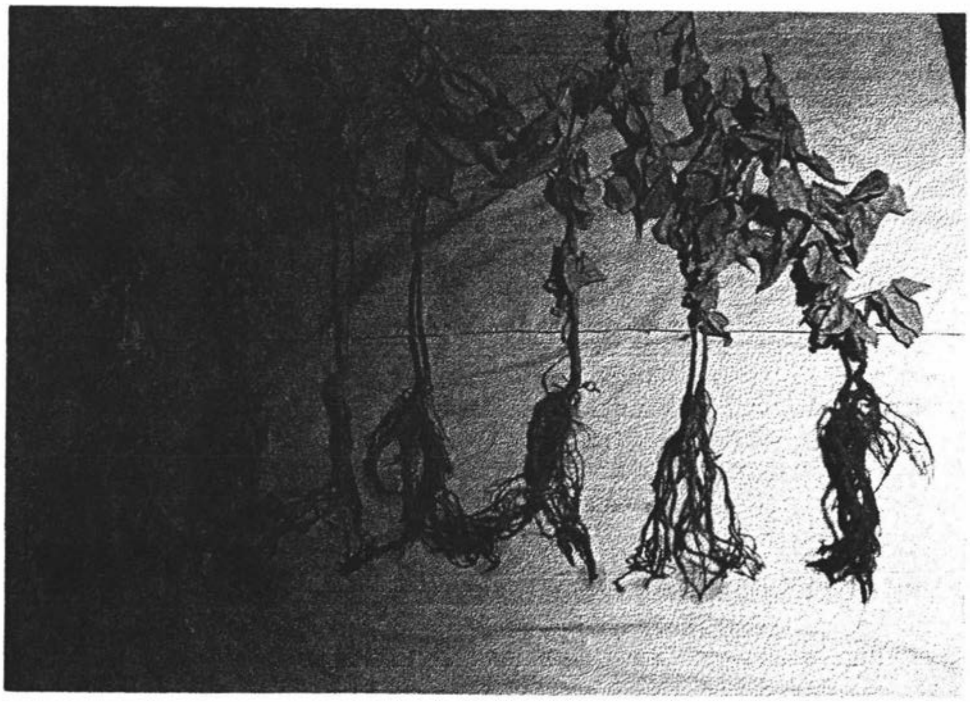
Seeds were extracted from ≈ 200 overripe fruit harvested from untreated experimental 'Sundrop' trees on 18th. January 1992. Seeds were stratified at 4°C within moist No 3 horticulture sand for 10 weeks. Germinated seeds were sown in 2.5 litre plastic pots of peat and pumice media (60:40 v/v) containing Osmocote base fertiliser. Seedlings were grown under glasshouse conditions at average 17°C using overhead misting irrigation (Plate 7.3 a.). Seedlings were sprayed with miticide (Neoron^{50}, 1.8 ml litre^{-1}) against spider mite on some occasions. When seedlings reached around 70 cm height (≈12 weeks after sowing) they were used for the preliminary studies.

A total of 24 seedlings with roots cleaned of potting medium were transplanted on 22nd July 1992 into 4 aeroponic tanks (6 plants tank^{-1}) placed in a greenhouse. The maintenance and nutrient solution of the system similar to those which will be described in the main experiment (section 7.2.4). Despite permanent misting a majority of the seedlings showed severe wilting after two days and did not recover, so were replaced with a new series of seedlings. When transferred to the tanks the shoots were trimmed to reduce transpiration, the temperature was reduced and the glasshouse was covered with paint to prevent direct sunlight (maximum temperature was 20°C). However, during this second preliminary study over ≈ 4 weeks more than 50% of the seedlings died (Plate 7.1 b.) and the remaining seedlings produced only small amounts of new roots and showed no increase in their shoot lengths.

Seedlings from the aeroponic tanks with new roots were transferred to a hydroponic system with 3 different levels of BAP (5, 10, and 25 ppm) applied in the flowing nutrient solution. After 4 weeks, visual examination (Plate 7.2 a.) showed the high concentration of BAP (25 ppm) had caused root damage and resulted in death of the plants (Plate 7.3 b.). No visible root damage was



a.



b.

Plate 7.1 a). Preliminary study with 'Sundrop' apricot seedlings in an aeroponic system under glasshouse conditions. **b).** A majority of seedlings failed to acclimatize with this system (after 4 weeks).

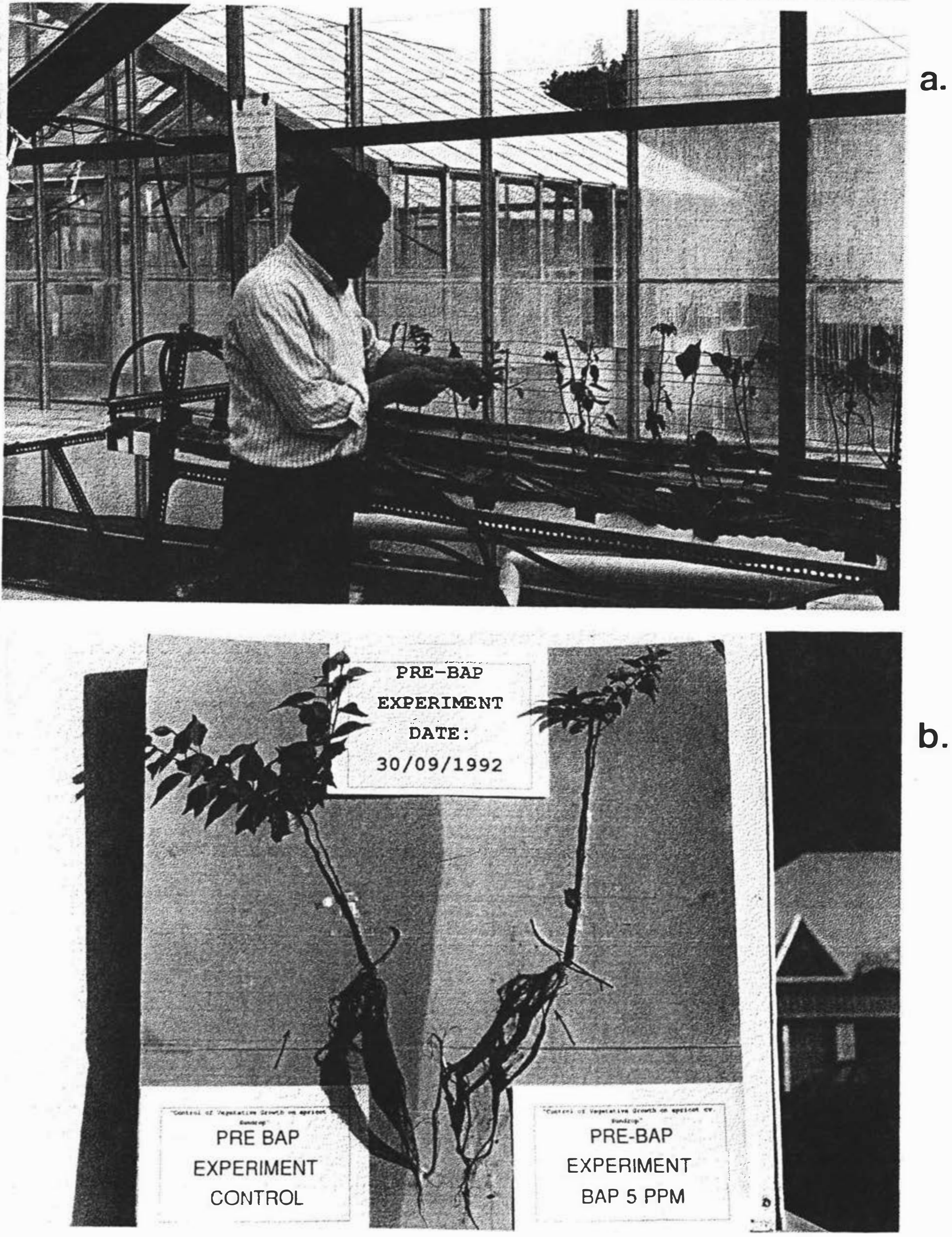


Plate 7.2 a). Visual checking of the 'Sundrop' apricot seedlings grown in a hydroponic system in the preliminary study with different BAP concentrations. **b).** After 4 weeks treatment (control and 5 ppm BAP).

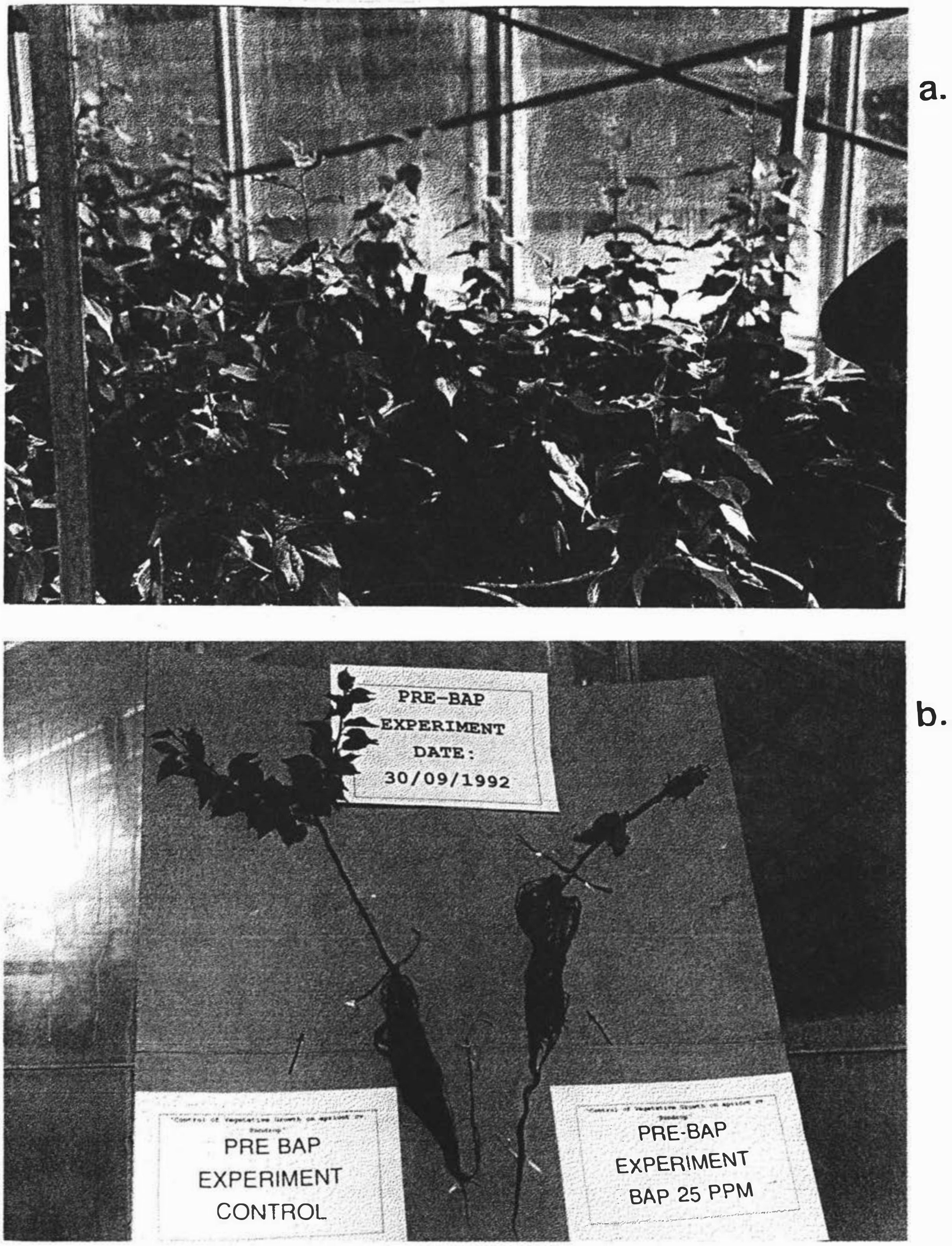


Plate 7.3 a). 'Sundrop' apricot seedlings as used for preliminary studies. **b).** After 4 weeks treatment in a hydroponic system, (control and 25 ppm BAP).

observed with 5 ppm treatment (Plate 7.2 b.). Seedlings given 5 ppm BAP had also produced new shoots, but those treated with 10 ppm BAP had not increased their shoot length. Thus 5 ppm BAP was used for the main experiment, although there was no physiological basis for the selection of this level of hormone or for the frequencies of application, other than to ensure a plant response without visible damage to the root or shoot tissues.

7.2.2 Plant material (Main experiment)

The material selected for this experiment was two years old apricot (*Prunus armeniaca L. cv. 'Trevatt'*) on plum rootstock. Two months before initiating the experiment, one hundred dormant uniform trees 15 months from budding were obtained from a commercial nursery (Pattullo's Nursery, Hastings), and stored in a cold room (4°C) until establishment of the experiment. During this period roots were kept in moistened straw to avoid drying the root system.

At the time of establishing the experiment (8th. September 1992) the trees' roots were washed and cleaned of soil and organic debris, and the root systems of all trees were reduced to a uniform size. Trees were cut back to 65 cm above the bud union and standardised to three branches. Before bud burst the plants were transferred to the aeroponic tanks located in two climate rooms. Trees were grown within 16 gauge steel wire frames mounted horizontally 130 cm above the aeroponic tanks, supported on vertical square aluminium bars. Each tree was supported by either cotton or plastic string, one end of which was tied loosely to the bud union base, from where it was spiralled along the stem length, and attached to the overhead steel wires (Plate 7.4 a.). The roots were suspended in the dark in the upper part of the tanks.

7.2.3 Experimental site and environmental conditions

The experiment was carried out in two controlled environment rooms (CE) at the National Climate Laboratory[1], Hort+Research, Crown Research Institute (CRI), Palmerston North, New Zealand.

The experiment was established at 20/18°C day/night temperature regime, this being gradually increased during the first 4 weeks to 28/24°C (Figure 7.1 b.) at the time of starting treatments (Plate 7.4 b.), this initial period being the acclimation time. In this account dates are stated, as appropriate, as days after establishment (dae) or days after treatments started (dats). Similarly the vapour pressure deficit (VPD) was increased gradually over 10 weeks to 18 mb (Figure 7.1 c.) from 7 mb at the time of establishment and 10 mb at the start of treatments. The objective was to apply a gradually increasing water stress by increasing VPD and limiting watering frequency. At the selected day/night temperatures this was achieved by adjusting room relative humidity (RH). Usually VPD was similar both day and night at the selected temperature regime, so RH (Figure 7.1 b.) was changed to maintain constant day and night VPD based on the method described by Kramer (1983). For example in this study at 10 mb VPD and 28/24°C temperature day/night RH was 73/66% (0 dats), changed to 50/37% at 18 VPD (50 dats) with the same temperature regime[2]. Xylem water potential (Plate 7.6 a. & Figure 7.3 a.) and shoot growth (Figure 7.4) was measured and an overall visual check (Plate 7.7) made of the trees' shoot and root systems to determine the need for any changes in environmental parameters or to increase the level of water stress. It had been

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[1] *This study ran for ≈12 weeks (Plates 7.4 & 7.5), using two CE rooms. Trees grown in an aeroponic system (soilless culture) with irrigation independent of the central irrigation unit. Some objectives of this experiment outlined in the 'National Climate Laboratory' newsletter No. 18, July 1993.*

[2] *According to the technical report from 51 - 55 days after treatments started (dats) dehumidifier failure occurred on some occasions boosting the RH up to 50-60%.*

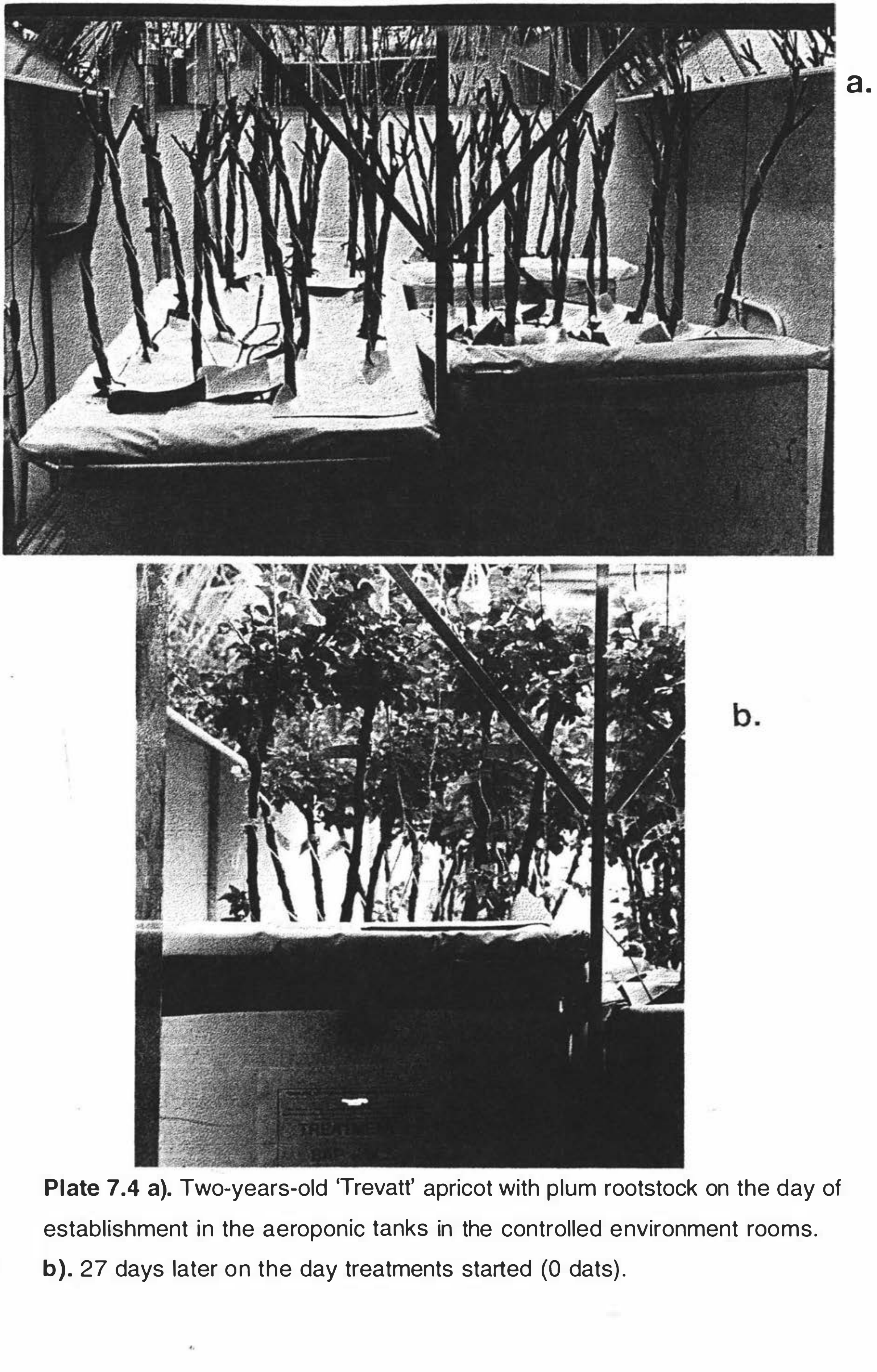

Plate 7.4 a). Two-years-old 'Trevatt' apricot with plum rootstock on the day of establishment in the aeroponic tanks in the controlled environment rooms. **b).** 27 days later on the day treatments started (0 dats).

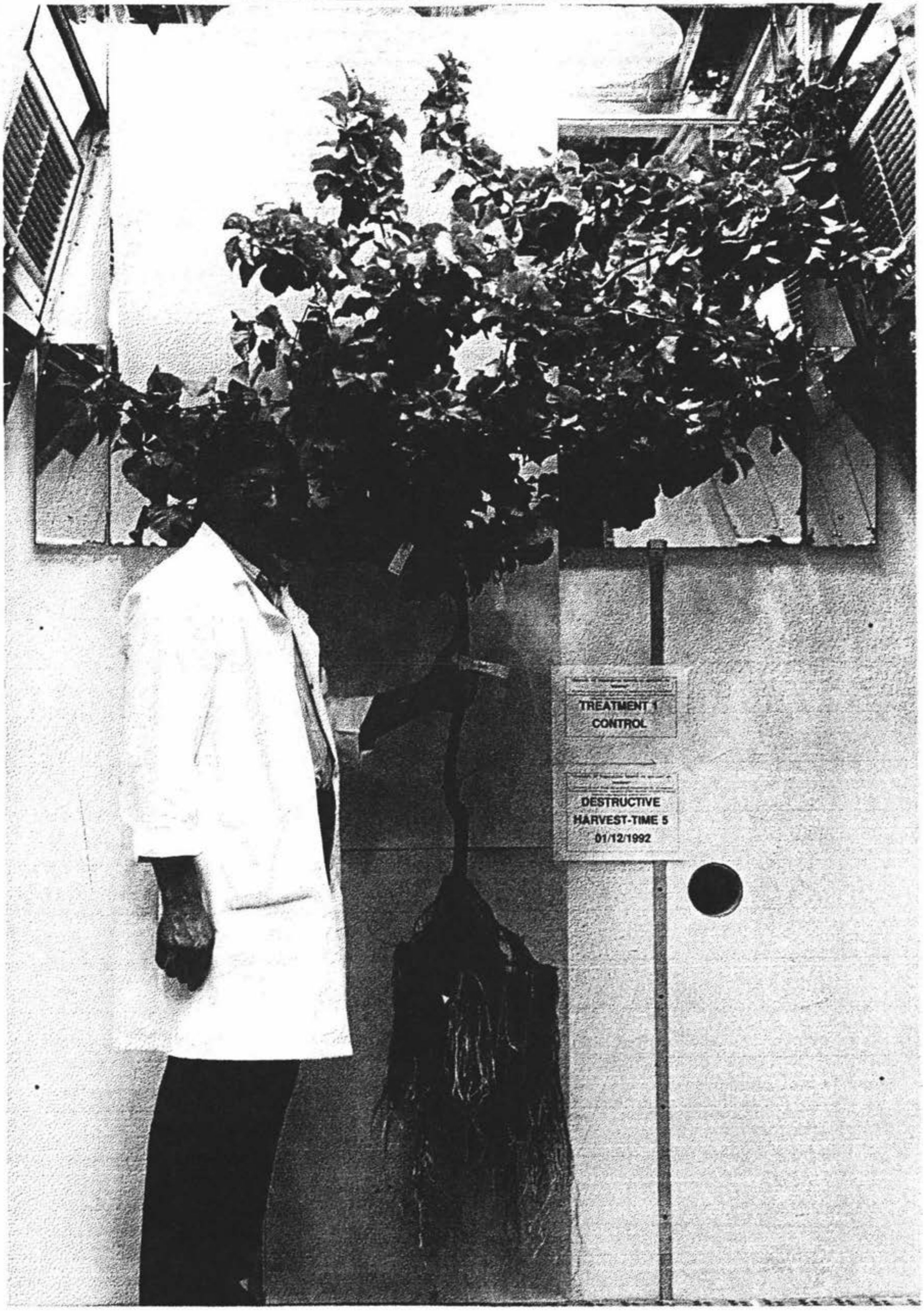

Plate 7.5 'Trevatt' apricot (control) grown in an aeroponic system under controlled environment conditions, 84 days after establishment in the tanks (57 dats).

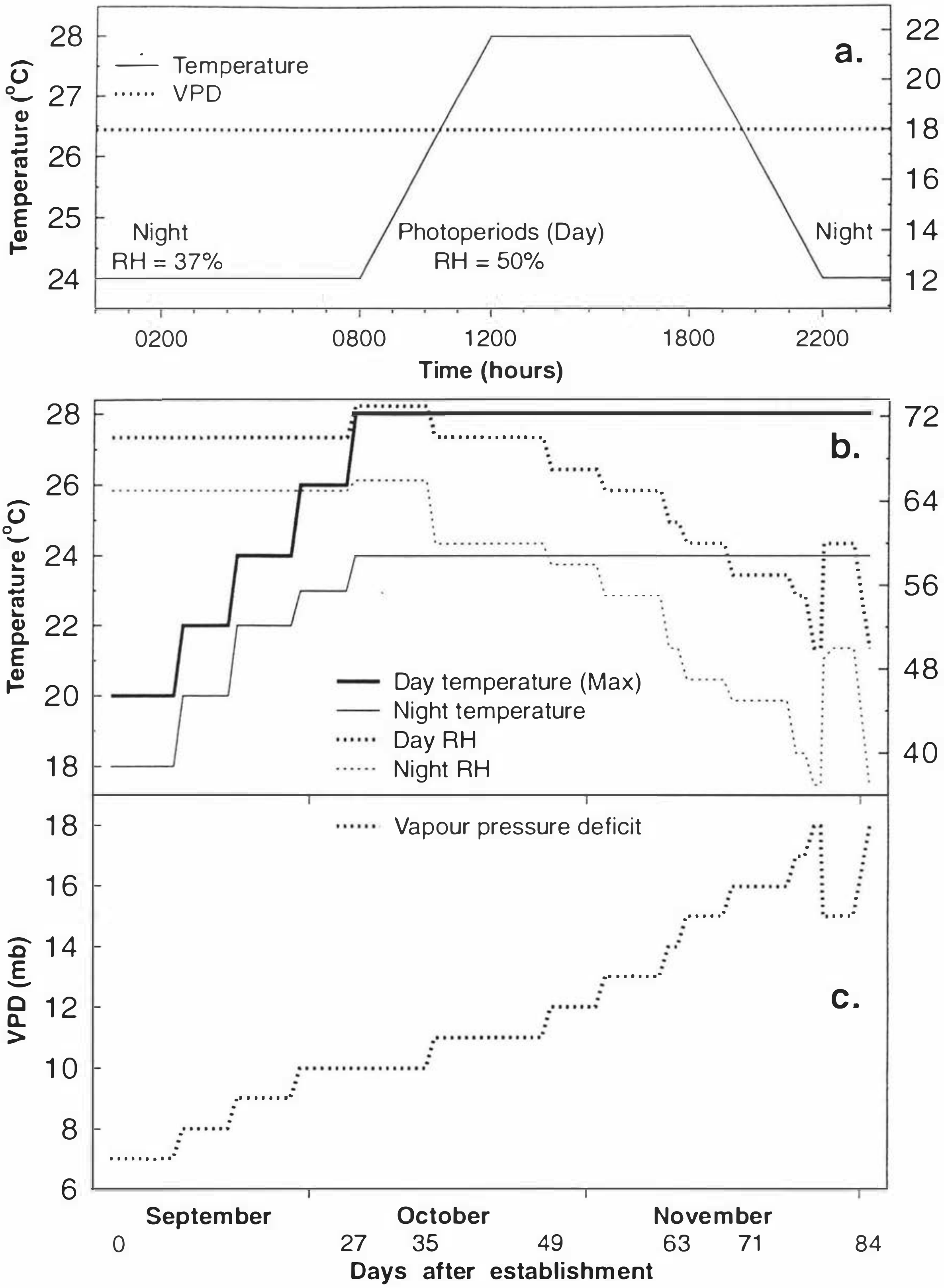

Figure 7.1 The climatic environment of climate rooms during the experiment. a). A typical pattern of day/night temperature (°C), RH and VPD (77 dae). 14 hour photoperiods (0800-2200 h) were maintained; b). Day/night temperature (°C) and relative humidity (RH); and c). Changes in vapour pressure deficit (VPD) during the experiment. For the first 27 days all plants were given graduating temperatures for acclimation purposes.

suggested that water stress be increased gradually and shoot growth checked frequently (Chalmers pers comm, 1992).

The photoperiod was 14 hours (0800 to 2200 hour) and the day/night, night/day temperature changeovers took 4 hours. Temperature was increased gradually in the course of the day and reached its maximum at 1200 hour and stayed at this temperature for 6 hours (Figure 7.1 a.). The photosynthetic photon flux density (PPFD) of 680-709 μmol m^{-2} s^{-1} at the height of the tree canopy was provided using 4 X 1000 W Sylvania 'Metalarc' high pressure Hg discharge lamps, together with 4 X 1000 W Phillips tungsten iodide lamps. Further details on the lighting system are provided in Warrington *et al.* (1978).

The CO_2 was maintained at ambient levels throughout the experiment in both rooms and was monitored daily. The CO_2 levels for daylight periods ranged from 300-526 ppm and for the night periods from 350-453 ppm. The CO_2 levels were on occasions higher than normal because of working in the room for extended periods for some measurements.

7.2.4 Specification of the aeroponic tanks and operation of the system

Eight aeroponic tanks (60 X 120 X 55 cm) were designed by Chalmers and used by Thuantavee (1991) for an experiment on tomatoes. Some modifications were made to the misting system and connecting tubing and to the supporting system to make it appropriate for apricot plants and the rooms used.

Microjet nozzles (14 $tank^{-1}$) were mounted immediately under the lid to provide a uniform mist environment within the tanks and over the root systems. This top placement resulted in a uniform foggy climate inside the tanks while plants were irrigated and helped prevent any nozzle blockage by roots with a floor placement. Tank lids were covered with aeroponic polythene sheet (with white colour outside and black colour inside the tanks) and sealed with staples to prevent any water loss and to keep direct light from inside the tanks. Inside the tanks were sealed with black polythene. A drainage pipe allowed solution to circulate back to reservoir nutrient tanks, which were 100 l black polythene tanks located inside each room, so that solution temperature was similar through the entire system. Each reservoir tank supplied two aeroponic tanks. Each aeroponic tank was supported on a galvanized iron pipe frame (front legs 37 and back ones 47 cm height off the floor) to give a 10 cm fall from back to front, enabling water to flow rapidly back to the reservoir tank. The nutrient solution within the circulation system was filtered twice, before passing through the pumps and before entering the reservoir tanks, to ensure particles did not cause blockage within the system. The pressure flow for each circulation system was provided by an electric pump Type CP11 (Mono Pumps, Limited, New Zealand).

The nutrient solution was prepared (Appendix 7.1) according to Cooper (1979) as cited and used by Saunders (1991). Cooper used this solution with the nutrient film technique (NFT). Stock solution was stored in the dark in a cold room at 4°C and diluted weekly for total nutrient solution replacement in each aeroponic system. Full strength nutrient solution at pH 6.5 was prepared from the stock solution, and used with or without BAP. Two separate reserve tanks located in the same room (not connected to the system) contained either nutrient solution or plain water for solution replacement or adjustment. The volume of nutrient solution in each aeroponic reservoir tank was maintained at 60 litres throughout the experiment. Adjustment for volume, monitored by inserting a previously calibrated dipstick into the nutrient solution, and concentration were carried out twice daily at 0800 and 1800 hours. Measurement and adjustment of pH (using 5% KOH and 2 molar HNO_3) and electrical conductivity (EC) was made to 6.5 and 2-2.5 mS respectively. When the EC dropped below 2.0 stock nutrient solution was added (full strength nutrient solution $\approx$2.5 mS) and when the EC rose above 2.5 mS plain water was added for adjustment. The added solution nutrient or water was calculated as plant water use (data not shown).

7.2.5 Experimental layout and treatments application

Uniform apricot trees were allocated randomly to 8 aeroponic tanks (≈13 trees tank^{-1}) located in two controlled climate rooms (4 tanks in each room) set to similar environmental conditions. All trees in all of the tanks received continuous misting with nutrient solution only during the acclimation period. After the acclimation period (4 weeks) each treatment was located in two diagonally opposite aeroponic tanks within a room, each tank being one block.

Treatments were arranged in a randomized complete block design (RCBD) with ≈13 trees block^{-1}. The treatments were:

I. Control with continuous misting
II. 5 ppm 6-benzylaminopurine (BAP) with continuous misting
III. Water stress (WS) with intermittent misting
IV. 5 ppm 6-benzylaminopurine with intermittent misting (BAP + WS)
V. Water stress b (WSb) with intermittent misting (see 7.2.5.2)

Because space allowed only 4 aeroponic tanks in each room, 2 controlled environment rooms (CE) were employed for this experiment. Irrigation was independent of the central irrigation system of the National Climate Laboratories facility. Because technical problems prevented connecting tubing between the two rooms treatments were not repeated in each room, so treatments were randomly allocated to rooms assuming that all environmental conditions were similar, which was subsequently confirmed from logs of room conditions.

7.2.5.1 Control and 6-benzylaminopurine (BAP)

These treatments received full irrigation as continuous misting with full nutrient solution throughout the experiment. The BAP treatment received 5 ppm (5 mg.l^{-1}) 6-benzylaminopurine (Sigma Chemical Company, USA) in the misting solution. 300 mg BAP were dissolved in 30 ml 95% ethanol by gently shaking in a 50°C water bath for 10 minutes. The dissolved BAP was made up to 60 litres with full strength nutrient solution and stirred to ensure good mixing. At the same time a similar quantity of ethanol (without BAP) was added and mixed into the nutrient solution reservoir tank of the control treatment.

7.2.5.2 WS ,WSb and BAP+WS

Irrigation was supplied to these stress treatments using intermittent misting. The intention was to start with low stress, then increase it slowly, and continue to increase stress until growth stopped by increasing VPD and off-time misting. However, the water stress treatments at the beginning appeared too severe as plants showed some wilting. The water stress was reduced to a minimum level and later, as a precaution another water stress treatment (WSb) was generated. Subsequently the water stress of all stress treatments was increased gradually until the end of the experiment. Stress was adjusted by the length and frequency of misting in the tanks (Figure 7.3 b.) using digital timers Model H_3CA (Plate 7.6 b.). WSb treatment started one week later than WS, using trees located within the WS tanks with different labels but receiving the same irrigation regime. WS and WSb received ethanol as for the control treatment at the time of weekly solution replacement, and 5 ppm BAP in ethanol was applied to the BAP+WS treatment as described for the BAP treatment.

7.2.6 Measurements

7.2.6.1 Environmental conditions

Temperature, RH, VPD, light intensity and CO_2 were monitored by the central computerised system operated by the National Climate Laboratory.

7.2.6.2 Xylem water potential (Ψ_{xylem})

Xylem water potential (Ψ_{xylem}) was recorded each 1-3 days (Plate 7.6 a.) as described in chapter 6 (section 6.2.1.3). Measurement was made during the period of maximum temperature between 1200 - 1400 hours on two fully expanded leaves on each of two trees per treatment at each date.

7.2.6.2 Shoot growth

Two shoots tree^{-1} were labelled at the start of treatments and shoot length was recorded at intervals. The number of recorded shoots decreased through the experiment, as destructive harvests reduced plant number.

7.2.6.3 Leaf photosynthesis (A) and stomatal conductance (g_s)

Net CO_2 assimilation rate (A) and stomatal conductance (g_s) were recorded (from 2 dats) at weekly intervals as described in chapter 6 (section 6.2.1.4). Measurements were made between 1200 - 1400 h on two leaves on each of 4 trees per treatment.

7.2.6.5 Destructive harvesting

Prior to starting the treatments at the end of the acclimation period (27 dae, 0 dats) 1 plant tank^{-1} was randomly selected for destructive harvest (a total of 8 plants). Destructive harvests (4 trees treatment^{-1}) were carried out to

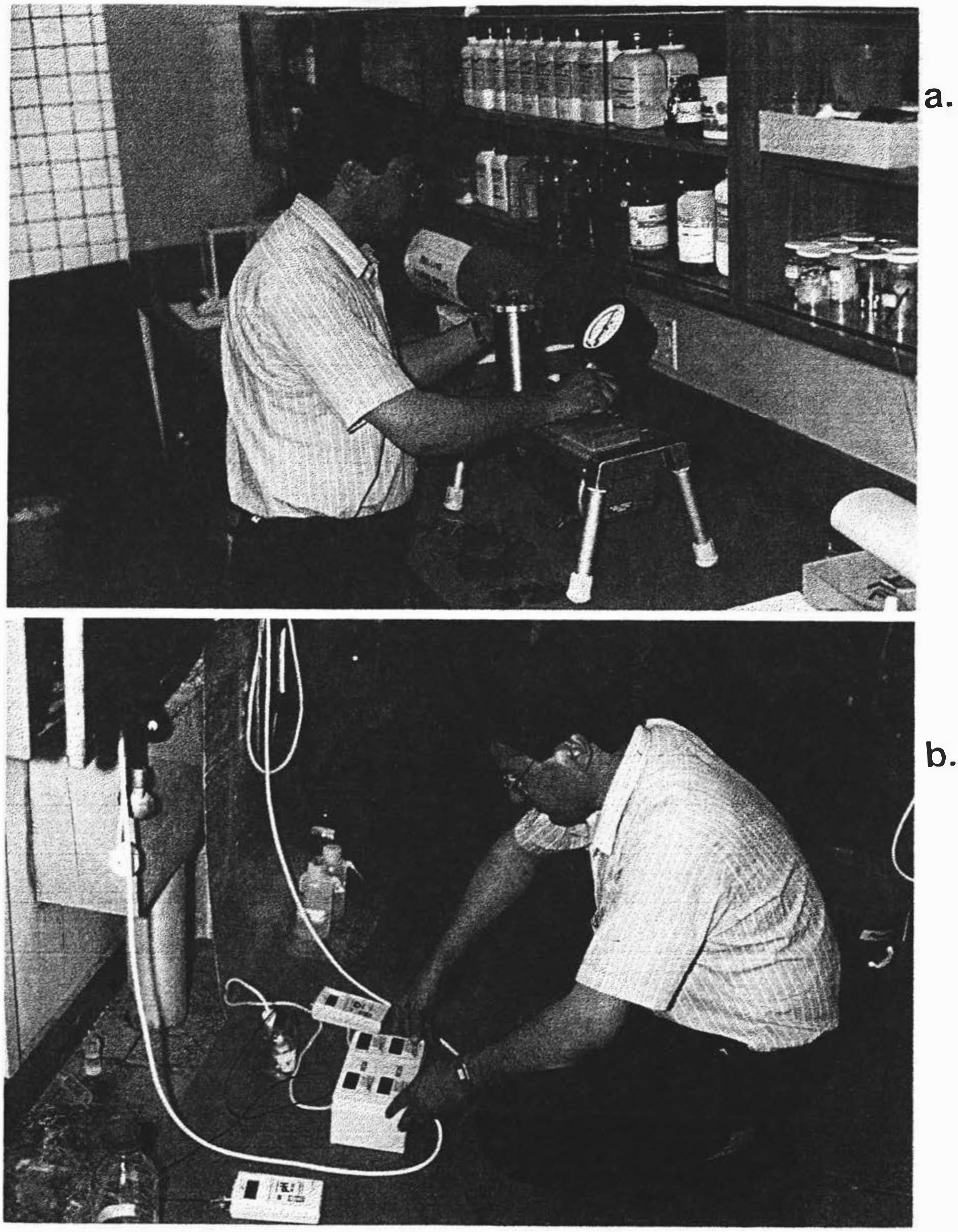

Plate 7.6 a). Xylem water potential (Ψ_{xylem}) measurement to check and adjust the level of water stress on 'Trevatt' apricot. **b).** Applying water stress using the intermittent misting system by adjusting on- and off-time using digital timers Model H_3CA.

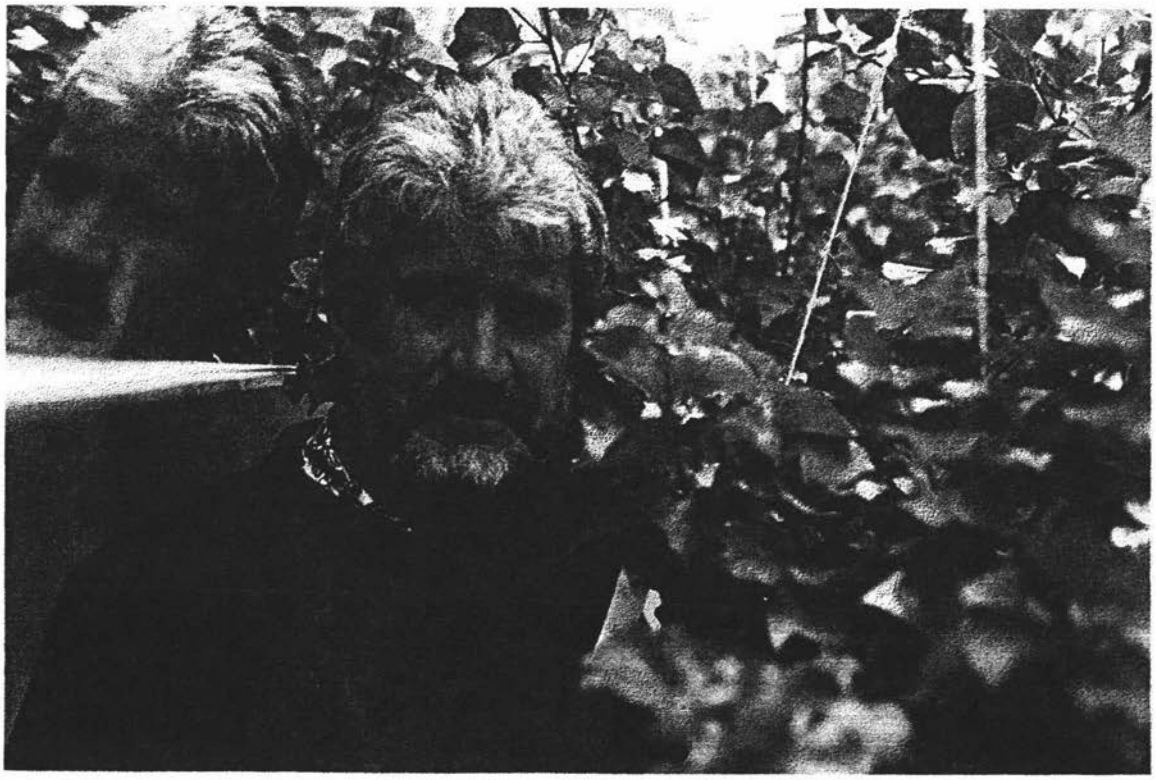

Plate 7.7 The response of trees was checked frequently by visually inspecting the shoots and root system. **a).** Four days after treatments started (Arzani and Lawes); **b).** Two weeks after treatments started (Chalmers).

determine the influence of treatments on the growth of different plant tissues, and were performed 8, 22, 36, 44 and 57 days after treatment started (dats). The first destructive harvest of WSb treatment was performed on 22 dats. Trees were separated into parts, being leaves, shoots and new roots (new tissues) and main stem and old roots (old tissues).

Root length of new roots was estimated as described in chapter 4 (section 4.2.1.5) with the exception that root length measured by the scanner was adjusted using the following formula, as suggested by the manual:

$$A = -0.2246 + 0.9655\,E + 0.00123\,E^2$$

Where A is adjusted estimate of root length (m) and E is root length measured by the scanner.

Total leaf fresh weight was recorded (data not presented) and leaf area measured as described in chapter 4 (section 4.2.1.2.2), but all leaves were recorded for each harvested plant. All plant parts were cut into small pieces, placed in tin cans and oven dried for one week using a vacuum oven (at 45°C). Plant parts dry weights were recorded and total plant dry weight calculated. Overall root:shoot ratio was calculated by dividing the total root dry weight (old and new roots) by the total top growth dry weight (main stem, shoots and leaves). The root:shoot ratio based on the new growth was also calculated by dividing the dry weight of the new roots by that of the new top growth (shoots and leaves). Each plant part dry weight was also calculated as a proportion of total plant dry weight.

7.2.6.6 Leaf water potential, pressure potential and osmotic potential

These parameters (Ψ_l, Ψ_p and Ψ_s) were recorded on both fully expanded leaves (mature leaves) and partially expanded leaves immediately below the shoot tip (young leaves) using the dew point method (Plate 7.9 a.) described in chapter 6 (section 6.2.1.5). The only difference was that liquid nitrogen was used for freezing the samples at the time of recording Ψ_s. All leaf samples were taken from trees between 1200 - 1400 hours. Measurements were made at weekly or biweekly intervals on one mature and one young leaf on each of four trees per treatment.

7.2.6.7 Leaf carbohydrates

Determination of simple carbohydrates in fully expanded leaves (Plate 7.8 a.) was performed prior to each destructive harvest. Between 2-10 g fresh weight of leaves were sampled and carbohydrates (CHO) determined as described in chapter 6 (section 6.2.1.6). The only difference was that during extraction chlorophyll was removed from the ethanol extract by chlorophyll partitioning prior to injecting into HPLC. Partitioning was performed on 4 ml (of 20 ml) sample extract with the addition of chloroform and distilled water (4:10:20 ml respectively). During the process an ultra-centrifuge (15000 RPM) was used for final partitioning and an automatic Speedvac concentrator (Savant, Model AS160, AS290, Savant Instrument, NY, USA) was used in two steps to dry off the sample extract for carbohydrate analysis.

7.2.6.8 Preparation of samples for mineral element analysis

At each of the 5 harvests after treatments started 2-10 g fresh weight of fully expanded leaves (Plate 7.8) was sampled from each harvested plant for analysis of some major elements (N, P, K, Ca and Mg). Sample fresh weight was recorded and the sample stored in a freezer (-20°C) in a 20 ml plastic vial. Samples were freeze dried for one week, then kept in air tight containers with silica gel in the freezer (-20°C). At the time of mineral analysis freeze dried samples were ground using a Glen Creston grinder (with 1 mm mesh).

Ground samples were stored at 40°C overnight to ensure they were free from

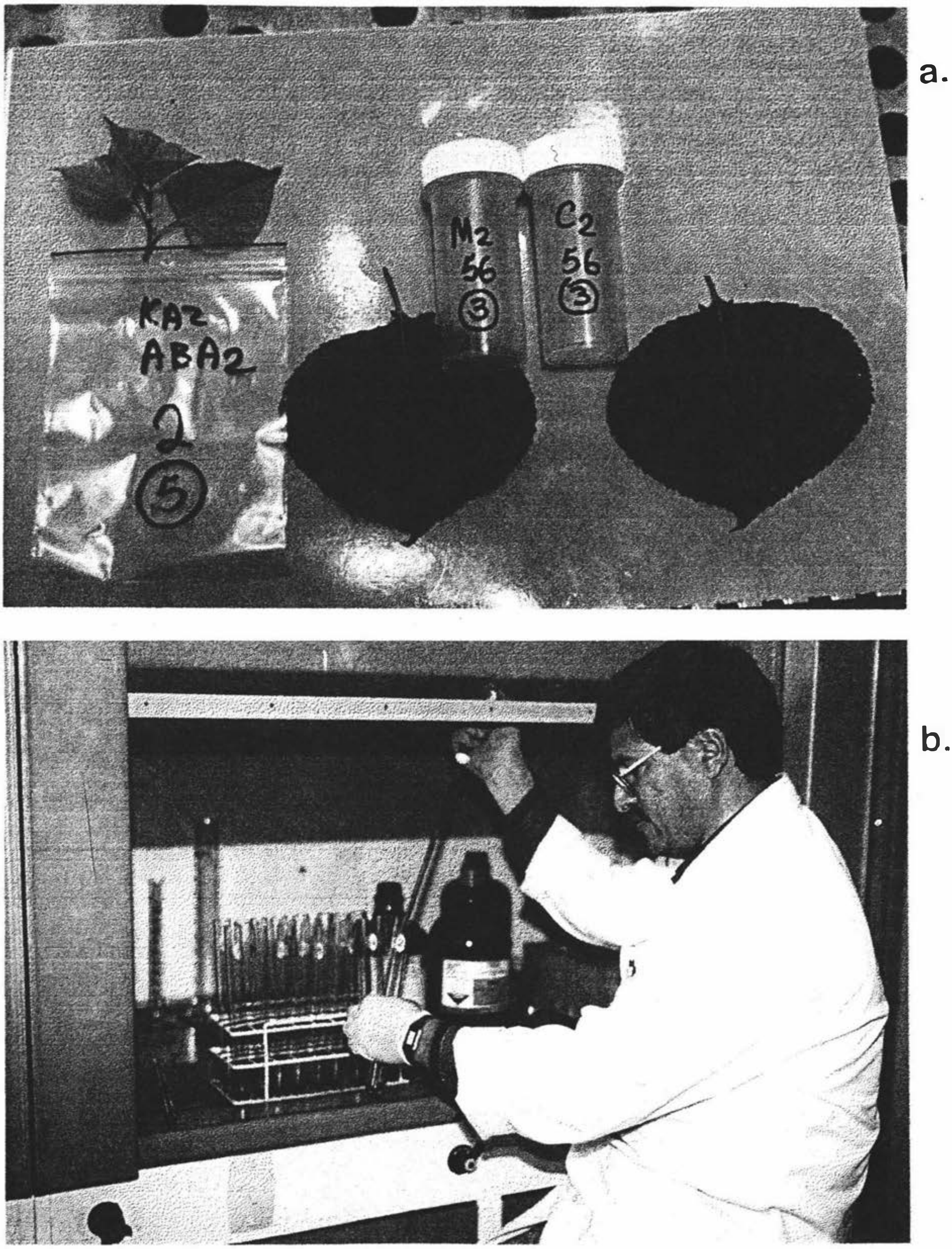

Plate 7.8 a). Sampling shoot tip and adjacent partially expanded leaves for ABA analysis, and fully expanded leaves for simple carbohydrates and mineral analysis of 'Trevatt' apricot. **b).** Mineral analysis of leaf samples.

moisture, then transferred to a desiccator with silica gel for cooling and preventing absorption of ambient humidity prior to analysis. Two separate extractions were carried out, each with different reagents and in different sets of digestion tubes.

For each series of analyses 0.1 g of dried sample was weighed in a labelled digestion tube. The heating block used held 22 digestion tubes, and therefore in each run 20 unknown plant samples and two known samples (standard, Wageningen No 3) were analysed. The results were presented as percentages of dry weight.

7.2.6.8.1 Analysis of K, Ca and Mg

All glassware used for this analysis was acid-washed in 2M HCl (made up with deionised water) prior to use. Four ml concentrated nitric acid (70%) was added to each weighed dried sample. Digestion tubes were set in a heating block with small funnels in the top to cause refluxing, and heated to 150°C until the solution became clear (after ≈10 hour). Then the funnels were removed and the temperature raised to 250°C and the extract reduced to dryness, which took 3 h.

Five ml 2 M HCl and 2 ml 25000 ppm Sr/Cs (30.19 g strontium nitrate, $Sr[NO_3]_2$ and 15.84 g caesium chloride, CsCl made up to 500 mls with deionised water) were added to each digestion tube whilst it was warm. The volume was increased to 50 ml by adding deionized water and well mixed using a vortex mixer. Sufficient volume (≈30 ml) was poured into labelled glass vials and K, Ca and Mg were analysed using atomic emission (K) or absorption (Ca, Mg) spectrophotometry (Department of Soil Science, Massey University). Extracts were diluted (0.2 M HCl and 1000 ppm Sr/Cs) as required to ensure results were within the range of the standard curve computed at the same time.

7.2.6.8.2 Analysis of N and P

For analysis of nitrogen and phosphorus a separate extraction was performed. The amount of N and P were determined by colorimetric autoanalysis methods following Kjeldahl digestion (Twine and Williams, 1971). Kjeldahl digestion solution was made in a fume cupboard by heating a mixture of 250 g:2.5 g:2.5 ℓ, K_2SO_4, selenium powder and concentrated H_2SO_4 respectively.

Four ml of digestion solution was added to digestion tubes containing pre-weighed 0.1 g plant or standard sample, then heated (at 350°C) for 4-5 h until the solution became clear. Two known standard samples were analysed with each series of digestions. The volume was increased to 50 ml using distilled water and mixed using a vortex mixer. A sufficient volume (≈30 ml) was poured into labelled glass vials and total N and P were determined by colorimetric autoanalysis methods within the range of a standard curve. Dilution was performed as required.

7.2.6.9 ABA analysis in shoot tip using ELISA method

7.2.6.9.1 Sampling and procedure prior to analysis

Prior to each destructive harvest of 4 plants treatment^{-1} (between 1200-1400 h) ≈2 cm long shoots tips with 2-3 adjacent partially expanded leaves (Plate 7.8 a.) were collected from each tree and transferred to 20 ml plastic vials and fresh weight recorded (2 samples tree^{-1}, 8 samples treatment^{-1}). Samples were immediately frozen in liquid nitrogen then freeze dried and stored in airtight plastic bags with silica gel and stored in a freezer (-80°C) until analysis. Samples were ground manually or with an electric grinder at the time of analysis.

7.2.6.9.2 Preparation of purification columns

Insoluble Polyvinylpyrrolidone (insoluble PVP) columns were prepared 2 days before use by suspending the required amount of PVP in an excess of distilled water. The PVP was allowed to settle a little, then the suspended fines fraction was decanted off to increase the flow rate of the columns. This was repeated five times. At the time of use most of the water was poured off to make a thick slurry, and this was poured into 10 ml plastic syringes with a filter paper disc at the bottom to make PVP columns of 5 ml volume.

The preparation of Sephadex columns was as follows. Five days before use the required amount of Sephadex (DEAE Sephadex A-25 - Pharmacia Fine Chemicals AB Uppsala, Sweden. Code No 17-0170-01 Anion exchanger Cap: 3.5 ± 0.5 mg/g Particle size 40-120) was suspended in excess 0.1M ammonium acetate (pH unadjusted). This buffer was replaced two days before use in the columns. To make each Sephadex column a sufficient amount of the slurry was poured into a 10 ml syringe with a filter paper disc at the bottom to make up a volume of 4 ml.

Both PVP and Sephadex columns were rinsed with 20 ml 1.0M ammonium acetate (pH unadjusted), followed by 30 ml of 0.01M ammonium acetate (pH unadjusted). Prepared columns were used for the purification of sample extracts or used as controls for checking recovery after adding internal standard (Plate 7.9 b.).

Sep-Pak cartridges (Plate 7.10 a., insert) were prepared before use by rinsing with 4 ml 100% MeOH, followed by 2 X 4 ml 0.1M acetic acid rinses.

7.2.6.9.3 Purification of sample extracts

Samples were purified (Plate 7.9 b. & 7.10 a.) following a method suggested by Woolley[3] (pers. comm., 1993). A series of preliminary studies was performed using spare samples to test the efficiency of the method for the main sample extraction. Both (±)-[^{14}C]ABA and (±)-[G-^{3}H]ABA were used as internal standards for this preliminary work. However, high specific activity tritiated ABA was used as the internal standard for the main sample extractions. An average of 89% recovery was obtained using (±)-[G-^{3}H]ABA (Amersham International plc, Buckinghamshire, HP7 9NA, England).

20 ml 80% methanol was added to 0.2-0.25 g dry weight samples in 25 ml plastic vials and homogenized on a shaker (179 RPM) overnight in the cold room (4°C). Homogenized samples were filtered through a sintered glass filter into 45 ml plastic vials and rinsed with 15 ml 80% methanol. Then 20 μl internal standard (tritiated ABA) was added to the sample extracts (20 μl ≈ 11000 dpm ≈ 20 pg ABA). Filtered samples (plus internal standard) were reduced to dryness (≈ 12 hours) using an automatic Speedvac concentrator (Savant). Dried samples were rinsed with 2 X 3 ml of 0.1 M ammonium acetate (adjusted pH 9 with ammonia) and after centrifuging for 1 min (4000 RPM) gently loaded using a Pasteur pipette onto the PVP column, mounted above the Sephadex column. Both columns were washed with 20 to 30 ml 0.01M ammonium acetate (pH unadjusted). The PVP column was removed and a Sep-Pak cartridge was placed under the Sephadex column. ABA was eluted from the Sephadex with 15-20 ml 1M acetic acid.

The Sep-Pak cartridge was removed and washed immediately with 5 ml H_2O and then eluted with 7 ml of 70% MeOH into a labelled glass vial. At this step 20 μl of eluted extract were added to 5 ml scintillation counter fluid and the internal standard measured using a Liquid Scintillation counter (Type Wallac 1409 Series, Wallac Oy, Turku, Finland) to check the recovery of each column. In each series of purifications 8 columns were used for plant samples

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[3] *Dr D.J.Woolley, Department of Plant Science, Massey University used this method for purification of samples in analysing ABA and cytokinins by radio-immunoassay (RIA).*

(with internal standard added at the beginning of extraction) and two columns used for internal standards using 20 µl tritiated ABA (specific activity described above). So, a total of 10 columns was used at each series of extractions.

Eluted extracts from Sep-Pak cartridges were reduced to dryness using the Savant concentrator (≈5.5 h). Dried samples were dissolved in 1 ml TBS and used for ELISA. A series of dilutions was performed as needed.

7.2.6.9.4 ELISA assay material

This procedure followed the method of Walker-Simmons (1987).
TBS buffer:
6.05 g Tris, 0.20 mg $MgCl_2$, 8.80 g NaCl per litre (pH 7.8)
Washing buffer:
TBS containing 0.05% (v/v) Tween 20, and 0.1% (w/v) BSA (ELISA grade, Sigma). Note that washing buffer was prepared by addition of the required amount of BSA to TBST (TBS plus Tween 20) on the day of use (1 mg BSA ml^{-1} of TBS).
ABA-4' -BSA conjugate:
The conjugate was prepared according to Weiler (1980, 1986) and lyophilized. Conjugate was suspended in 0.05 M $NaHCO_3$ (pH 9.6) at a concentration of 7 mg ml^{-1}, and stock aliquots of 30 µl were frozen at -20°C. Before ELISA assay a 60 µl (2 X 30 µl) aliquot was thawed and diluted with 0.05 M $NaHCO_3$ (pH 9.6), to final volume of 16 ml (2 X 8 ml), which was sufficient to coat the usable assay wells of a microtitration plate.
Monoclonal antibody (MAb):
MAb (Phytodetek® ABA Monoclonal Antibody [2 mg]) to free cis, trans(+)ABA was purchased from Idetek, Inc., 1245 Reamwood Avenue, Sunnyvale, CA 94089, USA. Two mg MAb was mixed into 135 ml TBS, containing 0.2% (w/v) BSA. Aliquots of 0.85 ml of the MAb solution were stored at -20°C. Before ELISA assay a 2.55 ml aliquot (3 X 0.85 ml) was thawed and diluted in TBS

buffer to a final volume of 12.75 ml, which was sufficient for the assay wells of a microtitration plate.

Second antibody:

Rabbit antimouse alkaline phosphatase conjugate (Sigma Chemical Co., USA) was diluted 1:1000 prior to use with TBS (16 µl in 16 ml for full plate).

Alkaline phosphatase substrate (PNP):

The substrate, *p*-nitrophenyl phosphate was prepared at a concentration of 1 mg ml^{-1} in 0.05 M $NaHCO_3$ (pH 9.6).

ABA standard:

(±)cis-trans ABA purchased from Sigma Chemical Co. was dissolved in TBS in concentrations ranging from 0.1 to 1000 pg 100^{-1} µl.

Microtitration plates:

Immulon 2 flat bottom, 96-well microtitration plates, Dynatech Laboratories, Inc., Alexandria, VA, USA were utilized. The upper and lower row of wells of the plate were not used.

7.2.6.9.5 ELISA assay procedure

All samples and solutions were kept in the dark during incubations.

1. Coating of well with ABA-4' -BSA conjugate

A 200 µl aliquot of the conjugate was added to each well of the microtitration plate. Plates were incubated at 4°C overnight.

2. Incubation of ABA samples with MAb

A 350 µl aliquot of either standard sample (ABA) or plant sample extract was pipetted into a labelled 1.5 ml Eppendorf tube, then 350 µl of MAb solution were added and mixed by vortex mixer and centrifuged. The solutions were incubated overnight at 4°C.

3. Addition of ABA samples incubated with MAb.

Plate wells coated with conjugate were washed four times with washing buffer. For this step only, the last two washing solutions were left in the plate for 5 and 10 minutes and then discarded. A 200 µl aliquot of the samples incubated with MAb was added to each well. Plates were incubated for 2.5 hours. This incubation and all the following steps were performed at room temperature.

4. Addition of the second antibody

Wells were washed four times with washing buffer. Rabbit antimouse alkaline phosphatase conjugate (200 µl) was added to each well. Plates were incubated for 2 hours.

5. Measurement of alkaline phosphatase

Wells were washed four times with washing buffer. *p*-Nitrophenyl phosphate substrate (200 µl) was added to each well. Plates were incubated for 2.5-3.5 hours. The sample absorbance was measured at 410 nm (Plate 7.10 b.) using a Dynatech Microplate Reader Model, MR 5000/7000 (Dynatech Laboratories Inc, VA 22021, USA). The absorbance of the sample was inversely proportional to the amount of ABA in the original sample incubated with MAb. All samples were assayed in 3 replicate wells within each plate (up to 12 assays replicated per treatment) and a series of dilutions performed as needed.

7.2.6.9.6 Estimating ABA content in plant samples

A linear regression analysis (*logit* B/B_o versus *ln* ABA standard) was computed using ABA standards [0.1 - 1000 pg well^{-1} (±ABA)](Figure 7.2), and the amount of ABA in the plant extract samples was calculated based on the coefficient of the ABA standard for each plate.

$$logit\ (B/B_o\ \%) = \ln\ [B/B_o/(100 - B/B_o)]$$

Where B_o is the absorbance in the absence of any ABA, and B is the absorbance of the ABA standard or sample.

7.3 STATISTICAL ANALYSIS

Data were analysed as described in chapter 4 (section 4.2.2).

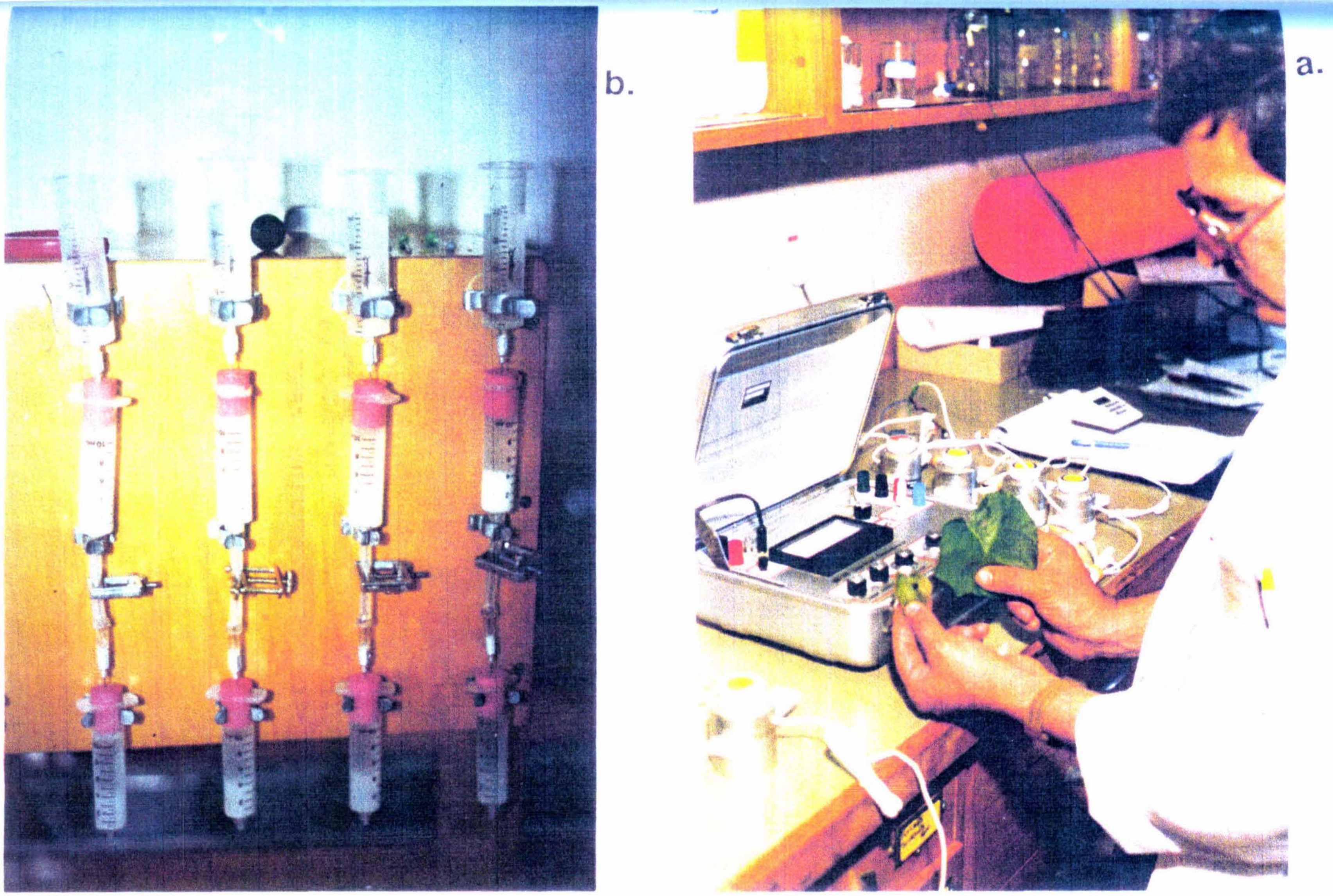

Plate 7.9 a). Measurements of Ψ_l and Ψ_s of partially expanded leaves below the shoot tips and mature leaves using the dew point method. **b).** PVP and Sephadex columns before purification of samples for ABA analysis.

Plate 7.10 a). Sephadex columns after purification of sample extracts for ABA analysis (insert. Washing Sep-Pak cartridge column with H_2O before eluting ABA). **b).** Measurement of sample absorbance at 410 nm using Dynatech Microplate Reader Model. MR 5000/7000.

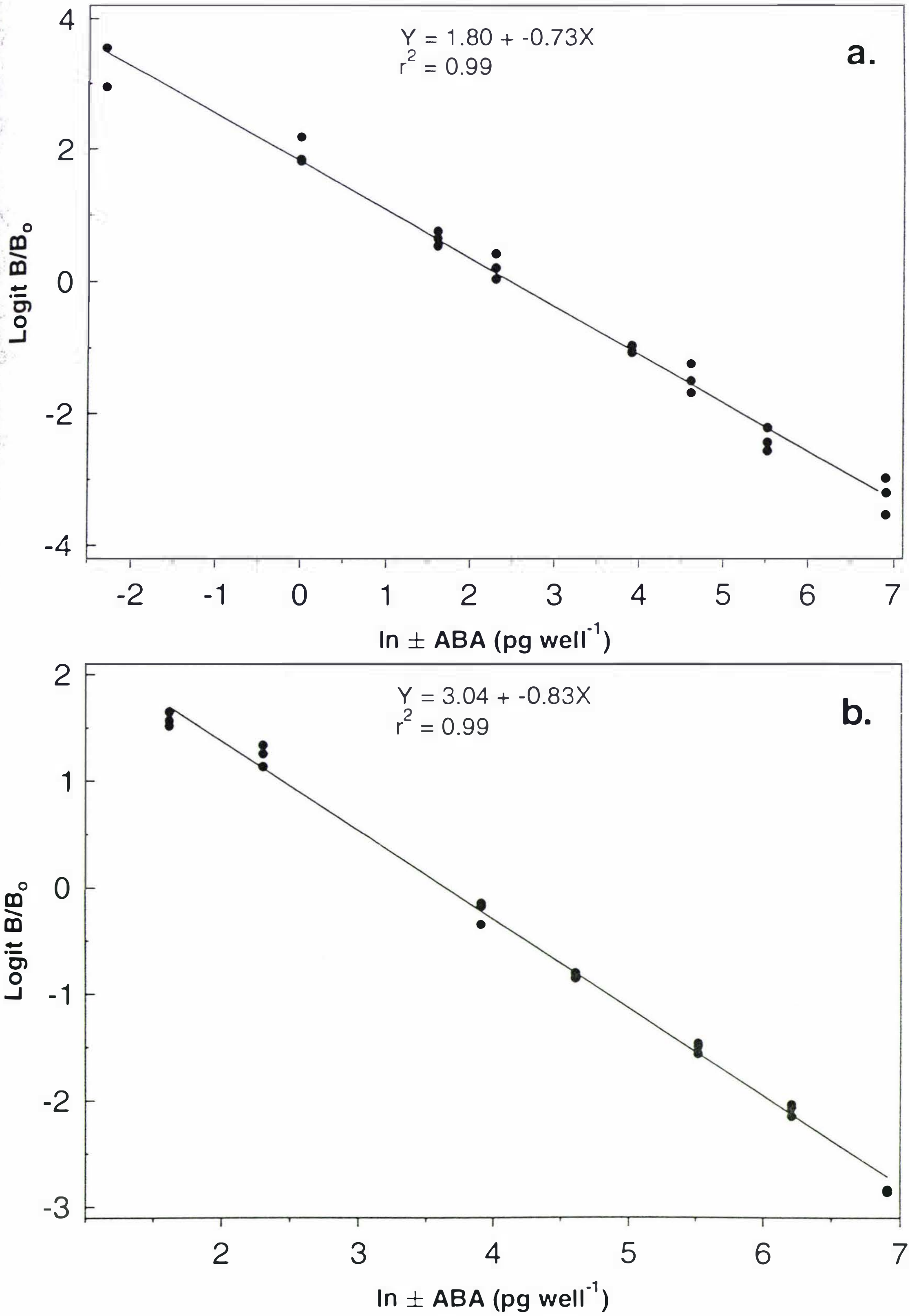

Figure 7.2 Typical standard curves used for determination of abscisic acid (ABA) in the plant extract samples using ELISA method. a). Logit (B/B$_o$) versus ln ±ABA (0.1-1000 pg well^{-1}) fitted to the linear regression; and b). Logit (B/B$_o$) versus ln ±ABA (5-1000 pg well^{-1}) fitted to the linear regression. Readings at 410 nm after 2.45 hours.

7.4 RESULTS[4]

7.4.1 Xylem water potential (Ψ_{xylem})

Xylem water potential (Ψ_{xylem}) was monitored from the time of applying the treatments (0 dats), and is recorded in Figure 7.3 a. In general, Ψ_{xylem} of control and BAP was higher than for all the water stress treatments during the experiment.

Following 28 days of continuous misting during the establishment period, treatments began on 0 dats. Both WS treatments (WS and BAP+WS) commenced with 30 minutes off-time and 1 minute on-time misting (Figure 7.3 b.). Six hours after commencing the water stress (0 dats) Ψ_{xylem} of WS and BAP+WS treatments had dropped considerably lower than control and BAP treatments ($P<0.05$). This unintentionally severe early water stress was responded to by immediately shortening the misting off time (Figure 7.3 b.). Over the next 7 days (WS) and 23 days (BAP+WS) Ψ_{xylem} returned to the level of the control plants. BAP Ψ_{xylem} dropped on 13 dats and recovered to the control level by 23 dats. With initial concern over the possible long term effects of the premature stress further control trees were allocated to the water stress treatment on 7 dats, identified as WSb treatment.

From 23 dats (28 October) when Ψ_{xylem} was similar in all treatments the misting off time and VPD were gradually increased (Figures 7.1 c. and 7.3 b.), and after 11 days Ψ_{xylem} started to decline in all water stressed trees. At both 36 and 44 dats control and BAP showed higher Ψ_{xylem} than the stress treatments ($P<0.05$). Ψ_{xylem} in stressed trees (WS and WSb) continued to fall rapidly to about -2.1 MPa on 51 dats when the plants made a rapid recovery.

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[4] *Leakage problems occurred in the aeroponic circulation tubing, causing interruption of the misting system for 1-2 hours during the following days and treatments: i. 12-15 dats BAP and ii. 12-14 dats BAP+WS (see also footnote 2). Effects will be considered in the discussion.*

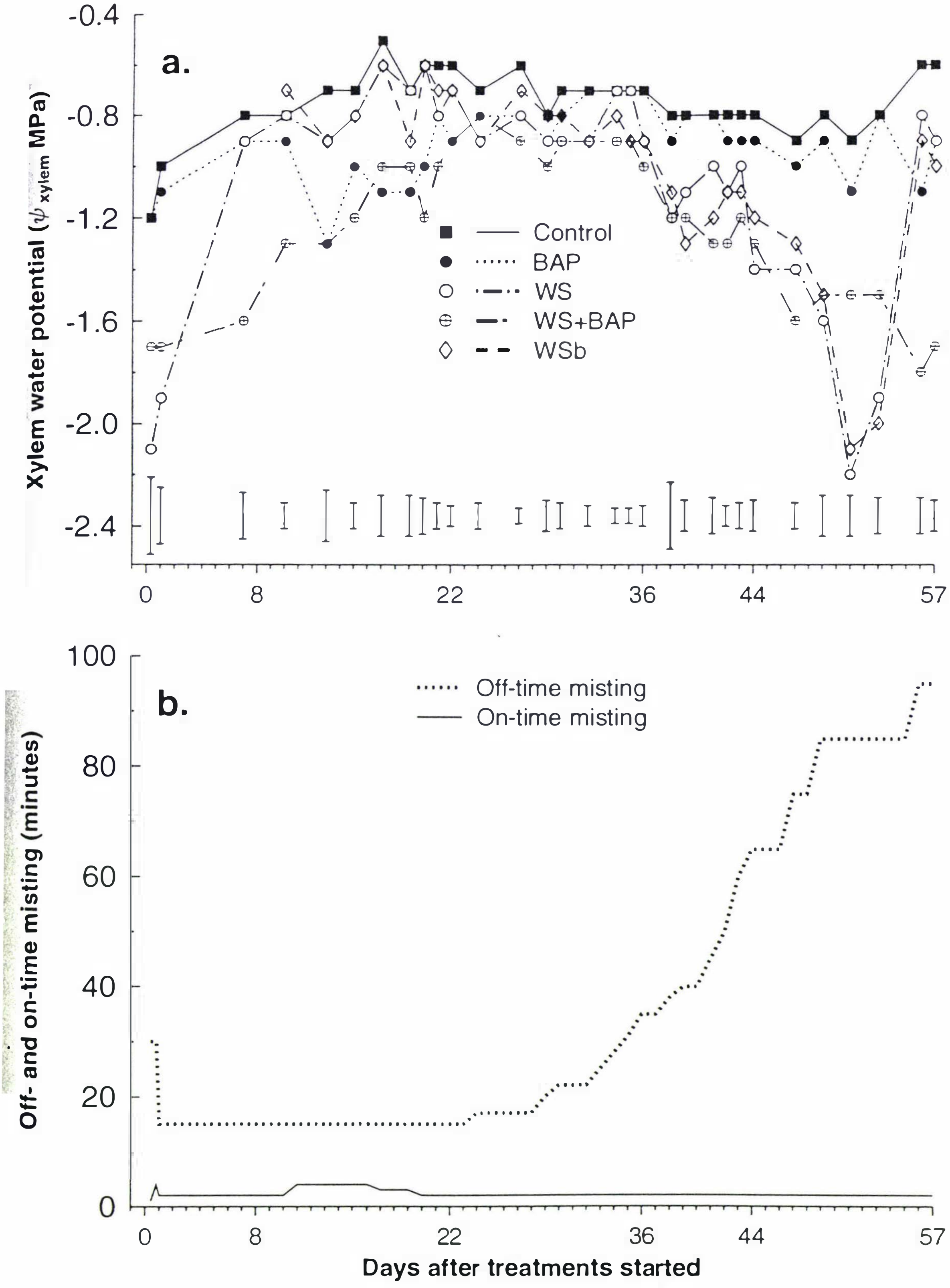

Figure 7.3 Xylem water potential response of 'Trevatt' apricot to different treatments under controlled environment conditions. a). Xylem water potential (ψ_{xylem}); Bars show the standard error of the means. Each point represents the mean of four leaves per treatment. b). Off-time and on-time misting (minutes) on water stress treatments (WS, WS+BAP & WSb).

In the presence of BAP, water stress trees Ψ_{xylem} fell only to -1.8 MPa. On the final day (57 dats), control had a higher Ψ_{xylem} than the other treatments ($P<0.05$).

7.4.2 Shoot growth

The shoot lengths and shoot growth rates are shown in Figure 7.4. In general control and BAP showed greater shoot length throughout the experiment than the 3 water stress treatments. From about 15 to 48 dats the shoot growth rate was similar in all treatments, but then it fell noticeably in the 3 stress treatments.

By 8 dats the shoot length of the WS trees which had been subjected to the brief but severe water stress in the previous week was significantly less than in the other treatments ($P<0.001$, Figure 7.4 a.). On trees receiving the same water deficit in the presence of BAP, shoot growth had not been reduced. Two weeks later (22 dats) both WS and BAP+WS showed shorter shoot length than control, BAP and WSb treatments ($P<0.001$ and $P<0.05$ for WS and BAP+WS respectively), but BAP+WS shoot length was significantly longer than WS alone ($P<0.01$). However at 35 dats only WS treatment showed less shoot length than the other treatments ($P<0.001$). At 44 dats WS and BAP+WS recorded shorter shoot length and their differences from the control were significant ($P<0.001$ and $P<0.05$ for WS and BAP+WS respectively). On the final day of experiment (57 dats), all stress treatments showed lower shoot length than control treatment ($P<0.001$, $P=0.084$ and $P=0.055$ for WS, BAP+WS and WSb, respectively). At this time (57 dats) BAP showed greater shoot length than WS treatment ($P<0.01$). During the experiment (after treatments were applied) generally shoot growth rate responded to environmental conditions and water stress level (Figure 7.4 b.). The shoot growth rate of stress treatments reached minima after 50 dats, while active shoot growth continued in both control and BAP.

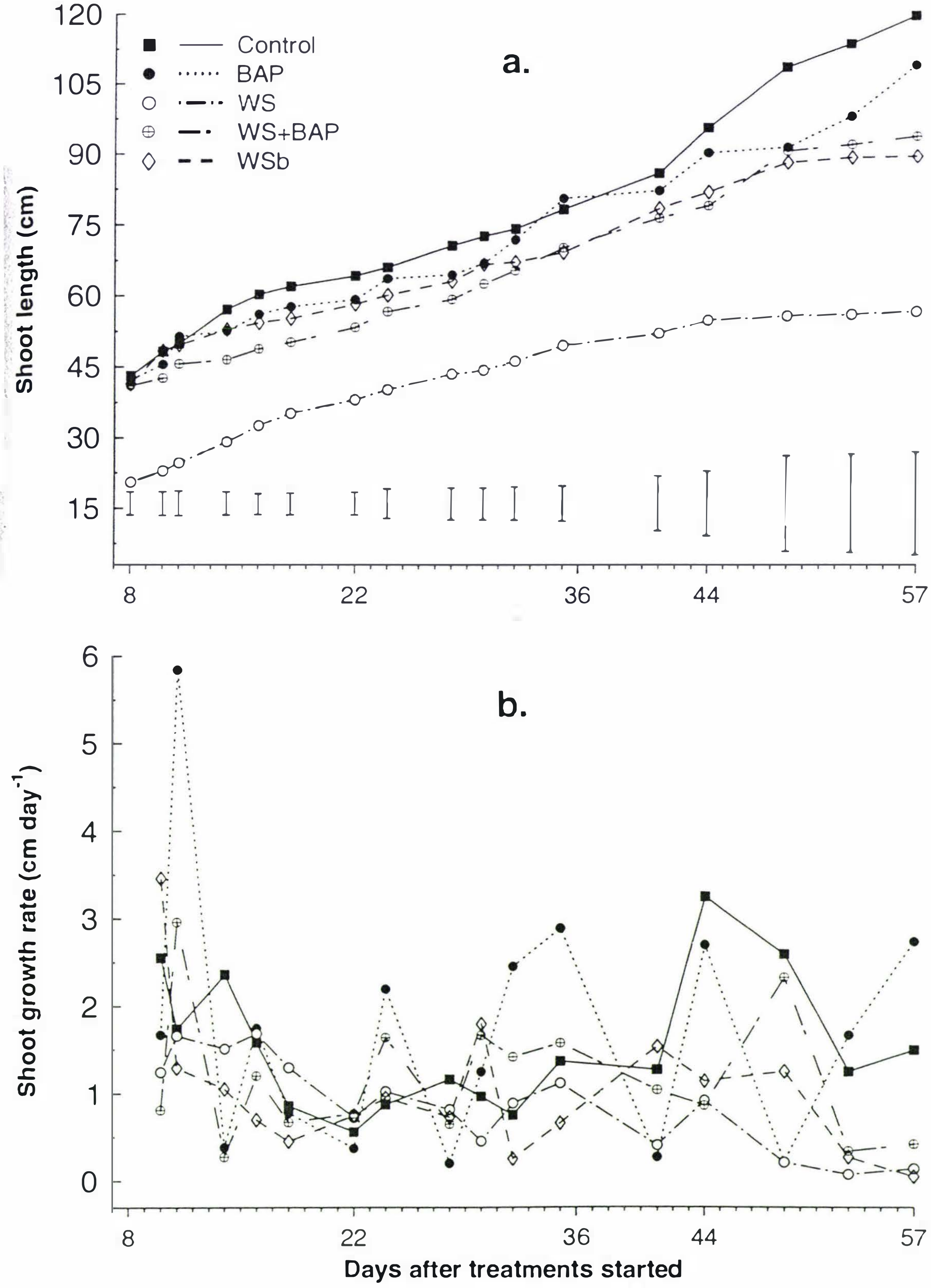

Figure 7.4 The effect of different treatments on shoot growth of 'Trevatt' apricot under controlled environment conditions. a). Shoot length (cm) and b). Shoot growth rate (cm day^{-1}). The number of recorded shoots for each treatment (n=34 at day 8) decreased as days after treatment increased (n=4 day 57). Bars show the standard error of means.

7.4.3 Net CO_2 assimilation rate (A) and stomatal conductance (g_s)

Net CO_2 assimilation rate and stomatal conductance measurements (Table 7.1) showed treatments were similar, values being high at the start then declining, but with a slight peak at 50 dats. Stomatal conductance continued to recover at the final date, but assimilation again fell.

Shortly after commencing treatments (2 dats) all treatments showed significantly lower A than control ($P<0.05$). The level of g_s at this time was higher in control than in WS ($P<0.05$). However, BAP showed higher and WS lower g_s than the other treatments ($P<0.05$). One week later (8 dats) control showed higher A and g_s than the other measured treatments ($P<0.05$). At 23 dats BAP showed higher A than control and higher g_s than all other treatments ($P<0.05$).

The differences between the net CO_2 assimilation rate values of treatments at 30 dats were not significant, but at this time control showed lower and BAP higher g_s than the other treatments ($P<0.05$). One week later (37 dats) all treatments A values were similar, but control and WS showed lower g_s than the other treatments ($P<0.05$). At 43 dats WS showed higher A than the other treatments ($P<0.05$), but at this time BAP recorded a bigger g_s than the other treatments ($P<0.05$). One week later (50 dats) control and BAP showed higher A than the other treatments, while BAP showed higher and WS lower g_s than the other treatments ($P<0.05$). At the end of the experiment (57 dats) BAP had significantly higher A than the other treatments ($P<0.05$), while showing higher g_s ($P<0.05$) than most other treatments (except WSb).

7.4.4 Dry matter partitioning (destructive harvest)

Understandably, the majority of dry matter was in the older tissue of the plants (old roots 18% and stem 77%) at the start of the experiment (0 dats), but the

Table 7.1. The effect of treatments on **a).** Net CO_2 assimilation rate (A) and **b).** Stomatal conductance (g_s) of 'Trevatt' apricot in the aeroponic system and controlled environment conditions.

dats[z]	**a).** A (μ mol $m^{-2}s^{-1}$)				
	Control	BAP	WS	BAP+WS	WSb
2	13.1 a[y]	9.6 bc	8.8 c	11.5 b	-
8	14.0 a	-	10.8 b	11.2 b	-
23	5.4 b	9.8 a	7.6 ab	8.7 ab	-
30	4.3 a	3.3 a	4.3 a	4.7 a	-
37	3.0 ab	3.0 ab	3.1 ab	4.5 a	2.1 b
43	3.3 b	2.2 b	5.5 a	3.6 b	2.5 b
50	8.8 ab	10.7 a	7.1 b	6.9 b	6.2 b
57	4.3 b	9.4 a	5.6 b	6.5 b	3.6 b
	b). g_s (m mol $m^{-2}s^{-1}$)				
2	224 b	306 a	87 c	180 b	-
8	278 a	_	177 b	-	-
23	145 b	271 a	118 b	159 b	-
30	97 c	187 a	142 b	154 b	-
37	157 c	263 b	149 c	228 b	311 a
43	76 b	124 a	88 b	98 b	98 b
50	112 bc	236 a	101 c	141 b	116 bc
57	122 b	256 a	117 b	161 b	228 a

[z] Days after treatment started (dats).

[y] Numbers in the same row with different letters are significantly different at $P \leq 0.05$ by Duncan's multiple range test.

proportion in new tissues (new roots, shoots and leaves) progressively increased to the end of the experiment (Figure 7.5). In general, BAP treatments produced more new roots than the other treatments (eg. Figure 7.5 f. and Plate 7.11).

At 8 dats total plant dry weight, area and dry weight of leaves and new root length (Figure 7.6) of all treatments were not statistically different. However, at 22 dats control produced more dry matter than the other treatments ($P<0.05$). Control showed larger ($P<0.05$) leaf area and leaf dry weight than the other treatments (Figure 7.6 a. & b.), but differences in root length were not significant at this date (Figure 7.6 c.).

Differences between treatments in total plant dry weight and new root length were not significant at 36 dats. However, both leaf dry weight and leaf area of WS treatment were reduced relative to control and BAP treatments ($P<0.05$). In addition, this treatment (WS) showed lower leaf dry weight than BAP+WS ($P<0.05$). BAP and BAP+WS leaf dry weight differences from control were not significant, but leaf area of BAP+WS was significantly less than control ($P<0.05$).

At 44 dats root length of the BAP treatments was higher than the other treatments, although only significantly different from WS and WSb ($P<0.05$). At the final destructive harvest (Figure 7.6 d.) total plant dry matter of BAP treatment was high similar to control, and significantly higher than WS and BAP+WS ($P<0.05$). Both control (Plates 7.5 & 7.12 a.) and BAP treatments at this time (57 dats) produced the highest leaf area and leaf dry weight, and their differences relative to WS (Plate 7.12 b.) and BAP+WS were significant ($P<0.01$). Control and BAP also had larger leaf dry weight than WSb ($P<0.05$), and control tended to have larger leaf area than WSb ($P=0.066$).

Strong new growth occurred in all tissues of control and BAP through the 57 days and it was much greater than in WS, and to a lesser degree greater than in BAP+WS. Plant growth in the latter two treatments was inhibited in comparison with control plants from about 36 dats, especially in shoots and leaves. WSb inhibited plant growth less than WS, and treatments including BAP had enhanced root growth.

7.4.4.1 Root to shoot ratio

The root to shoot ratio determined on both new and total dry matter bases are shown in Figure 7.7 c. & d. respectively. The differences between treatments were not significant during the first week of treatment (at 0 and 8 dats). In general, all treatments increased the root:shoot ratio, which was highest on both bases for BAP+WS.

At 22 dats the new root:shoot ratios (Figure 7.7 c.) of both BAP treatments were higher than the other treatments ($P<0.01$), but their total root:shoot ratio was only higher than control ($P<0.05$). At 36 dats these differences remained, and at this time WS also showed higher new and total root:shoot ratios than control ($P<0.05$). Treatment differences at 44 dats were not significant.

At the final destructive harvest (see also Plates 7.5, 7.11 & 7.12) on both dry weight bases the control root:shoot ratio was lowest ($P<0.05$ relative to BAP and WSb and $P<0.01$ to WS and BAP+WS). BAP+WS showed the highest value on both bases at this time (57 dats), significantly greater than WS and WSb ($P<0.05$), which were higher than control on both bases ($P<0.05$).

7.4.5 Water potential (Ψ_l), turgor potential (Ψ_p) and osmotic potential (Ψ_s)

Plant leaf water relations (Figure 7.8) were recorded to determine if osmotic adjustment occurred. This was done on mature leaves as sampled for CHO and mineral analyses (section 7.2.6.7 and 7.2.6.8) and also on partially

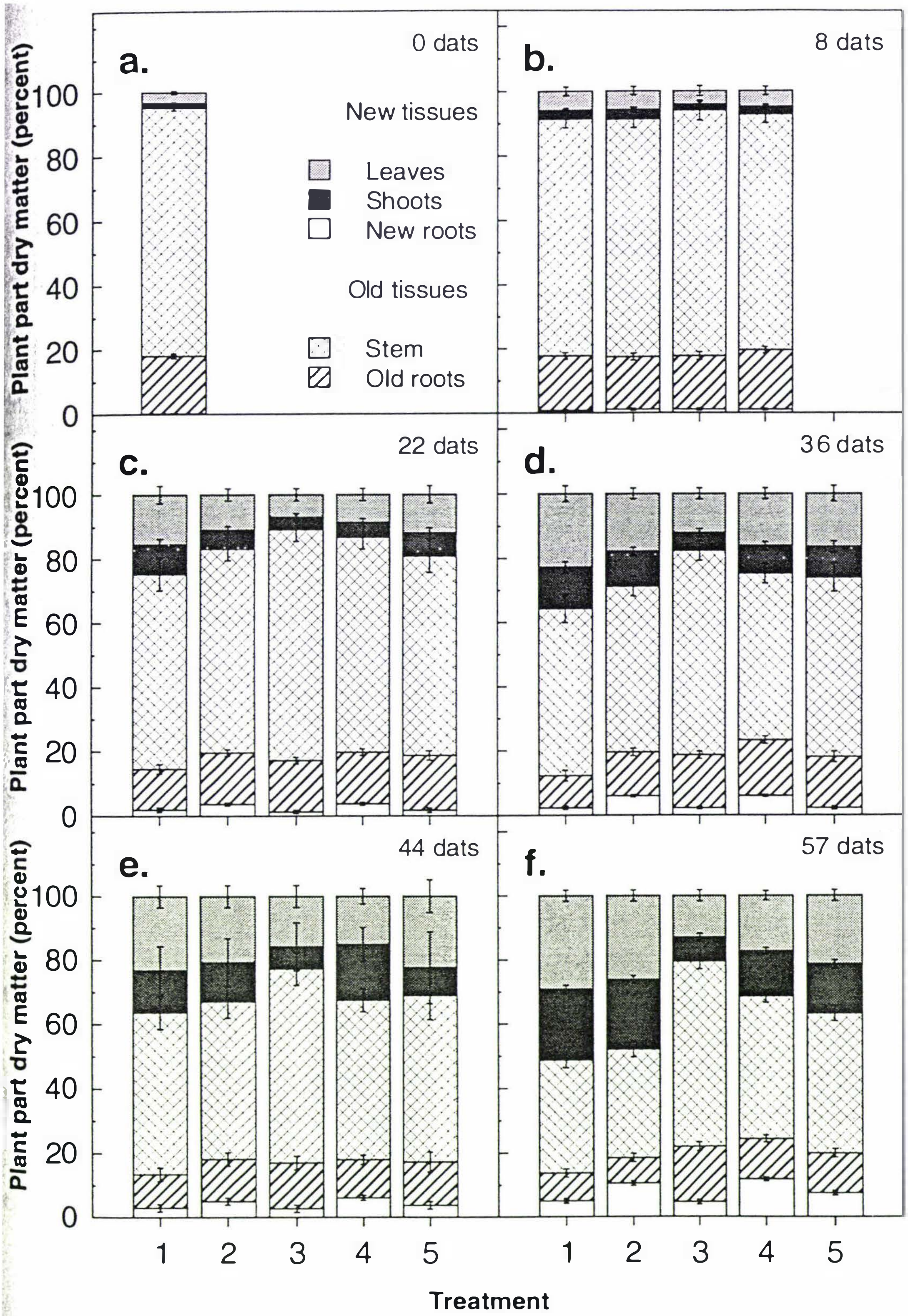

Figure 7.5 The influence of treatments on distribution of plant dry weight of 'Trevatt' apricot grown in an aeroponic system. a). Destructive harvest prior to of start treatments (4 weeks after establishment, n=8); b). 8 days after treatments started (dats); c). 22 dats; d). 36 dats; e). 44 dats; and f). 57 dats. Treatment: 1=Control; 2=BAP; 3=WS; 4=BAP+WS and 5=WSb. (n=4 treatment^{-1} harvest^{-1}).

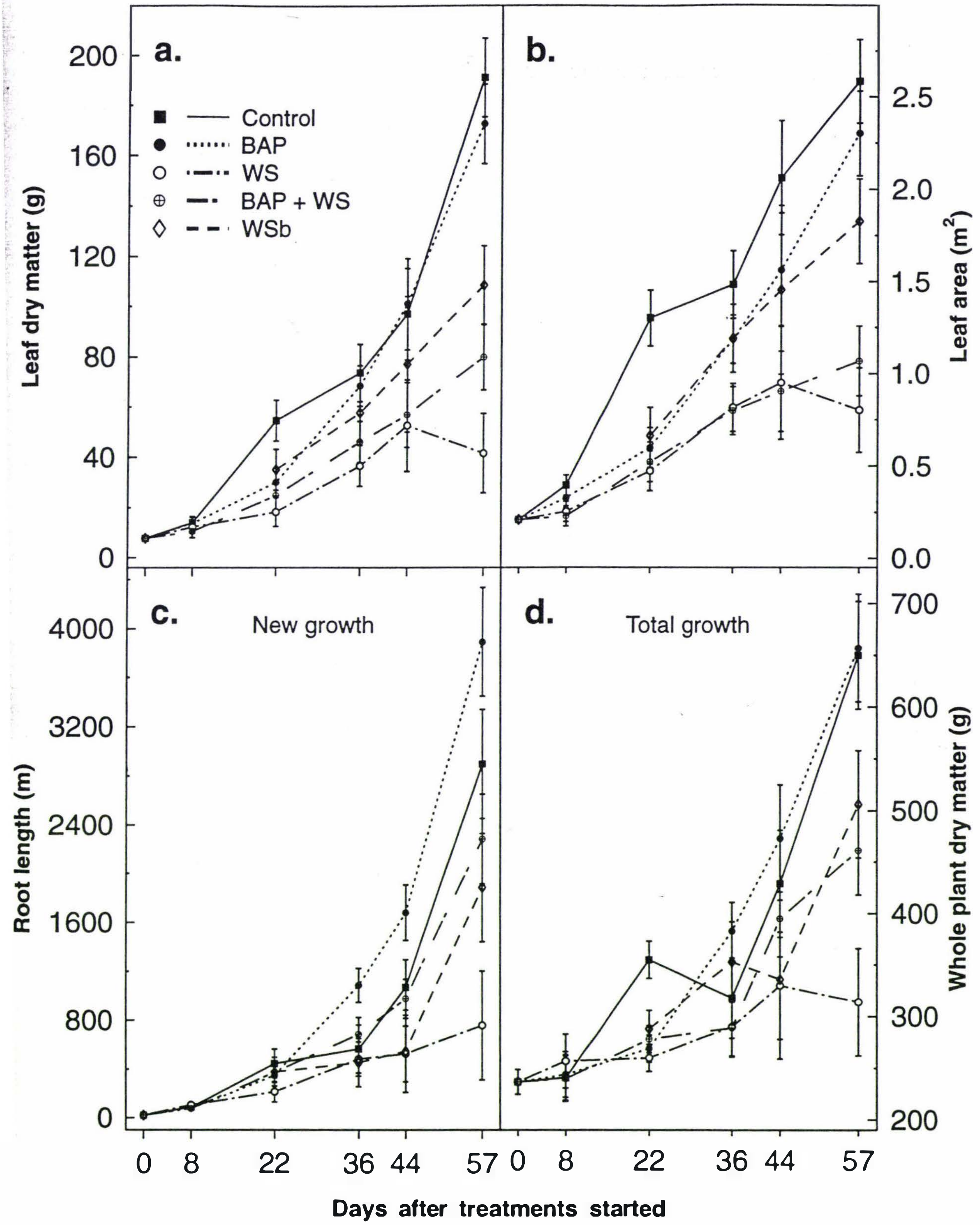

Figure 7.6 The effect of treatments on plant parameters of 'Trevatt' apricot grown in an aeroponic system. a). Leaf dry matter (g); b). Leaf area (m^2); c). Root length (m); and d). Whole plant dry matter (g). Bars show the standard error of the mean. Each point represents the mean for two plants on each replicate per treatment (n=8 for day 0 and n=4 for all other dates for all treatments).

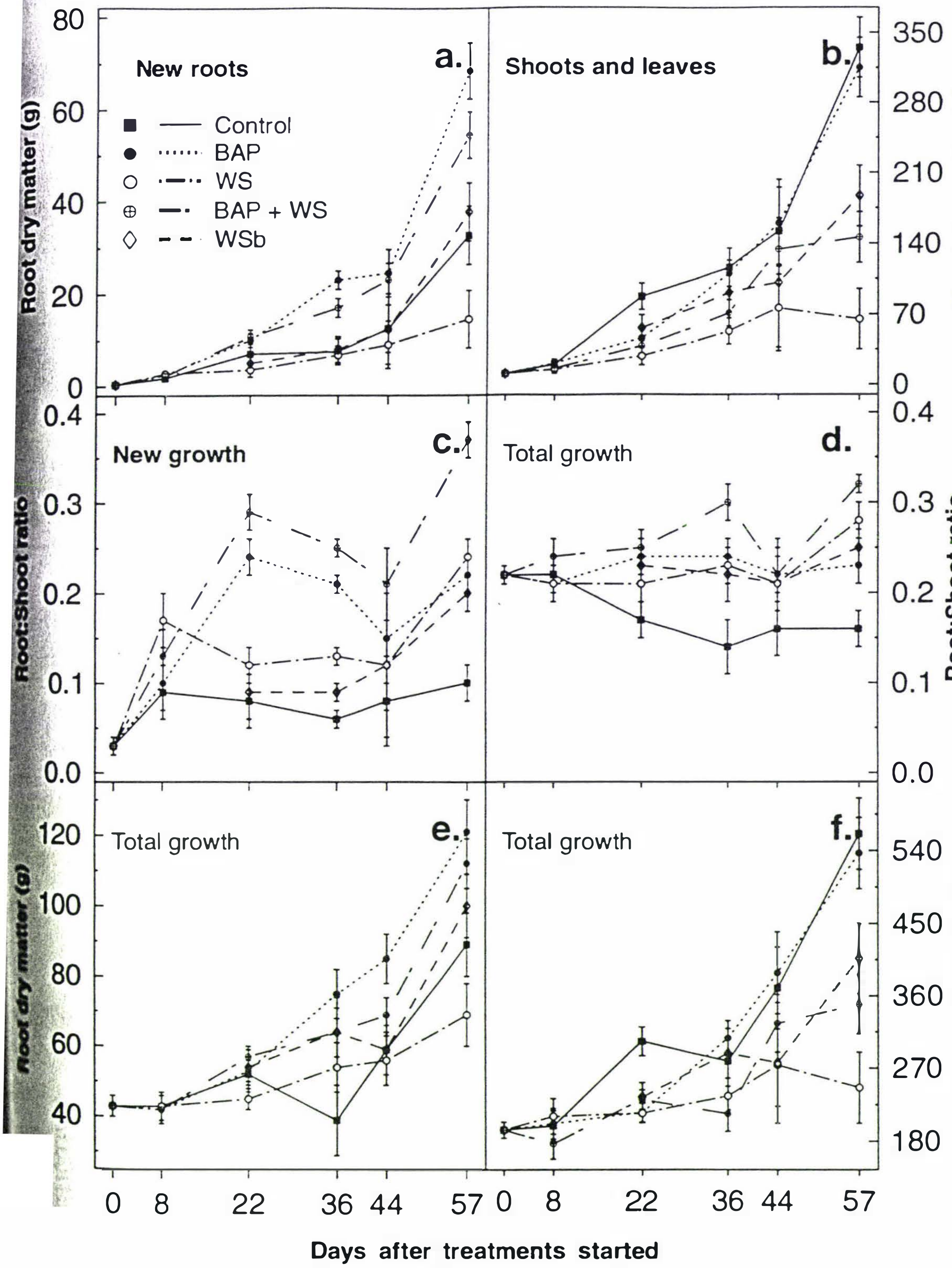

Figure 7.7 The effect of treatments on dry matter partitioning in 'Trevatt' apricot grown in an aeroponic system. a). New roots dry matter (g); b). New shoots and leaves dry matter; c). New root to shoot dry weight ratio; d). Overall root to shoot dry weight ratio (new and old parts); e). Root dry matter (new and old roots); and f). Top dry matter (stem and new shoots and leaves). Bars show the standard error of the mean. Each point represents the mean of two plants in each replicate (n=4 treatments^{-1} harvest^{-1}).

Plate 7.11 Final destructive harvest, 84 days after initiating the experiment (57 dats), of aeroponically grown 'Trevatt' apricot under controlled environment conditions. **a)**. BAP+WS and **b)**. BAP treatments.

Plate 7.12 Final destructive harvest 84 days after initiating the experiment (57 dats) of aeroponically grown 'Trevatt' apricot under controlled environment conditions: **a)**. control and **b)**. WS treatments

expanded leaves as sampled for ABA analysis (section 7.2.6.9.1, Plate 7.8 a.).

7.4.5.1 Fully expanded leaves (mature leaves)

During the experiment generally, Ψ_p of all treatments remained at similar levels to control, although on 42 dats, control dropped briefly with Ψ_p values lower than BAP, WS ($P<0.05$) and BAP+WS ($P=0.07$)(Figure 7.8 d.).

At 21, 42, 49 and 56 dats BAP+WS had lower Ψ_l ($P<0.05$, $P<0.01$, $P<0.05$ and $P<0.001$, respectively) and Ψ_s ($P<0.01$) than control. BAP had lower Ψ_l and Ψ_s than control on 21 and 56 dats ($P<0.05$, and $P<0.01$ for Ψ_l and Ψ_s respectively). No significant differences occurred between WS and control, but at 49 and 56 dats WSb had lower Ψ_l and Ψ_s than control ($P<0.05$).

7.4.5.2 Partially expanded leaves (young leaves)

Generally, Ψ_p of all treatments was maintained at similar levels to control, although at the end of the experiment (56 dats), WS had a higher Ψ_p than control ($P<0.05$, Figure 7.8 c.). In comparison with control, only BAP+WS had lower Ψ_l and Ψ_s at 17 and 35 dats ($P<0.05$, Figure 7.8 a. & e.). No other differences in Ψ_l and Ψ_s were significant.

7.4.6 Leaf simple carbohydrates (CHO)

The major simple carbohydrate observed in fully expanded leaves of all treatments was sorbitol (Figures 7.9 & 7.10).

At the first sampling date (8 dats), the only significant difference observed was in leaf glucose which was higher in BAP+WS than in the other treatments ($P<0.05$, Figure 7.9 b.). This treatment also showed higher sucrose at 22 dats than all other treatments ($P<0.05$). At this time (22 dats) sorbitol and total

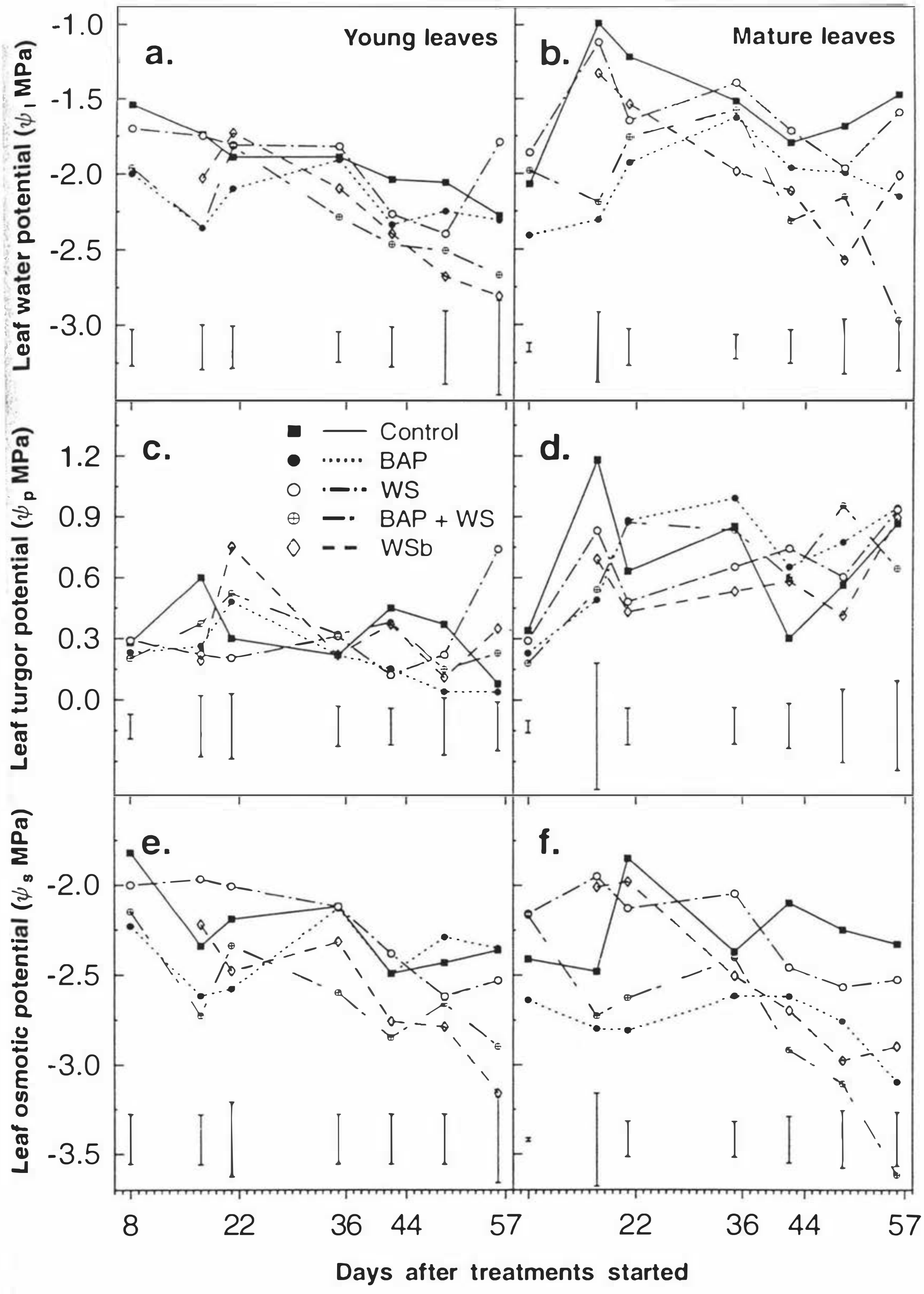

Figure 7.8 The effect of treatments on leaf water status of 'Trevatt' apricot under controlled environment conditions. a). and b). Leaf water potential (ψ_l); c). and d). Turgor potential (ψ_p); e). and f). Osmotic potential (ψ_s); a., c. & e. recorded on young leaves and b., d. & f. on mature leaves. Bars show the standard error of the mean. Each point represents the mean of two leaves on each replicate treatment^{-1} (n=4 for all treatments at each date).

CHO significantly increased in BAP+WS relative to BAP, WS and WSb ($P<0.05$), but not to control.

At 36 dats no significant differences were observed among treatments in any simple carbohydrate. However, at 44 dats the amounts of all simple carbohydrates declined in control treatment, although differences with the other treatments were significant ($P<0.05$) only in sucrose and glucose. In contrast, on the last day (57 dats) all simple carbohydrates in control treatment were higher than in the other treatments, although significant differences only occurred in sucrose and glucose relative to WS and WSb treatments ($P<0.05$).

7.4.7 Minerals analyses

The results of the major ions analyses are shown in Figure 7.11.
At the first sampling date (8 dats), the concentration of Mg was lower in BAP+WS leaves than in control and WS treatments ($P<0.05$) and lower in BAP than in control ($P<0.05$, Figure 7.11 a.). At 8 dats in control leaves the concentration of K, Ca and P was higher and of N lower than in WS and BAP+WS treatments ($P<0.05$, Figure 7.8 d.).

Two weeks later (22 dats) Mg was higher in WS than in the other treatments ($P<0.05$). Both WS and WSb at this time showed higher Ca, although the difference was significant only with both BAP treatments ($P<0.05$) and not with control. WS treatment showed higher P relative to both BAP treatments and control, but WSb was only significantly higher in P than the BAP treatment (all $P<0.05$). At 36 dats both WS and WSb showed more Mg than the other treatments ($P<0.05$) and higher Ca, although their differences were only significant relative to BAP treatment. Control showed a higher K value than the other treatments ($P<0.05$). The P value of control was higher than WS and WSb. No significant differences occurred in N value at this date.

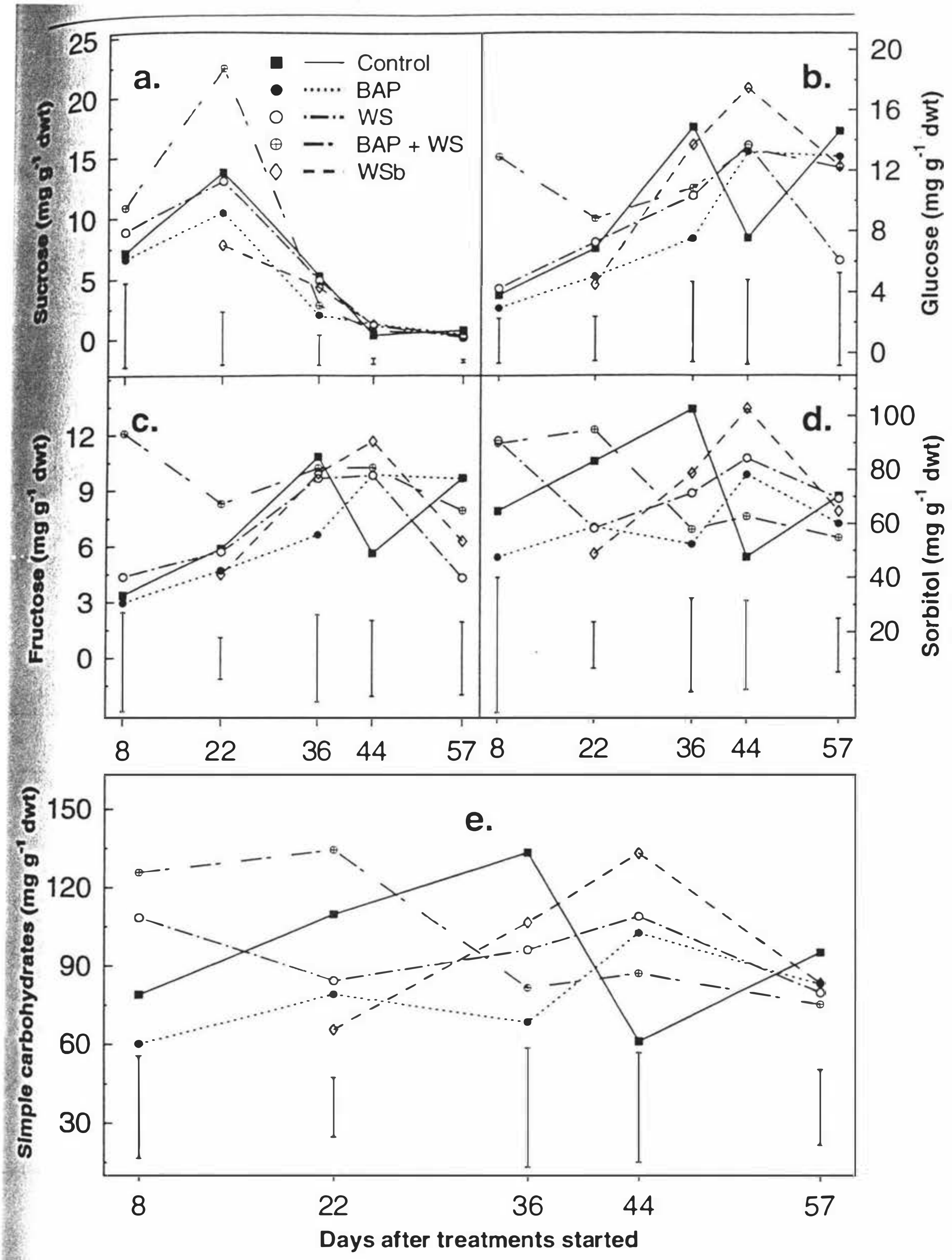

Figure 7.9 The effect of treatments on fully expanded leaf simple carbohydrates of 'Trevatt' apricot grown in an aeroponic system under controlled environment conditions. a). Sucrose; b). Glucose; c). Fructose; d). Sorbitol; and e). Total simple carbohydrates (sucrose + glucose + fructose + sorbitol). Each point represents the mean of two samples on each replicate per treatment (n=4 treatment^{-1}). Bars show the standard error of the mean.

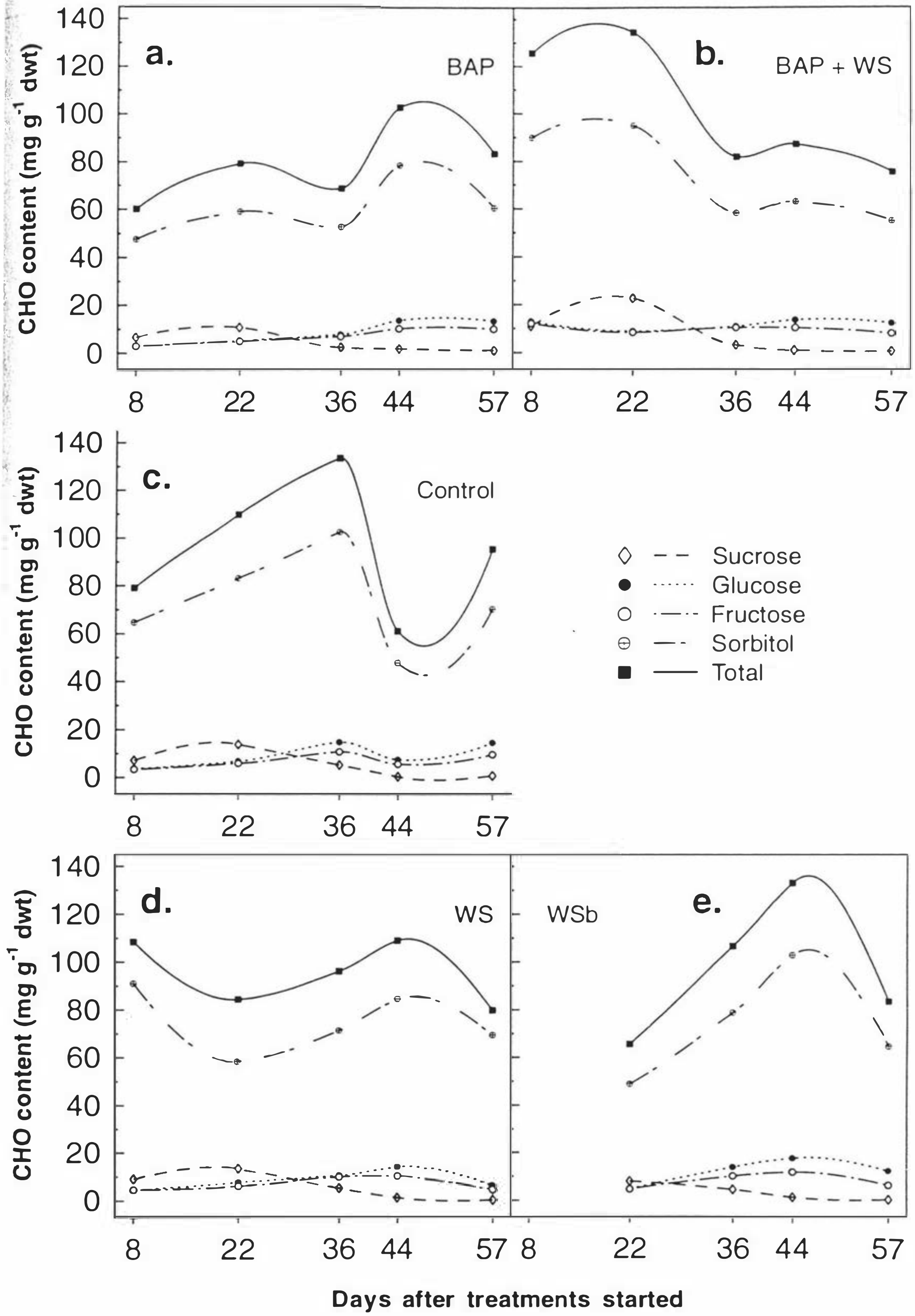

Figure 7.10 The influence of treatments on simple carbohydrates of 'Trevatt' apricot fully expanded leaves grown in an aeroponic system under controlled environment condition. a). BAP; b). BAP + WS; c). Control; d). WS; and e). WSb.

Each point represents the mean of two samples on each replicate (n=4 treatment^{-1}). Total simple carbohydrates is the sum of (sucrose + glucose + fructose + sorbitol).

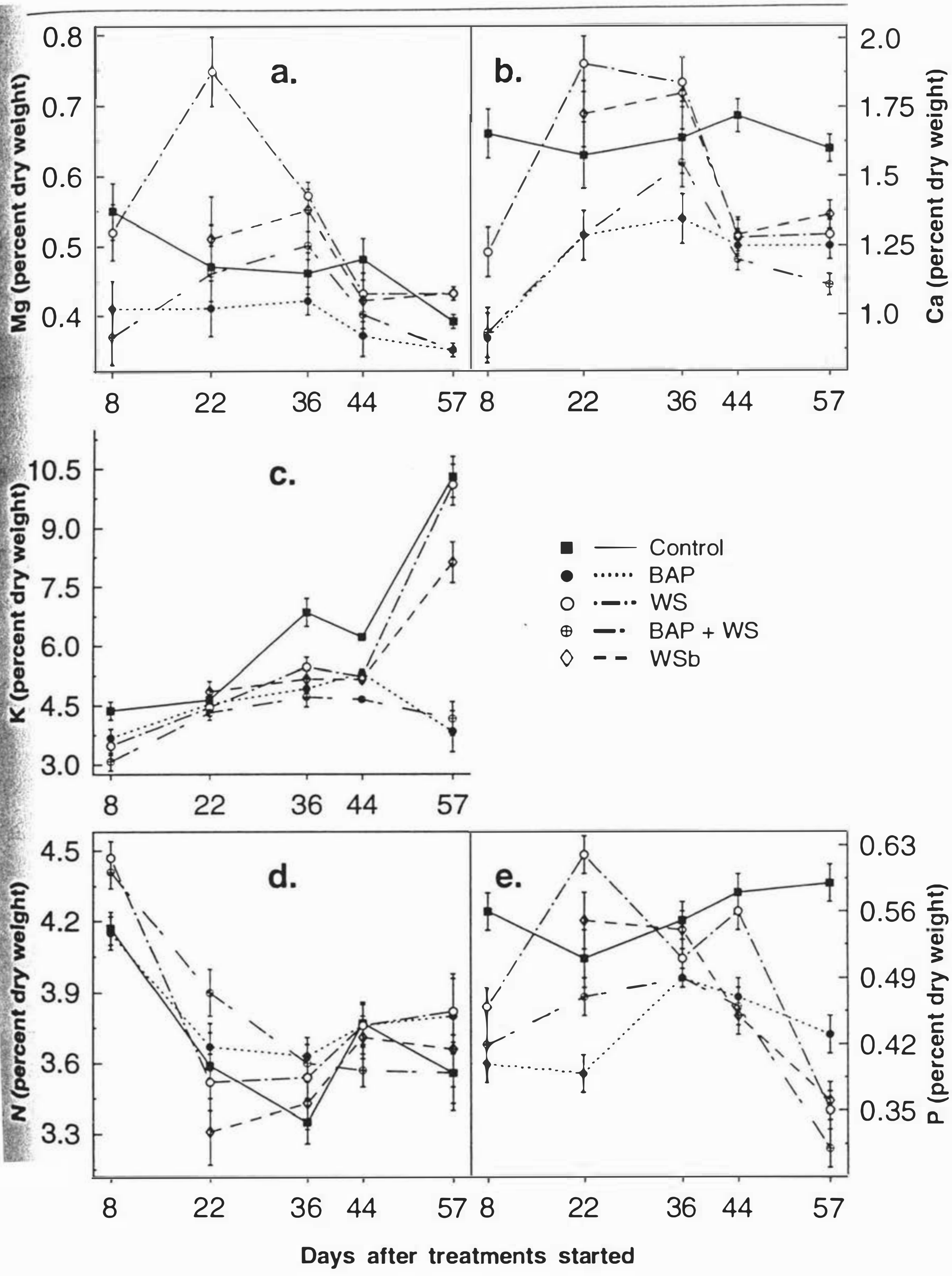

Figure 7.11 The influence of treatments on major elements of fully expanded 'Trevatt' apricot leaves grown in an aeroponic system under controlled environment conditions. a). Magnesium (Mg); b). Calcium (Ca); c). Potassium (K); d). Nitrogen (N); and e). Phosphorus (P). Bars show the standard error of means. Each point represents the mean of two samples on each replicate (n=4 for all treatments).

The concentrations of Mg, K, Ca and P declined in the BAP treatment at 44 dats relative to control ($P<0.05$). WS and WSb levels of K and Ca fell relative to control ($P<0.05$). Again no significant differences in N occurred. At the final sampling date (57 dats), the leaf Mg of WS and WSb treatments was significantly increased above control ($P<0.05$). In contrast, the concentration of Ca and P in WS and of K, Ca and P in WSb declined relative to control. At this time the Mg, K, Ca and P were lower in both BAP treatments than in control ($P<0.05$). Treatment differences in N were not significant.

7.4.8 ABA determination in shoot tip and partially expanded leaves using ELISA

A strong relationship was consistantly obtained (ie. $r^2=0.99$ in Figure 7.2 a. & b.) between absorption and ABA over a wide range of concentrations in the ELISA assay. The changes in ABA concentration (ng g^{-1} dry weight) in the shoot tip during the experiment are shown in Figure 7.12 a. (except WSb). In general, WS had a higher concentration of ABA than the other treatments from 22 dats with a maximum achieved at 44 dats, the final date sampled.

At the first sampling date (8 dats) there was no significant treatment effect (Figure 7.12 b.). From 22 to 44 dats (Figure 7.12 b., c. and d.) ABA concentration was higher in the WS treatment ($P<0.05$). No significant differences occurred between BAP or BAP+WS and control.

7.5 DISCUSSION

It is generally considered that a great part of plant cytokinin synthesis takes place in the roots. Cytokinins produced in the roots are translocated to the shoot through the vascular tissue (Moore, 1979; Seeley, 1990), being mobilized along an auxin gradient in the active growing regions towards the shoot tip and there used for cell division. Reduction in shoot growth occurs

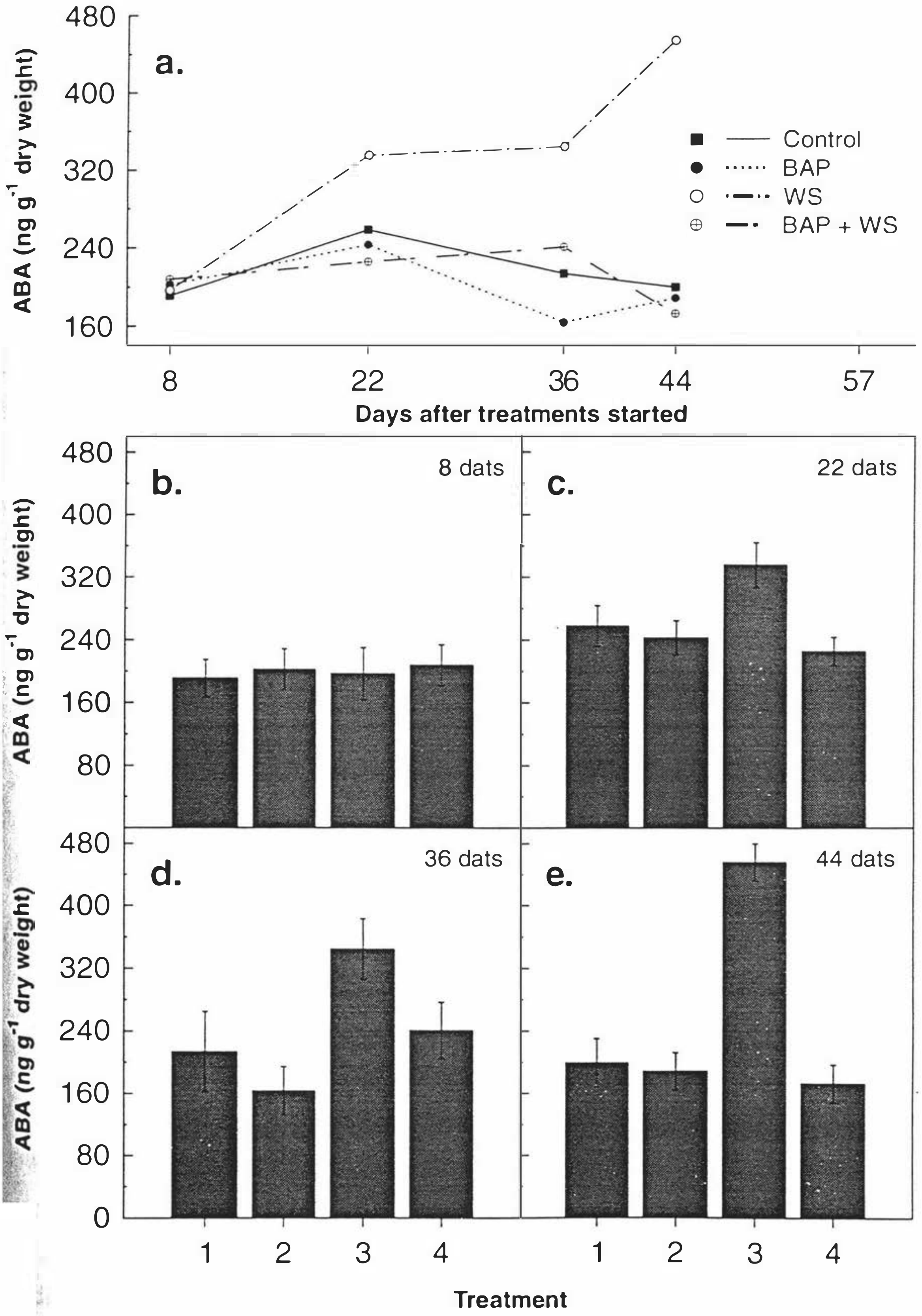

Figure 7.12 The influence of treatments on abscisic acid (ABA) of terminal buds and two adjacent partially expanded leaves in 'Trevatt' apricot grown in an aeroponic system and controlled environment conditions. a). Changes after treatments started; b). 8 dats; c). 22 dats; d). 36 dats; and e). 44 dats. 1=Control; 2=BAP; 3=WS and 4=BAP+WS. Each data point represents 4-12 replicates treatment^{-1} (n=4-12).

when IAA and cytokinin levels are low and ABA levels are enhanced by stress (Seeley, 1990). An objective (section 7.1) was to ascertain if drought induced shoot growth inhibition could be ameliorated by application of a synthetic cytokinin. Simultaneous ABA determination sought to establish a role for shoot ABA in mediating a water stress induced growth reduction.

The experiment was performed under controlled environment conditions to minimize the possible interactions which usually exist between environmental conditions and plant growth in field conditions. Seeley (1990) demonstrated that environmental variables such as temperature, radiation, precipitation, humidity, wind and soil factors interact with water stress to affect growth and development.

In addition, the controlled environment produced similar conditions to those in which apricot culture normally exists in arid and semi arid environments (see sections 7.1 & 7.2). The aeroponic system provided a precise and controllable tool for applying irrigation, hormones and water stress (see section 7.2). This was an air water culture technique in which the apricot roots were suspended in air and sprayed with nutrient solution. This system provided access to clean intact roots for direct observation, measurement and other experimental purposes such as application of precise levels of exogenous cytokinin (BAP) and water stress. Krizek (1985) reported that regulating water deficit in plant tissues is perhaps one of the most difficult of all environmental factors to control experimentally, because of the dynamic nature of water in the plant and surrounding substrate. He stated that using some osmotic agents such as PEG has the advantage of applying a precise level of water stress, but often causes undesirable side effects. Thus the physiological responses of plants to the application of water stress by withholding water or by PEG are quite different. Withholding water from the soil is the most obvious and natural method of inducing water stress in plants, but is also one of the most difficult to control. In the present study the aeroponic system with intermittent misting gave good control of water stress in the apricot plants throughout the experiment. However, this was achieved using continuous attention to the system technology and frequent checking of plant condition, shoot growth and Ψ_{xylem} measurements of the apricot trees. Saunders (1991) successfully used the aeroponic system with intermittent misting to generate mild to moderate water stress on tomato and Asian pear under glasshouse conditions. In the present study the aeroponic system also proved useful by maintaining and growing apricot trees for 12 weeks successfully under conditions of relatively high temperature and VPD. It provided an easy way to supply and maintain known levels of nutrients and BAP to the trees, and was very satisfactory for visual observation and harvesting of clean good root systems.

The level of stress applied to the water stress treatments at the beginning of the experiment was high (day 0, Figure 7.3 b.) and the plant response was visible wilting and a drop in Ψ_{xylem} (Figure 7.3 a.). At that time plants had a very small amount of new roots and were possibly not able to absorb sufficient water during the short (1 minute) misting on-time. Another explanation might be that non-adapted plants with succulent new shoots could not adequately control their water loss in the long (30 minute) misting off-time set initially. The temperature applied at this time was 28°C (day) which was relatively high, and it became apparent within a few hours that the plants were not able to tolerate the stress level created. Kramer (1983) suggested that plants subjected to frequent water stress accumulated modifications with adaptive value, such as thick cuticle, extensive root system and low osmotic potential. In the present study, when the degree of stress was reduced (to 15/2 minutes misting off/on time at VPD 10 mb) and subsequently increased gradually (up to 90/2 minutes misting off/on time at VPD 18 mb) the plants were able to adapt and maintain turgor in both fully and partially expanded leaves (Figure 7.8). Turner and Jones (1980) suggested that the rate of stress development can be an important factor for water stress adaptation, because rapid stress development may not give sufficient time for adaptive mechanisms such as osmotic adjustment. The degree of response to water deficit is a plant related characteristic (Kramer, 1983), although knowledge of the degree of water stress that can be tolerated by a plant has important management consequences (Lakso *et al.*, 1984).

When the initial water stress was reduced (to 15 minutes off-time, and 2 minutes misting on-time) 6 hours after treatments started (Figure 7.3 b.), both WS and BAP+WS treatments started to recover their Ψ_{xylem} (Figure 7.3 a.). The speed of recovery in WS was faster than in BAP+WS. Over the next 7 days (WS) and 13 days (BAP+WS) Ψ_{xylem} returned to the level of control. The low Ψ_{xylem} in both BAP treatments between 12 and 15 dats might be a result of leakage occurring in the misting circulating system (see footnote 4), and it may have continued to reduce these treatments' Ψ_{xylem} until 23 dats. While this may be explained by the leakage problem, it could be caused by higher transpiration from BAP+WS treatment, as suggested by the higher stomatal conductance of this treatment (eg. at 2 dats Table 7.1 b.). Mansfield (1987) suggested that cytokinins act as important regulators of stomatal movements, and the plant can use cytokinins from the root system as a chemical signal to the shoot to modify stomatal behaviour according to the availability of soil water. Whilst water stress reduced stomatal aperture of maize plants, application of cytokinins reopened the stomata (Blackman and Davies, 1985).

The initial high stress level severely checked shoot growth of WS, but after the stress was reduced (by 8 dats) its Ψ_{xylem} and rate of growth were similar to control and other treatments (Figures 7.3 a. & 7.4). Steinberg *et al.* (1990) suggested that in peach trees reduction in dry matter production under water stress was related to the decline in shoot extension growth and leaf production. In the present study, with a similar initial water deficit level, but in the presence of exogenous root applied BAP, the reduction in (BAP+WS) shoot growth was much less than in the WS treatment. This result was consistent with the other results of plant dry matter, root and shoot growth and dry weight (Figures 7.5 to 7.7). This suggested that in water stress conditions the presence of BAP (BAP+WS) reduced the negative effect of water stress on vegetative growth and total plant dry matter. This might be explained by lower g_s or A value in the WS treatment, although higher g_s on BAP+WS relative to WS treatment led to higher A only early in the treatments (Table 7.1 a. & b.). It has been reported that endogenous ABA concentration in leaves of water stressed plants plays a role in controlling stomatal aperture of many plant species. The increase in ABA level in leaves could readily inhibit stomatal opening in water stressed plants (Mansfield, 1987). Another possibility might be that applied BAP increased assimilate supply to the new roots and shoots on water stressed plants. Thomas (1986) suggested that hormones may be involved in the production and movement of assimilates to the growing organs, and generally cytokinins, and possibly gibberellins and auxins, may all be involved in initiating sink activity.

Destructive harvesting revealed that 4 weeks after establishment of the experiment (eg. at the end of the acclimation period), total new growth was only 2% of total plant dry matter (Figure 7.5 a.). However, the proportion of dry matter in the new growth increased up to the end of the experiment (57 dats), with significant treatment effects on the proportions to each plant part. For example, control and BAP treatment showed higher new growth in all tissues at 57 dats (Figure 7.6), more growth than BAP+WS, and much more than WS. WSb was less inhibiting to plant growth than WS. This is presumably because WS received a severe water stress at the beginning which markedly checked growth (before the plants recovered) while WSb experienced low water stress for the first 36 days. When Ψ_{xylem} in WSb dropped after 44 dats from -1.2 MPa to -1.6 MPa, and even to -2.2 MPa, extension growth on this treatment stopped. The longer term inhibitory effect of WS treatment in controlling shoot growth might be explained as a result of long term reduced cytokinin and IAA levels (Seeley, 1990) and increased ABA in the shoot tips of this treatment (Figure 7.12).

In the present study, the number of lateral shoots was not recorded, but visual observation during the experiment revealed that generally both BAP treatments produced a higher number of laterals with shorter internode lengths. This might be explained by movement of applied BAP via the xylem sap toward the lateral buds where it decreased the inhibitory effect of apical dominance. Increase in branching of apple trees following BAP application has been reported by Greene and Autio (1990). Moore (1979) suggested that auxin produced at the shoot tip and translocated basipetally produces apical dominance over the lower buds. The inhibition of buds by apical dominance is antagonized by cytokinin, some of which he suggested is synthesized in the suppressed lateral bud itself, and some of which is transported past the bud in the xylem sap. An increase in lateral shoot number of peach trees treated with foliar BAP application was reported by Richards and Rowe (1977a & b) and shoot:root ratio increased as a result of promotion of leaf and shoot number by foliar BAP application. The reduction of root growth rate in their study, they suggested, was caused by current season assimilates being preferentially used for top growth. However Richards (1980) reported that when BAP was applied either to the shoot or to the roots of apple trees the resultant growth was dependent on the site of application, and root-applied BAP reduced the top:root ratio. In the present study BAP application to the root system increased root growth, although in wheat Stenlid (1982) suggested that cytokinins should be considered as possible natural root growth inhibitors.

The highest new root length was produced by BAP (≈4 km at 57 dats, Figure 7.6 c.). Both BAP treatments produced the greatest new and total root dry weight (Figure 7.7 a. & e.). Visual assessment showed more branching occurred in the root system as a result of BAP application. Increased root growth might have resulted from more demand by the increased shoot growth for nutrients and water uptake, which can also be interpreted as the need for plants to re-establish their root:shoot ratio. Brouwer and De Wit (1969) suggested the ability of plants to re-establish their root:shoot ratio to its "functional equilibrium". In the present study, on both new and total dry weight bases both BAP treatments produced higher root:shoot ratios than control (Figure 7.7 c. & d.) from 22 dats. Both WS treatments also produced higher root:shoot ratios than control. Another explanation for increased root growth might be an increase in the sink strength of roots for assimilates, due to cytokinin application to the roots. Moore (1979) reported that cytokinin creates new source-sink relationships, causing mobilization of metabolites from an untreated to a treated portion of a leaf. Seeley (1990) suggested that the relative production of cytokinins and auxins by roots and shoots regulates the root:shoot ratio. Reduction in shoot growth by water stress resulted in an increase in the cytokinin:auxin ratio. He concluded that drought caused reduction in shoot growth, and high ABA levels occurred concurrently with low IAA, GA, and cytokinins levels under drought conditions. In the present study, the level of ABA in the shoot tips of WS treatment from 22 dats was also significantly higher than in the other treatments (Figure 7.12). Watts *et al.* (1981) found that ABA limits the growth of the shoot and enhances the growth of the roots. This might explain the reduced shoot:root ratio that is often found in plants subjected to water stress.

Another possibility to explain the increased root growth of BAP treatments in the present study might be that root applied BAP increased endogenous auxin production and this increased root growth. Kinetin promoted increase of auxin production in rape leaves was demonstrated by Engelbrecht and Conrad (1961) and Conrad (1961) as cited by Hemberg (1972). When aerial parts or excised shoots of *Coleus blumei* plants were treated with kinetin growth was stimulated (Hemberg, 1972). This was associated with more bound auxin in stems of kinetin treated plants. He concluded that the breakdown of endogenous auxin might be decreased by kinetin treatment, or that the kinetin treatment caused new formation of auxin. Torrey (1976) questioned whether auxin played a role in root elongation. He suggested that auxin formed in the shoots is continuously supplied to the root system in amounts related to its fluctuating levels in the shoot. Seeley (1990) reported that auxin moves basipetally from growing shoot tips and expanding leaves and acropetally from root tips and, through effects on mobilization and directed transport of other hormones, nutrients and metabolites, maintain control of where growth occurs in the roots or shoots. Auxin is one factor affecting root and shoot elongation and when its production and basipetal movement are reduced by water stress, allows it to exert long-term control over tree growth and development. Torrey (1976) suggested that roots and root systems play an important role in the overall hormonal physiology of the plant. Environmental influences which affect the root system such as water stress, act not only on water uptake, and transport of organic substances, but also on the hormonal flow from root to the shoot and vice versa.

Both WS and to a smaller extent WSb reduced top growth, leaf area, dry matter and total dry matter, but increased the root:shoot ratio (Figure 7.6 & 7.7). The levels of net CO_2 assimilation rate (A) and stomatal conductance (g_s) at 2 and 8 dats were relatively higher than later, but the levels were lower in WS and BAP+WS than control until 8 dats for A and lower in WS for g_s (Table 7.1). The decrease in vegetative growth of WS treatment after 8 dats might be related to this decline in A value, although thereafter the control also showed low values of A and g_s similar to the WS treatment. However, the control had a larger leaf area. The leaf area ratio (LAR) was not calculated, but Figure 7.6 b. and d. demonstrate that control had larger leaf area and LAR than WS treatment. Wilson (1988) suggested it is not clear whether water deficit has a greater effect on net CO_2 assimilation rate or growth of the plant, although an adequate supply of water is necessary for growth of shoots and roots. Landsberg and Jones (1981) reported that both stomatal closure and reduction in leaf area expansion of plants are important water stress responses.

In the present study, g_s of BAP generally was higher than in control and water stress treatments, supporting the regulatory effect of cytokinins on stomata and enhancement of stomatal opening reported by Davies (1987) and Mansfield (1987). Stomatal closure in water stress plants might be caused by reduction in cytokinin supplied by the roots and by increased ABA in the leaves (Kramer, 1983). However, he reported that stomata sometimes remain open in leaves with high ABA concentration, may close before the concentration increases, or stay closed or partly closed after the concentration of ABA has been reduced, causing difficulties in interpreting the role of ABA in stomatal closure. In the present study, the differences in ABA concentration in the shoot tips were not significant between either of the BAP treatments and control. The ABA concentration in mature leaves was not recorded in the present study, but the work on apricot trees by Loveys *et al.* (1987) suggested that ABA was relatively unimportant in the control of stomatal conductance in apricot grown in the conditions of South Australia. However, Schulze (1986) suggested that stomata may directly respond to humidity or to root cytokinin, which may open stomata and counteract the effect of ABA in the leaves. Also, he illustrated that the metabolism of ABA and the content of this hormone in the bulk leaf were not related to its action on stomata, in which only a small amount of the ABA content needed to be released into the apoplast of the guard cells for a significant response of the stomata. It has been suggested (Milborrow, 1983) that stomata do not make ABA and must import it from the mesophyll cells, although active stomata invariably contain chloroplasts and they could be the site of ABA synthesis or storage.

In the present study, the high concentration of ABA in the shoot tips and partially expanded leaves of WS treatment was associated with reduction in shoot growth and leaf area. ABA increased as the severity of stress was raised through the experiment. This suggested that the rate of shoot growth when treatments started was higher than at later dates as greater water stress developed. This conclusion is supported by the shoot growth rate results (Figure 7.4 b.). Westwood (1993) reported that ABA is a natural growth inhibitor which inhibits shoot growth. Robinson and Barritt (1990) found that reduced shoot growth rate and leaf expansion rate of apple seedlings subjected to water stress was associated with increase in ABA concentration in response to severity of water stress. In the present study, the ABA concentration was the same in all recorded treatments at 8 dats (Figure 7.12 b.). At the other recorded dates the ABA in both BAP treatments was not significantly different from control, but was lower than in WS treatment (Figure 7.12 c., d. & e.). This result suggested that when the severity of water stress increased, the level of ABA also increased in WS treatment, but when BAP was combined (BAP+WS) the level of ABA was not affected by water stress. Zeevaart (1977) found that water stress caused an increase in the ABA concentration in the shoot tip of 'castor' bean (*Ricinus communis*), and illustrated that both young and mature leaves have the capacity to synthesis ABA from an early stage of development. Although mature leaves are a primary site of ABA synthesis, other plant tissues are also known to synthesise ABA (Westwood, 1993).

As described previously (see section 7.2), the environmental conditions applied in the climate rooms (28/24°C day/night temperature and with VPD up to 18 mb) normally produced a higher water stress than in the orchard experiment. In both expanding and mature leaves generally Ψ_l declined through the period of experiment (with the possible exception of BAP), and the Ψ_l of mature leaves of WSb and BAP+WS were significantly lower than control from 44 dats. The water status study of both mature and expanding leaves obtained by the dew point method (Plate 7.9 a.) revealed that the apricot plants were able to maintain their turgor throughout the experimental period, through the mechanism of osmotic adjustment, which occurred even in control plants (Figure 7.8). Generally, at the low degree of Ψ_l in young leaves (less than -1.5 MPa) and low Ψ_s ($\approx$ -2.0 to -3.5 MPa), without significant differences in Ψ_p relative to control, turgor maintenance can be explained as a result of osmotic adjustment occurring. When mature leaves of all treatments were compared the results revealed that BAP (at 21, and 56 dats) and BAP+WS (at

21, 42, 49 and 56 dats) showed lower Ψ_l and Ψ_s than control. However, at the same time their Ψ_p was maintained at the level of, or higher than, control. At 17 and 35 dats the Ψ_l and Ψ_s of BAP+WS was significantly reduced but not the Ψ_p, indicating osmotic adjustment had occurred in the young leaves. Lakso *et al.* (1984) found osmotic adjustment in mature apple leaves, but not in the shoot tips. The work on apricot by Loveys *et al.* (1987) suggested that apricot had the ability to maintain its turgor at low water potential because the osmotic potential was also low at the same time.

Analysis of simple carbohydrates of the mature leaves revealed that sorbitol was the major simple carbohydrate in apricot leaves, ranging in all treatments between ≈45-100 mg g^{-1} dry weight (Figures 7.9 & 7.10), which is consistent with the results of Bieleski and Redgwell (1985). They reported that sorbitol is the major translocatable carbohydrate in apricot leaves, and that cannot be metabolized by mature leaves. In the present study, no significant differences occurred between control and the other treatments in sorbitol concentration, but the amount of glucose was lower in control than in BAP+WS (at 8, and 44 dats). Mineral analysis revealed that generally both BAP treatments showed lower minerals content on a dry weight basis (Figure 7.11). Significant differences relative to control occurred later in the experiment, with lower Mg, Ca, K and P concentrations in BAP (44 and 57 dats) and BAP+WS (57 dats) treatments. Decreases in Ca, Mg, N and P following foliar application of BAP to peach trees were reported by Richards and Rowe (1977 a). However, Richards (1980), following application of BAP to apple trees through the nutrient solution, found leaf levels of Mg and Ca increased, K and P decreased, while P levels were unaffected. In the present study control generally showed a higher K value than the other treatments, differences being significant relative to WS (at 8, 36, 44 dats) and WSb (36, 44 and 57 dats). The level of Mg (at 22, 36 and 57 dats) was higher in WS than control. However, control leaves showed a higher Ca concentration than WS at 8, 44 and 57 dats. It therefore seems unlikely that the minerals recorded were involved in the osmotic adjustment occurring in BAP treatments, but the occasional significant increase in glucose in BAP+WS may have been. The increase in K in control, WS and WSb at 57 dats might have had an effect in osmotic adjustment occurring in these treatments at this time. The levels of osmotic adjustment which occurred at 57 dats were 0.86, 0.93 and 0.88 MPa in control, WS and WSb respectively. The potassium level was higher than of the other recorded minerals throughout the experiment (Figure 7.11). This result is consistent with that reported by Loveys *et al.* (1987) on apricot.

Summary

The preliminary work revealed that the emergence of new roots in seedling apricot plants was slow at the time of transplanting into an aeroponic system, and suggested they were not able to supply the plants transpiration demand, so that more than 50% failed to acclimatize to the system. The climate rooms experiment showed that it is important to use larger dormant plants which are able to acclimatize to the misting system gradually, with less than 5% failing to acclimatize. The stage of growth and size of apricot plants, together with careful environmental control are key factors for the establishment of plants under aeroponic culture.

The aeroponic system provided access to clean intact roots for direct observation, measurement and other experimental purposes such as the application of precise levels of BAP and water stress. The use of intermittent misting gave good control of water stress on the apricot plants throughout the experiment. However, the system needed extensive care to make sure that it was working properly, as the roots were suspended in air within the aeroponic tanks at temperatures higher than usually exists in the soil in field conditions. Any leakage within the circulation system or any blockage of the nozzles might interfere with the response to applied treatment or might cause death of the plant. Proper working of the system was achieved by extensive visual and manual checking of the circulating system to prevent any unexpected interruption within the system. For future work, installing an electronic device within the circulating system may be desirable to warn about any leakage problem.

The levels of misting on- and off-time need to be carefully adjusted when water stress is first applied, as plants can very rapidly dehydrate. So gradual increase of the level of water stress was an important factor. When water stress was gradually developed the apricot plants were able to maintain their turgor despite high internal water deficit (-2.2 and -3.0 MPa of Ψ_{xylem} and Ψ_l respectively), with misting off-time of up to 1.5 h and with high environmental stress of up to 18 mb VPD. It appeared osmotic adjustment occurred in both partially and fully expanded leaves throughout the experiment. Sorbitol was the major simple carbohydrate recorded in apricot leaves in all treatments, ranging between ≈45-100 mg g^{-1} dry weight. Potassium was the most abundant of the recorded minerals, although substantial amounts of Ca and N were also found in mature leaves.

When BAP and water stress were applied together the response of trees to water stress was moderated, giving higher dry matter production, root and shoot growth. Also compared with water stress BAP+WS caused reduction in ABA concentration, and showed bigger osmotic adjustment than WS and control in both fully and partially expanded leaves on some occasions throughout the experiment. However, ABA increased in WS treatment as the degree of water stress increased. There were no significant differences between BAP and control in ABA, and in particular no significant differences between BAP+WS and control in this even though without BAP water stress resulted in a considerable elevation of ABA as expected. Both BAP treatments increased overall and new root:shoot ratios and increased branching both in the top and root portions of the apricot plants. Water stress caused reduction in leaf area in WS and BAP+WS and increased the root:shoot ratio.

CHAPTER EIGHT

GENERAL DISCUSSION AND CONCLUSION

Orchardists are concerned with supplying good quality fruit to the market with low production cost. The management of the vigour of tree canopies is important in determination of yield, fruit quality and production cost. Factors such as cultivar, soil type, tree spacing and environmental conditions influence tree management decisions aimed at maintaining the correct balance between vegetative and reproductive growth. Understanding the interacting complex of factors influencing tree performance to optimise orchard productivity is an important task.

It is obvious that vegetative growth is necessary for maintaining vigour and developing new bearing wood in fruit trees (Forshey and Elfving, 1989; Martin, 1989). However, excessive vegetative growth adds to production cost as removing it by pruning is expensive (Martin, 1989). With the lack of dwarfing rootstocks for apricots, other vigour control techniques would be of great value in the apricot production industry at the present time. This study has examined three possible vigour control techniques for their potential to control vigour of mature 'Sundrop' apricot trees grown on Tatura trellis at two spacings. This variety of apricot was chosen because whilst it has attractive fruit and potential for export (Glucina *et al.*, 1990), this cultivar grows tall, upright and vigorously. The primary aims of the orchard experiments were:

- To examine whether PBZ, root-pruning and RDI have potential for controlling the vigour of the cultivar without having undesirable effects on yield and fruit quality.
- To examine the effects of these treatments on the internal water relations of this apricot cultivar.
- To evaluate whether the mechanism of response to root-pruning appeared similar to the mechanism of response to water stress.
- To examine the mechanism of water stress adaptation in apricot, such

as the possible occurrence of osmotic adjustment in fruits and leaves.

Essentially, these aims have been achieved, although the comparison between the two spacings was not fully explored due to an insufficient number of healthy trees at the wide spacing. The orchard study revealed the water stress type response to root-pruning, however the degree of internal stress was low under the conditions of the field study. It has been suggested (Randolph and Wiest, 1981) that root-pruning may induce water stress as a result of limited water absorption, and that it may reduce the source of cytokinin supply from the root system. The reduction of cytokinin supply as a result of water stress was reported by Kramer (1983). So it was decided to carry out the controlled environment experiment (chapter 7) with the following aims:

- To test if root-supplied cytokinin has an effect on shoot growth and whole plant physiology (i.e. root:shoot ratio, dry matter partitioning and plant dry weight).
- To determine concentrations of ABA in shoot tips to examine whether ABA played a role in controlling shoot growth under water stress conditions.
- To further examine the mechanism of stress adaptation in apricot under higher water stress conditions than in the orchard study, and determine whether osmotic adjustment occurred in fully and partially expanded leaves.

In order to avoid climatic variation this experiment was conducted under controlled environment conditions. It was also desirable to have access to clean, intact root systems, allowing direct observation and measurement, and the application of precise levels of water stress and of exogenous cytokinin (BAP). This was arranged by growing the trees in an aeroponic system. The experiment provided information relevant to the application of vigour control techniques such as RDI and root-pruning in the orchard.

In both the orchard and controlled environment studies a number of other areas of inquiry were also developed and discussed in previous chapters. This chapter will discuss the beneficial and physiological aspects of controlling vegetative growth based on the overall findings obtained through both the orchard and the controlled environment experiments.

8.1 Practical and beneficial aspects of controlling vigour

In the orchard conditions of this study 'Sundrop' apricot produced almost 50% of its growth in the top canopy region. All treatments reduced growth by 10 to 40% in the top canopy region of close spaced trees in the first season. The best performance was achieved by PBZ application, which resulted in an average 54% reduction in total year's prunings and increased fruit size ≈6 cm^3. The reduction of vegetative growth resulted in the allocation of more assimilate to reproductive growth so that this treatment had a significantly increased P-index in both seasons. On a commercial scale PBZ treatment would be able to give substantial benefits to apricot growers by saving in pruning costs and by producing larger fruit size. PBZ treatments also tended to advance fruit maturity which may be beneficial as the supply of early fruit to the market usually obtains higher returns. Yoshikawa *et al.* (1987) as cited by Martin (1989) suggested PBZ applied to peach orchards increased grower returns by an average of US$ 2018 ha^{-1}. Somerville (1994) has reported that PBZ reduced vegetative growth of cherry trees and caused early and consistent cropping without detrimental effects on fruit quality. Jacyna *et al.* (1989) reported PBZ inhibited vegetative growth and increased flowering of sweet cherry.

Generally all treatments used in the orchard study reduced vegetative growth. Root-pruning and RDI were more effective in reducing vegetative growth in the first season. The lower inhibitory effect of root-pruning in the second season might have been due to the removal of only a relatively small part of a root system which had proliferated within the pruning cut, as root-pruning was performed in the same position as in the first season. This was supported by the root study (chapter 4), as RLD was higher in the upper layer of the soil within the root-pruning cut region. Thus the lower inhibitory effect of this treatment in the second season resulted from a higher potential for water uptake closer to the tree trunk.

The low inhibitory effect of the RDI treatment in the orchard study can be explained by the deep soil which was fully water charged by high winter rainfall. Relatively low temperatures and humid conditions in spring prevented fully effective water deficits being created. These were limiting factors at this experimental site for effectively applying RDI as a vigour control technique. Girona *et al.* (1993) reported that in a deep and fertile California soil there was no reduction in vegetative growth of peach trees by RDI. They suggested a relatively long time was needed to achieve a moderate water stress in RDI treatments on a deep soil. The root study (chapter 4) showed root growth in the deeper soil zone, roots being observed down to 1.6 m in the soil. Under these conditions it was very difficult to dry the soil adequately in the early season, and this did not result in a sufficiently high water stress in the second season. This reduced the potential effect of the RDI treatment in reducing vegetative growth in the second season. In neither season was any detrimental effect of water stress observed on fruit growth and there was a tendency for an increase in TSS and advanced maturity on some occasions in both seasons. Huslig *et al.* (1993) showed that irrigation before stage III of peach fruit growth did not affect yield and fruit size compared to trees irrigated at the onset of stage III. Ebel *et al.* (1993) found that RDI on 'Delicious' apple trees caused a reduction in fruit size at harvest, but increased soluble solids. They stated that RDI was severe under their experimental conditions.

In RDI the application of a controlled water stress to the trees early in the season when vegetative growth predominates is important (Chalmers *et al.*, 1981; Mitchell and Chalmers, 1982; Mitchell *et al.*, 1986; Mitchell *et al.*, 1989; Proebsting *et al.*, 1989). In Palmerston North the climate is characterised by high rainfall during winter and early spring. Plastic soil covers and plastic lined trenches were not a complete answer in the deep soil, and are hardly commercially practical. If the supply of soil water during the early stage of fruit growth were controllable, then commercially it would be possible to use this technique. Under controlled environmental conditions and precise control of water supply, a water stress treatment showed that apricot trees' vegetative growth is very sensitive to water stress (chapter 7). Caspari *et al.* (1994) noted that as removing winter rainfall from the root zone is difficult early in the season in such a climate, growing herbaceous cover crops below the cropping trees might have potential to withdraw the excess water and induce sufficient water stress early in the season. In their research in Marlborough, New Zealand on grapevines, ryegrass (*Lolium perenne L.*) or chicory (*Chicorium intybus L. var. sativum* DC., cv. Puna) were used to reduce soil moisture early in the season to make application of RDI feasible. In the more humid climate of Palmerston North lucerne ground cover in conjunction with RDI was used to reduce vegetative growth of 'Royal Gala' apple without detrimental effects on fruit growth and quality (Durand, 1990). In her work midday Ψ_{xylem} fell as low as -1.5 MPa (RDI plus lucerne and control plus lucerne) and -1.3 MPa (control plus full irrigation, and control plus RDI), with predawn Ψ_{xylem} of $\approx$-0.3 MPa for the first 60 days after full bloom. However, in the present study in neither seasons was midday Ψ_{xylem} lower in RDI than -0.8 MPa with predawn Ψ_{xylem} of $\approx$-0.4 MPa, and in the first 60 days stress was lower. In this experiment the Ψ_{xylem} of root-pruned trees was at a similar level or lower than the RDI treatment and they showed a water stress type response. It might be possible to combine RDI with root-pruning to increase the inhibitory effect on vegetative growth early in the season, although further research would be needed to determine the responses to such a combined treatment.

The orchard study of the water relations of experimental trees was carried out during the fruit growth period (full bloom until harvest). However, the postharvest water relations of fruit trees are also important, for example, careful irrigation application and scheduling have direct effects on the subsequent year's flowering and fruiting. Uriu (1964) reported that discontinuing irrigation after fruit harvest decreased flower bud formation, fruit set and yield of apricot in the desert areas of California. However, fruit production in a humid climate with more rainfall and low temperatures such as at this experimental site needs a different approach to irrigation scheduling than that suited to desertic or semi-arid climatic regions. In the present study 'Sundrop' apricot were harvested by ≈130 dafb (during January), and for the rest of summer evaporation usually is higher than rainfall (Figures 3.1, 6.1 & 6.3). During this period irrigation scheduling after consideration of soil water content, rainfall and evaporation is should ensure that high water deficit does not reduce next years flowering and fruiting, but excessive irrigation may stimulate an undesirable late growth flush.

In this study the PBZ and root-pruning treatments were applied during tree dormancy and the RDI treatment was applied during stages I and II of fruit growth, with irrigation being resumed at the beginning of stage III. These times of treatment have been reported effective in controlling vegetative growth for other crops. For instance, dormant season application of root-pruning to apple trees (McArtney and Belton, 1992; Schupp and Ferree, 1989) and of PBZ to peach trees (Erez, 1986) were effective in controlling vegetative growth. If RDI is employed as a vigour control technique it is important to be aware of the timing of shoot and fruit growth. The majority of shoot growth occurred during stages I and II of fruit growth (eg. Figure 4.5a. & b.), whilst a sharp increase in fruit size occurred during stage III (Figure 4.6 d. & 5.4). So irrigation was resumed at the start of rapid fruit growth (stage III). Chalmers *et al.* (1981) pointed out that RDI is based on the idea that vegetative growth of fruit trees can be restricted if RDI is applied during the period of slow fruit growth and rapid shoot growth (Figure 2.3). This irrigation strategy has greatly facilitated the management of pear, cherry, peach and nashi orchards established on vigorous rootstocks under high density plantings in low summer rainfall areas of Australia (Jerie *et al.,* 1989 b.).

In this study PBZ was the most useful vigour control technique under the climatic conditions of this experiment, as was reported earlier by Arzani *et al.* (1992). RDI had less inhibitory effect on vegetative growth. However, in areas with arid and semi arid conditions RDI has been very successful in controlling vigour of fruit trees without undesirable effects on fruit. The spring climate in most parts of Iran is dry (Plate 8.1 b.), for example, the monthly average rainfall is ≈18 mm during spring (April to June) in the big fruit production area to the east of Tehran (Arzani, 1989). However, many regions of Iran have deep soils, a difference from the shallow soils where the technique was developed in Australia. Nevertheless, high transpiration, higher temperature and low rainfall would allow the application of a more precise and effective RDI strategy in Iran than in the humid climate and deep soil of Palmerston North.

The relationship between shoot and fruit growth and when they occur during the season are critical factors in determining the time of applying water deficit within the RDI strategy. It is assumed that when shoot growth is suppressed early in the season by a vigour control technique then more assimilates are available for fruit growth. Chalmers *et al.* (1983) showed that fruit growth sensitivity changed with the growth stage. In the present study the highest proportion of early season aboveground growth was in shoots, but this proportion then decreased in favour of fruits (Figures 4.6 a. & b.), and fruit growth occurred rapidly during stage III (after 92 dafb) until harvest. Chalmers *et al.* (1985) showed that vegetative growth of deciduous fruit trees was greatest in the spring, at which time fruits grow slowly (Figure 2.3). Li *et al.* (1989) found it was possible to reduce vigour of peach trees without affecting fruit size and quality if water stress was imposed at a particular stage of fruit development, normally in stages I and II of fruit growth. When PBZ is used for

a.

b.

Plate 8.1 Mature sweet cherry orchards in two different climates and planting systems. **a).** A high density orchard using PBZ as a vigour control technique (South Island, New Zealand). **b).** A typical Iranian wide planted orchard (≈50 Km east of Tehran, Iran)(Arzani, 1989).

vigour control Lever (1986) suggested that reduction in vegetative growth is a direct morphological effect of PBZ, and the secondary effect is alteration in sink strength within the plant, allowing greater partitioning of assimilates to reproductive growth. Generally in the present study a greater reduction in vegetative growth tended to give more positive effects on fruit growth, especially on close spaced trees. PBZ treatments reduced vegetative growth throughout the season and enhanced fruit growth. However, the reduction of fruit size in the middle canopy zone of the root-pruned treatment at the time of harvest (Figure 5.5 b.) might be explained by increased shoot growth later in the season on this treatment, as shown by summer pruning and shoot growth data (Figure 4.2 c.). This increase in vegetative growth on root-pruned trees later in the season might have resulted from regenerated root which reduced the overall inhibitory effect of this treatment. In wide spaced trees, root-pruning had a longer term effect, possibly caused by a higher proportion of the root system being removed, which resulted in a significant reduction in fruit size (Figure 5.12).

George and Nissen (1992b) suggested that higher fruit growth on peach trees after soil applied PBZ might have been due to control of vegetative growth after bud break which allowed more current assimilate to be partitioned into fruit and not vegetative growth. H-PBZ increased fruit growth in all regions, and was more inhibitory to vegetative growth in the year of application. Besides PBZ increasing fruit growth by reducing competition for phytoassimilate between fruits and shoots by reducing vegetative growth, there may have been a secondary effect. In this study in both seasons summer pruning was performed on all treatments prior to or early in stage III to remove excess shoot growth in order to allow better light penetration within the Tatura trellis training system. However reduced shoot growth resulting from treatment may also have given an indirect effect of increased light penetration. Light measurements (chapter 4) generally revealed that light decreased as canopy depth increased. This was reflected in fruit growth within the various canopy zones, and generally fruit growth was greater in the top canopy zone than in the lower region (Figure 5.7). However, PBZ treatments tended to increase the light penetration on some occasions. Palmer (1989) suggested that high yields of good quality fruits can only be produced in systems having high light interception enabling high dry matter production, and that light well distributed within the canopy is needed to maintain fruiting and fruit quality.

8.2 Physiological aspects of controlling vigour

From the practical point of view, understanding the physiological responses of fruit trees to vigour control techniques is essential and a prerequisite for using a technique at the correct time on a specific plant and in a specific geographic region. One of the aims of this study was to monitor the degree of internal water stress produced by treatments. The results showed that PBZ did not increase the internal water stress of the plants relative to control and on some occasions moderated the degree of internal stress during periods of high transpiration. It has been reported (DeJong, 1986) that when vegetative growth of peach trees was inhibited by PBZ application, trees showed higher Ψ_{xylem} and stomatal conductance (g_s) during stage III of fruit growth. Wang and Steffens (1985) found the rate of water loss was much slower from leaves on PBZ-treated apple trees than on untreated plants. Biasi *et al.* (1989) found that under non-water stress conditions PBZ increased stomatal conductance of peach, but when PBZ was combined with low soil water availability stomatal conductance was reduced. Jones (1990) suggested the decrease in Ψ_l under water stress conditions might cause stomatal closure tending to decrease transpiration, and stabilizing Ψ_l. In the orchard study, the response of g_s to internal water stress was not consistent and may primarily reflect ambient humidity. However, at higher internal water stress (i.e. Ψ_{xylem} of ≈-2.0 at 2 dats, chapter 7) in climate rooms the g_s of water stress trees was significantly lower than control (chapter 7, Table 7.1 b.). The response of apricot stomata to humidity was reported by Schulze *et al.* (1972) who suggested that stomata may directly respond to humidity or to root cytokinin, and thus over-ride the effect of ABA in the leaves which may otherwise cause stomatal closure (Schulze, 1986). In the orchard study generally g_s was increased by PBZ during stage III of fruit growth (chapter 6), although there was an increase in net CO_2 assimilation rate (A) value only in the case of L-PBZ treatment on close spaced trees in the second season. The increase in g_s and A values in this period might be explained as the effect of higher fruit growth rate. The effect of fruits increasing net CO_2 assimilation rate of peach trees was reported by Chalmers *et al.* (1975), and DeJong (1986) reported that fruits influenced stomatal regulation of mature peach trees.

Soil moisture was not recorded in the PBZ treatments, but it might be assumed that PBZ reduced water consumption, because this chemical reduces vegetative growth by it's antigibberellin effect and less vegetative growth will tend to consume less water. This explanation agrees with Wang and Steffens (1985) who found that PBZ reduced water loss of apple seedlings, and Biasi *et al*. (1989) found greenhouse-grown peach seedlings treated with 0.1 g soil applied PBZ decreased their water consumption up to 60% compared with control at any level of FC. However in the present study, root-pruning caused water stress, which probably contributed to a reduction in vegetative growth. In spite of soil moisture content within the several regions of the root zone of root-pruned trees being similar to, or higher than that of other treatments, root-pruned trees showed higher internal stress than RDI and other treatments. It is likely this was caused by reduction of the root system giving reduced absorbing capacity and a reduction in water uptake, thus the greater difference between water loss and supply would give lower leaf and xylem water potential in this treatment.

However, the water stress level induced in the orchard by either root-pruning or RDI was low and this might explain the low inhibitory effect of these treatments on vegetative growth, especially in the second season of the experiment. In controlled environment conditions when the level of induced water stress was higher than in the field experiment it resulted in a large reduction of vegetative growth (53% in shoot length at 57 dats) and thus of total plant dry matter (chapter 7). This suggests RDI is a good technique for reducing vegetative growth if water can be withheld in early spring so as to produce a reasonable degree of internal stress. It has been reported that many aspects of plant growth are sensitive to water stress. At the cellular level, low Ψ_l affects cell expansion, stomatal regulation, photosynthesis, translocation and cell wall synthesis (Hsiao, 1973; Kramer, 1983). These changes on the tissue or at the whole plant level include reduced growth rates and changes in carbohydrate partitioning (Kramer, 1983).

The greatest difference between midday Ψ_{xylem} of field irrigated trees and RDI trees was less than 0.2 MPa, which resulted in a small reduction in vegetative growth. Chalmers *et al.* (1986) found that during the water withholding period 'Bartlett' pear reduced it's dawn and midday Ψ_{xylem} by about 0.1 and 0.5 MPa respectively compared to the control. They reported that when dawn and midday Ψ_{xylem} declined to -0.57 and -2.22 MPa, respectively shoot growth declined to zero. In the controlled environment study in aeroponic culture, differences of 1.0 MPa between fully irrigated and water stress trees at the beginning of the experiment (0 dats, Figure 7.3 & 7.4) stopped shoot growth almost immediately. Later, when water stress was developed gradually, shoot growth remained active at higher internal water stress, but showed a lower shoot growth rate than control, this declining more rapidly at a difference of -1.2 MPa between control and water stress treatment (at 50 dats). This was presumably because the apricot plants had time to adapt with a gradual increase in drought conditions, hence they performed better and were able to continue to grow. In the first season of the orchard experiment RDI reduced new growth and total year's pruning, and in the controlled environment study water stress reduced shoot growth, leaf area and total plant dry matter but did not reduce root growth. When the water stress was high (i.e. -2.2 MPa Ψ_{xylem}

at 50 dats) leaf dry matter, shoot dry matter and total dry matter declined in the stress treatment while an increased proportion of dry matter was partitioned to the root system, thus the root:shoot ratio increased. The root:shoot ratio was increased by all root applied BAP and water stress treatments. Steinberg *et al.* (1990) found that when young peach trees were subjected to 100, 75, 50 and 25% of full water requirements, total dry matter production was reduced with each incremental decrease in applied water, and the root fraction of total dry matter increased as the level of water stress increased. They found that the difference in Ψ_{xylem} between the wettest and driest treatment was not greater than 0.6 MPa. George and Nissen (1992b) found that at higher stress level the Ψ_{xylem} of two-year-old potted peach trees declined to -3.2 MPa, which caused wilting. The work on aeroponically grown sunflower by Hubick *et al.* (1986) showed that leaf area of water stressed plants decreased even 2 days after applying water stress. They also found that drought conditions increased root:shoot ratio, so root growth was less affected than shoot growth by water stress.

ABA analysis of the shoot tip and adjacent partially expanded leaves (chapter 7) revealed that as water stress developed the concentration of this hormone increased dramatically. However root applied BAP reduced the accumulation of ABA in the tips of water stressed plants and thereby possibly moderated the inhibitory effect of ABA in reducing vegetative growth. The negative effect of the water stress treatment on dry matter partitioning to new tissues was related with the ABA concentration in its shoot tips. In the presence of BAP, shoot growth was increased in water stressed plants, and the inhibitory effect of water stress on dry matter partitioning to each plant part was moderated. This result suggests water stress may reduce endogenous cytokinin levels and hence have considerable effect on shoot growth, as proposed by Davies *et al.* (1986), Kramer (1983) and Seeley (1990). Also ABA may play a role in reducing shoot growth and dry matter production. The increase in ABA level in water stressed plants (Davies *et al.*, 1986; Davies, 1987; Robinson and

Barritt, 1990) and also in plants subjected to higher temperature (Abass and Rajashekar, 1993) has been reported previously. Hubick *et al.* (1986) found that when aeroponically grown sunflower were subjected to water stress the level of ABA increased significantly in both roots and shoots, and shoots of stress plants had 6.7 times more ABA than control plants. They found the level of ABA in water stressed roots was 46% greater than in stressed shoots, and that water stress reduced the level of cytokinins in the stressed shoots by 53%. Jones (1980) suggested the possibility that the production and export of ABA is greater for roots in dry soil than roots in wet soil, and this may cause stomatal closure. Alternatively, it may be that the root tips produce a chemical signal that counteracts the effects of ABA and keeps the stomata open, but only as long as there is adequate water flow through the root system for its transport to the shoot. Blackman and Davies (1985) found that cytokinins may play such a role. Other hormones have been reported to counteract the effect of ABA in reducing shoot length. The work by Makus and Guinn (1992) showed that when the ABA concentration in asparagus spears was high it was associated with a low level of IAA concentration. High concentration of IAA in green spears was consistent with greater elongation, higher ABA concentration was not. They suggested cytokinins or gibberellins also might have effects in spear elongation in relation to ABA.

However, the present results do not suggest the reduced vegetative growth and increased ABA in water stress treatment was associated with stomatal closure or a decline in net CO_2 assimilation rate. Factors other than ABA may have an impact on stomatal closure. Seeley (1990) proposed that the balance of ABA, IAA, and cytokinins may be responsible for precise rapid control of stomatal movements in response to changing environmental characteristics. Hsiao (1973) suggested that different plant processes respond differently to water stress, and cell expansion which depends upon turgor is most sensitive, while net CO_2 assimilation rate and translocation are less sensitive. Schulze (1973) suggested that the stomata of some plants may be more closely linked to the water status of the atmosphere than to that of the mesophyll tissue, and found stomata of apricot trees remained open at high relative humidity even under drought conditions (Schulze, 1972). Later Schulze (1986) suggested that the production of plant dry matter is not only a function of carbon metabolism, but is highly determined by concurrent fluxes of water and nutrients and the process by which these resources are partitioned. He concluded that carbon flux is dependent on both CO_2 uptake through the stomata and its assimilation during photosynthesis. When assimilates enter the assimilation pool they are easily accessible and mobile in the phloem in the form of various sugars, which alternatively may be stored in specialized organs in condensed form like starch or other polysaccharides. Mosi (1960) as cited by Schulze (1986) suggested that the partitioning of assimilates during growth into either green leaves or supporting organs such as stem or roots is important in plant performance.

In the present study although high ABA concentration in shoot tips was associated with reduction in shoot length and thus vegetative growth, the mechanism of ABA effect on elongation may have been other than through controlling stomata. It has been reported (Seeley, 1990) that under water stress conditions ABA increases and IAA and cytokinins decrease, which is associated with control of shoot growth. Little and Wareing (1981) found a continuous supply of basipetally transported IAA has an important role in active growth, while water stress caused an increase in endogenous ABA and reduced basipetal movement of IAA (Little, 1975). In the present study BAP ameliorated the inhibitory effect of water stress on growth and had lower ABA in the shoot tips. As described (see chapter 7) one possible reason for increased root:shoot ratio in BAP treatments might be explained as BAP increased shoot growth and thus increased auxin production and basipetal movement of this hormone to the roots which caused greater root growth. The increase in auxin level by foliar kinetin application (Hemberg, 1972) was discussed earlier. Richards (1980) reported that the effect of BAP on root:shoot ratio was dependent on the site of application, and root-applied BAP reduced the top:root ratio. Wilson (1988), in his review on the evidence of hormone involvement in the control of shoot:root ratio, pointed out that the shoot:root ratio response to BAP applied to the shoot was the opposite of that when it was applied to the root. In addition, the nature and type of growing system is another possibility which might change the hormonal pattern in either roots or shoots. Yang *et al.* (1990) found that when the hormone contents of tomato plants grown under either aeroponic or NFT systems were compared by organs, roots from the aeroponic contained more GA_3 and zeatin but less ABA and IAA than those from NFT, while the leaves from the aeroponic contained a higher amount of only zeatin than those from NFT. Salisbury and Ross (1992) after reviewing the effect of cytokinin on root and shoot growth suggested in summary, that exogenous cytokinins application to the plants can promote cell expansion in young leaves, cotyledons, wheat coleoptiles and watermelon hypocotyles. They concluded that much remained to be learned about the normal role of cytokinins in cell expansion, especially in shoots and roots, and as usual, even less is known about trees.

Under field conditions during high transpiration demand when Ψ_l or Ψ_s dropped trees maintained their turgor through the mechanism of osmotic adjustment in leaves or fruits on some occasions (chapter 6). Under the more severe water and environmental stress of the controlled environment experiment, apricot trees were able to maintain turgor in both fully and partially expanded leaves through this mechanism. The response to drought is dependent on the stage of plant development (Wardlaw, 1990), and osmotic adjustment has been suggested as a mechanism of drought tolerance by plants under shortage of water supply (Morgan, 1984). Lowered osmotic potential in response to water deficit stress is proposed to play a significant role in turgor maintenance and regulating plant growth processes (Morgan, 1984). When irrigation was reduced in high-density peach orchards during the pit-hardening stage (Chalmers *et al.*, 1981), shoot growth was terminated with little or no effect on fruit growth during the applied water deficit. They found that after supplying full irrigation fruit growth continued and competition for water and assimilates from shoots was reduced. Fruit growth was faster on RDI trees in stage III when trees were fully irrigated (Chalmers *et al.*, 1981; Mitchell *et al.*, 1984), which they proposed was the result of osmotic adjustment in the fruits in the previous water stress period (Chalmers *et al.*, 1986). Faster fruit growth on RDI trees against lower Ψ_{xylem} suggested RDI fruit had an increased turgor potential. When RDI fruit adjusted osmotically early in the season fruit growth was maintained, and the higher osmotic potential could have enhanced fruit growth when the water status of plants was improved when irrigation was resumed at 120% E_{ps}.

Study of the simple carbohydrates of fruits from the field experiment and of fully expanded leaves from the controlled environment experiment revealed that glucose and sorbitol were the major simple carbohydrates in fruit and leaves respectively. The concentrations of these simple carbohydrates increased at the time of osmotic adjustment (chapters 6 & 7). In apple, within the soluble-carbohydrate fraction, the sugar alcohol sorbitol was reported to be the main component in all tissues, although sucrose, glucose, and fructose might be found in fair amount (Hansen and Grauslund, 1973). Sorbitol is the main end product of photosynthesis in apricot leaves (Bieleski and Redgwell, 1977), and conversion from sorbitol into glucose in known to occur in apple leaf tissue (Yamaki, 1980).

8.3 Some possible directions for future research related to this study

The study in both orchard and controlled environment conditions raises a number of suggestions for further research to clarify horticultural and physiological aspects of tree performance:

The results of root-pruning showed it has potential for controlling vigour, however under conditions of deep soil and close planting the root system was able to grow into deeper soil regions. Also as time elapsed after root-pruning more roots were produced closer to the tree trunk, which reduced the effect of this treatment and the potential for repeated root-pruning. Possibly combining root-pruning with RDI may give better control of vegetative growth in deep soils in this climate, which would need further investigation.

In controlled environment conditions results revealed that reduction of shoot growth by water stress was moderated by root applied cytokinin. In the water stress treatment ABA concentration increased in the shoot tips as the degree of water stress increased and vegetative growth was also increasingly inhibited. The results suggest that ABA reduced shoot elongation possibly by an effect on cell expansion rather than by closing stomata, although both stomatal conductance and net CO_2 assimilation rate were decreased by water stress. The reduction in stomatal conductance may have occurred by volume changes in mesophyll cells during drought, or releasing ABA in apoplast to promote stomatal closure. These possibilities and related physiological processes need further investigation.

Does concentration of ABA in shoot tips determine the rate of shoot elongation, and is its effect opposed by cytokinins? These questions need further investigation. Alternatively, the reduction in shoot elongation by high ABA concentration might be explained as a change in the ratio of this hormone to other hormones, gibberellin or auxin as well as cytokinins, which also needs further investigation in apricot trees.

The increase of ABA concentration in the shoot tip of WS plants and its possible effect in controlling shoot growth was discussed previously (see sections 7.5 & 8.2). For instance, i. drought caused reduction in shoot growth, and high shoot tip ABA levels occurred, presumably concurrently with low IAA, GA and cytokinins as proposed by Seeley (1990); ii. ABA may have limited the growth of shoots (Kramer, 1983; Robinson and Barritt, 1990), and enhanced the growth of roots and thus increased root:shoot ratio (see Figure 7.7 c. & d.). iii. Shoot ABA was not well correlated with stomatal aperture at greater levels of water deficit (Table 7.1 b. & Figure 7.12). This inconsistency in the effect of ABA on stomatal aperture was reported in a review by Kramer (1983). iv. High ABA concentration was associated with reduction in shoot growth, leaf area and dry matter.

It has been reported that under water stress the amount of ABA in the roots increased (Hubick *et al.*, 1986), the level of cytokinins decreased (Kramer, 1983; Seeley, 1990), and activity of other hormones was also affected. Under the conditions of the present study, in the presence of BAP the negative effect of water stress on vegetative growth was moderated. Possibly the shoots received a chemical signal from roots which facilitated their growth. In BAP, BAP+WS and control treatments cytokinins from the roots might have acted as a chemical signal promoting shoot growth, and ameliorating the effect of water stress in BAP+WS, suggesting that shoot activity was controlled by root cytokinin. In the WS treatment possibly root cytokinin production was suppressed, which could account for the reduced shoot growth. Cytokinins have been found in the xylem sap of a wide range of plants (Skene, 1975) and it has been suggested that roots exert control over shoot growth by synthesis and/or translocation of growth regulator(s), possibly cytokinins (Richards and Rowe, 1977a).

In the present study the level of cytokinins was not measured either in the shoots or in the roots. The response of apricot plants to BAP+WS treatment revealed that the inhibitory effect of ABA in droughted plants (WS treatment was ameliorated by exogenous application of cytokinin to the roots giving low ABA concentration in the shoot tip. So the present results support the hypothesis that root cytokinins act as a chemical signal to the shoot tips and in drying soil decrease in their export results in a reduction in shoot growth.

Two or more hormones may interact under drought conditions. Although ABA and cytokinins have received the most attention all five classes of plant hormone may have an effect in a plant's response to drought. The leaves of even well watered plants can synthesise ABA and contain a lot of ABA. There is also strong evidence for accumulation of ABA in roots in drying soil (Davies and Zhang, 1991). Makus and Guinn (1992) suggested that high ABA concentration in the asparagus spear was associated with low IAA level. They suggested other plant hormones may be involved in spear elongation under water stress conditions, such as cytokinins and gibberellins. During water stress in addition to the absence of cytokinins possibly ABA acts as a chemical signal to the shoot tip, being synthesised in the roots and transported to the shoot tip. Walton (1987)[1] reported that in woody plants the combination of synthesis, metabolism, import and export are probably involved in determining the ABA levels in plant tissue. These mechanisms would require further investigation in apricot plants to determine what amount of accumulated ABA in the shoot tips related to those processes mentioned by Walton (1987). Davies *et al.* (1994)[1] suggested that an increasing degree of soil drying leads to increased production of root produced ABA and an increasing suppression of shoot activity. They noted that dehydration of leaves caused accumulation of ABA, and suggested roots also synthesize the compound in increased amounts when exposed to dry soil. Davies and Zhang (1991) suggested that the physiology of shoot growth can be modified as a function of soil drying, even when shoot water relations are not altered. They suggested plants have

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[1] *see supplemental references on page 314-d.*

a means of communication between roots and shoots other than by reduction in the flux of water to the shoots, indicating that chemical signals do have a role in the regulation of the physiology, growth and development in droughted plants. The experiment of Gowing *et al.* (1990)[2] with apple trees having a split-root system gave evidence of root-shoot communication and its effect on growth in drying soil. They divided the roots of small apple plants into two containers. Soil drying in one container, whilst the other remained wetted, restricted leaf expansion and leaf initiation, though there were no obvious effects on shoot water relations. When roots in contact with drying soil were severed from the plants, leaf growth rate recovered to that of plants in two well watered containers. They proposed that restriction of leaf growth rate was caused by supply of an inhibitor originating in roots that were in contact with drying soil, the supply of which was removed when the roots were severed. They mentioned that their result showed a reduction in leaf area expansion of plants in drying soil in spite of the fact that the bulk water potential of the leaves was not affected by treatments. This finding is in agreement with the present results obtained from WS treatment, which showed a reduction in leaf area and dry weight and shoot growth. For example, as discussed in chapter 7, shoot growth decreased and reached its minimum level at the end of the experiment (after 50 dats), when it did not respond to increased Ψ_{xylem}. Gowing *et al.* (1990) proposed that their growth data provided strong evidence that shoot growth was controlled by a chemical signal. Initiation and production of leaves was more sensitive than leaf elongation to soil drying. They suggested that their data on growth recovery after severing roots in dry soil strongly reinforced the case for a chemical inhibitor being involved in the root-to-shoot signalling of drying soil. Gowing *et al.* (1990) acknowledged that their experiment did not provide evidence as to the identity of the chemical involved, but they proposed that the most obvious candidate would be ABA.

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[2] *see footnote 1*

The evidence for ABA as a root generated signal in drying soil is now very strong. Neales *et al.* (1989)[3] after reviewing the literature suggested that in drying soil, i. ABA is produced by roots; ii. ABA transportation occurs in the xylem; and iii. root produced ABA appears in the leaves.

The present study supports the hypothesis that this signal might be ABA produced in WS roots and accumulated in the shoot tip. However, root ABA production and its transport to the shoots was not measured. Further research is needed to confirm the hypothesis that ABA is produced in the roots in drying soil (or WS treatment) and transported to the shoot tip of apricot plants where it exerts its effect of reducing shoot growth and changing physiology. If this is confirmed, it may help to explain the mechanism of RDI in reducing vegetative growth. In an RDI strategy in the field, reduction of shoot growth at the beginning of the season is desirable, and provided the root produced ABA signal is sent, this may be achieved with only a slight reduction in Ψ_l, so that a very low Ψ_l induced by a severe water stress is not required. If the reduction of vegetative growth by the induced ABA signal in drying soil is the objective, then knowledge of the threshhold soil water content, root water potential and root ABA level are other areas of investigation on apricot trees.

As referred to earlier in this discussion, root produced cytokinin also appears to function as a signal to the aerial parts of the plant, promoting growth and development. So further research on ABA as a chemical signal needs to recognise the opposing nature of the cytokinin signal.

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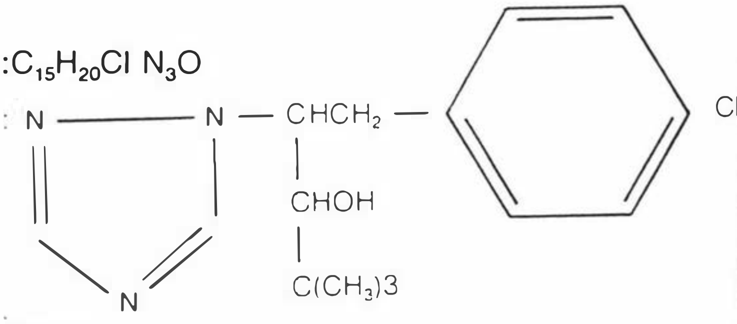
APPENDICES

Appendix 3.1. Chemical and physical properties of PBZ (from Technical data sheet 1982. Plant Protection Division, ICI, England)

Chemical name (IUPAC)	:(2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimmethyl-2-(1H-1,2,4-triazol-1-yl)pentan-3-ol
Common name (BSI approved and ISO proposed)	:Paclobutrazol
Empirical formula	:$C_{15}H_{20}Cl\ N_3O$
Structural formula	
Molecular weight	:293.5
Appearance	:White crystalline solid
Melting point	:165-6°C
Density	:1.22 g/cm^3
Solubility	:In water 35 ppm, methanol 15%, propylene glycol 5%, acetone 11%, cyclohexanone 18%, methylene dichloride 10%, hexane 1% and xylene 6%.
Vapour pressure	:1×10^{-6} pa at 20°C
Stability	:Stable at all temperatures up to 50°C for at least 6 months

Appendix 7.1 The composition of the aeroponic nutrient solution.

Salt	Final solution			Stock solution	
	g 100^{-1}		ppm	g $2l^{-1}$	ml^z l^{-1}
potassium phosphate	26.3	K	78	131.5	4
(KH_2PO_4)		P	62		
potassium nitrate	58.3	K	254	291.5	4
(KNO_3)		N	91		
calcium nitrate	100.3	Ca	168	501.5	4
($Ca(NO_3)_2.4H_2O$)		N	117		
magnesium sulphate	51.3	Mg	49	256.5	4
($MgSO_4.7H_2O$)		S	65		
sequestrene	7.9	Fe	5.6	158.0	1
(NaFe chelate)					
manganous sulphate	0.610	Mn	2.2	12.20	1
($MnSO_4.H_2O$)		S	3.8		
boric acid	0.170	B	0.32	3.40	
(H_3BO_3)					
copper sulphate	0.039	Cu	0.065	0.78	
($CuSO_4.5H_2O$)		S	0.130		
ammonium molybdate	0.037	Mo	0.007	0.74	
($(NH_4)_6Mo_7O_{24}.4H_2O$)		N	0.001		
zinc sulphate	0.044	Zn	0.075	0.88	
($ZnSO_4.7H_2O$)		S	0.037		

[z] ml l^{-1} final nutrient solution for aeroponic system.

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