WATER RELATIONS OF ACACIA

WITH SPECIAL EMPHASIS ON OSMOTIC ADJUSTMENT

by

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SUMMARY

Water relations of Acacia with special emphasis on osmotic adjustment

In spite of their importance in the Australian flora, acacias in Australia have received limited attention in research apart from taxonomic studies. This project was designed to study one physiological aspect, namely water relations at the critical seedling stage and more specifically, osmotic adjustment.

Osmotic adjustment was defined as the decrease of osmotic potential of cell sap to more negative level as a result of the accumulation of osmotically active solutes in protoplasm rather than the concentration of cell contents due to loss of water.

The aims of this study were

1. To demonstrate the existence, magnitude and variability of osmotic adjustment in *Acacia* especially South Australian species.

2. To investigate some of the solutes which accumulated when the plants were water stressed, particularly inorganic ions K^+ and Na^+ and organic compounds proline and others.

3. To seek correlations between osmotic adjustment patterns and the degree of water stress species might experience in their natural areas of distribution.

This study included glass-house, field and laboratory experiments. Ten species were selected for a pot droughting experiment namely *A.anceps*, *A.aneura*, *A.gillii*, *A.longifolia*, *A.myrtifolia*, and *A.saligna; A.cyclops*, *A.iteaphylla*, *A.leiophylla* and *A.rivalis*. From measurements of water and osmotic potentials it was found that osmotic adjustment occurred in the first group but not the second. There was not a strong relationship between osmotic adjustment and the rainfall in the area of distribution of a species.

Five of the species (*A.anceps*, *A.aneura*, *A.gillii*, *A.iteaphylla* and *A.myrtifolia*) were then grown in the field and monitored over summer. Four of the five showed stronger osmotic adjustment than in the pot experiment. Magnitudes were *A. anceps* 1.10 MPa, *A. aneura* 1.09, *A. gillii* 0.69, *A. iteaphylla* 1.07 and *A. myrtifolia* 1.11 MPa.

Proline concentration increased significantly in the stressed plants of most field grown species, while other organic solutes were not detected, except in *A.iteaphylla*, which unexpectedly contained phenyl-ethylamine (PEA). However, no evidence was found of a significant relationship between drought treatment and PEA concentration.

Potassium and sodium did not fluctuate with the fall of water potential in the field. For potted *A. iteaphylla* seedlings fluctuations in K^+ were also absent when the K supply was varied in Hoagland's nutrient solution. However the osmotic contribution of K and Na was between 29% and 52% of total osmotic potential in the field grown plants, a significant contribution. Which solutes did generate the observed osmotic adjustment was not determined.

The study suggested that osmotic adjustment is important in the survival of these acacia seedlings, although other factors may also be involved. The great diversity in Australian acacias and the tolerance of many to water stress may lead in future to their use as a genetic resource in developing drought-tolerant varieties.

STATEMENT

This thesis contains no materials which have been accepted for the award of any degree or diploma in any university. So far as I know and believe, no part of it has already been published or written by another person, except where due reference is made in the text of the thesis.

I consent to this thesis being made available for photocopying and loan if accepted for award of the degree.

B. PAUL NAIOLA

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CHAPTER 1

INTRODUCTION

1.1. *Species and distribution*

The genus *Acacia* includes a large number of species. It is not known exactly but it is estimated that more than 1200 species are distributed throughout the world (Hopper and Maslin, 1978; Simmonds, 1981). *Acacia* is distributed in both northern and southern subtropics and is associated with tropical regions as well (Simmonds, 1981).

Within this genus which occurs throughout the world, not less than 700 species are native to Australia, ranging from herbs to trees (Simmonds, 1981).

The genus in Australia is distributed throughout the continent with a few species in rain forest while more concentrate in the south-west of western Australia and south-east Australia regions (Simmonds, 1981). At least 97 species are distributed or originated in South Australia (Whibley, 1980).

Acacia's endemic status in Australia is interesting. The south-west region possesses the greatest number of endemic species in the world. Not less than 17 species are endemic to South Australia (Whibley, 1980).

1.2. *The role and uses of* Acacia

Australian acacias possess enormous economic significance and have been used in many parts of the world for timber, tanning, animal feed, fuelwood, agroforestry, ornamental, horticulture, soil stabilization, soil enrichment and essential oil production (Sedgley, 1987). Among the great variety in Australia some have a characteristic role.

Mulga (*A.aneura*) plays an important role as a main element in grassland pasture in many drier parts of the country. Brigalow (*A.harpophylla*) is the same. The occurrence of this species has a positive relationship with the total nitrogen content and phosphorous availability in the soil (Johnson, 1981). The firewood "prickly Moses" (*A.pulchella*) is a common understorey component of forest ecosystems throughout the south-west of Western Australia. The species shows the characteristics of a "fireweed", germinating abundantly only after its habitat has been subjected to a hot summer burn (Monk et al, 1981). In many forests, *Acacia* spp. germinate in large numbers following the regeneration burn and these include several species of *Acacia* (Adams and Attiwill, 1984). Due to their ability to colonize poor or disturbed sites, they are used in dune reclamation (Nakos, 1977) and restoration in mining operation areas (Langkap et al, 1979). In these situations they help to stabilize the soil structure, hence its conservation.

1.3. *The reason for studying Australian* Acacia

In spite of the important role which many of them play, with the exception of taxonomic studies, *Acacia* in Australia has received no great attention in research especially in regard to water relations. This is a contrast to *Eucalyptus* (for example Sinclair, 1980; Myers and Neales, 1984, 1986), one of the other plant groups dominating the Australian flora. To the best of my knowledge, intensive water relations studies have been reported for only two species, namely mulga, *A.aneura* and brigalow, *A.harpophylla* (Slatyer, 1967b; Connor and Tunstall, 1968; Tunstall and Connor, 1975; Pressland, 1976). A recent compilation by Ferrar and Vranjic (1988) regarding the water relations of Australian native plants revealed the lack of study in acacias especially their water relations.

One of the most limiting factors for the growth of *Acacia* in arid environments is soil water availability. Their physiological behaviour is a result of the interaction between two

main factors i.e. their genetic potential (inheritance) and the main environmental components of Australian desert namely a long-term water deficiency (caused by disequilibrium between precipitation and evaporation) and high temperature. Thus they are available to be employed in breeding programs due to the possibility of carrying drought resistance genes.

1.4. *The study of seedling water relations*

Seedlings are a critical stage in the continuation of a species. The transition from germination to the established seedling is perhaps the most profound transition in the life of an individual plant (Osmond et al, 1980). It seems that seedling characteristics are similar in some respects to the adult individual plant in tolerating environmental fluctuations. However, in several aspects they are more vulnerable. The spread and depth of roots are inadequate to seek water and mineral salts in a large volume of soil. A hazardous problem for seedlings of arid zone species is how to adapt to high temperature of the soil surface with inadequate water. For example, as recorded by Cloudsley-Thompson (1968) at a desert site in northern Africa, when T_{air} was 40.5^0 to 43.5^0 C, the $T_{surface}$ of soil reached 83.5^0 to 84^0 C.

Not all seedling mortality is caused by desiccation, other factors include grazing, harsh winters, pests and disease (Fenner, 1987). However, water shortage may be the most limiting factor for seedlings' survival in the field (Wellington and Noble, 1985).

Presumably the properties of *Acacia* in Australia such as wide distribution, ability to colonize as a pioneer species in poor soil and resprouting after fire, are related to their ability to control their internal water shortage. In concentrating on the water relations of *Acacia*, this study is aimed at understanding the behaviour of seedlings in terms of the fluctuations of their water relations in both glass-house and field conditions. Particular topics investigated were, how they regulate their internal water balance during water stress, to what extent they accumulate solutes, and how they respond to nutrient and other environment factors. The

main question is whether osmotic adjustment takes place in successful *Acacia* species under very harsh conditions.

The investigation started with a glasshouse trial to study osmotic adjustment within 10 selected species mainly South Australian endemic species. A drying cycle was applied, and the relationship between water potential and osmotic potential was interpreted to classify the adjusting and non-adjusting species. This osmotic adjustment study was then expanded to a more realistic field environment. Osmotic adjustment in 5 of the original species was examined throughout a summer season concentrating on the seasonal summer fluctuations of osmotic adjustment during 7 harvests. Solutes which probably were accumulated such as proline, betaines, potassium and sodium were determined during the season. This solute study was also extended to investigate a new solute which possibly accumulated due to drought stress. The effect of environmental factors such as nutrient, soil type and light on particular solute concentration, also become the object of this study.

CHAPTER 2

WATER RELATIONS AND PLANT GROWTH - GENERAL THEORY AND INTRODUCTION TO THE METHODS

2.1. *The role of water in plant growth*

Water occupies 60 to 90% of the plant body made up to 95% in aquatic plants (Sutcliffe, 1979). It is involved in various ways during plant growth. Some of the very basic forms of involvement (Craft, 1968; Slatyer, 1967a; Meidner and Sheriff, 1968) are in metabolic processes, as solvent in association with the absorption and transport of solutes, as a regulator, and in germination.

In metabolic processes, water takes part as a substance at a very basic level, as a central part of the photosynthetic machinery, the evolution of oxygen from water. It is involved in photosynthetic electron transport where the process begins with water being oxidized in the O_2-evolving centre in photosystem II.

Other artificial chemicals have been demonstrated to be capable of being photo-oxidized and acting as an electron donor to photosystem II, for examples hydroquinones, cysteine, ascorbate, Mn etc. (Hauske, 1977). Nevertheless, none of them will be capable of replacing water in its position as photosynthetic oxidative substrate, due to its natural occurrence: abundant and anywhere!

As a solvent, in association with the transport of solutes, all metabolic reactions which proceed in the protoplasm, do so in the aqueous phase (Sutcliffe, 1979; Richter, 1978). Water is also actively involved around the rhizosphere in the process of solute absorption from the soil solution. Solutes in the soil are not always readily available for absorption by the plant root unless being carried by the flow of water (Craft, 1968).

The affinity of water for most solutes and ions is due to the natural dipole property of its molecules which is an extraordinary contribution to its characteristics as a universal

solvent (Richter, 1978; Kramer, 1983). This is especially important during solute movement across the root into the xylem.

Once solutes reach the xylem after passing through resistances such as the casparian band and plasma membrane of the root cells (Drew, 1987), the long distance travel to the leaves is accompanied by water flow. This flow is driven by the transpiration stream (passive transport) and the gradient of water potential between xylem and leaf cell sap, thus expressing the role of water in solute transport.

As a regulator, water is vital because of its high latent heat, which through transpiration leads to the cooling of the leaf. In conjunction with radiation absorption, this property of water allows plants to absorb an enormous amount of solar radiation without the cells being collapsed by the increase of temperature (Sutcliffe, 1979).

The involvement of water in the *germination events of seeds* starts from the pre-germination period i.e. from the process of dormancy breakage to the early and further metabolic activities inside the seeds. For seeds which occur in the field, early rainfall would lead to imbibiton and allow the leaching of inhibitor substances out of the seed (Bryant, 1985).

Three stages of development can be distinguished in the process of seed germination i.e. - imbibition, lag phase and germination (Bewley and Black, 1978; Simon, 1984). Water is already involved from the first stage. Imbibition promotes and activates some metabolic event in the seed soon following the rehydration of enzymes and their substrates (Bewley and Black, 1978; Bryant, 1985).

The flow of water through the imbibition process also carries dissolved oxygen into the seed. This in an important contribution to the respiration of the embryo (Come, 1975, as quoted by Simon, 1984), in the state of development after imbibition.

2.2. *The effect of water shortage on plant growth*

Without doubt, water is essential to plant life. The shortage of water will reduce or bring to a halt some essential processes within the plant.

Plants may face water stress at any stage during their life cycles, either as germinating seed, seedling or mature plant (Kramer, 1983). The injury that they might suffer depends on the level of stress and the age of plants.

Water stress can produce direct or indirect negative effects on the physiological, biochemical and physical processes within plants. These impacts might be summed up in four main points:

a. *morphological/ anatomical mal-formation*

b. *physiological disintegration*

c. *biochemical degradation, and*

d. *physical relationships*.

Morphological/anatomical mis-formation due to water stress covers a wide range of aspects within plant growth. Some examples: fruit size may be reduced (Hales et al, 1968); the elongation of expanding wheat leaves was severely inhibited and leaf tissue died at -3.5 MPa (Barlow et al, 1977); the formation of leaf primordia was inhibited under water stress (Hussain and Aspinall, 1970); the average leaf sizes and number of leaflets per plant was smaller in non-irrigated than in irrigated soybean (Sivakumar and Shaw, 1978).

Physiological disintegration will result in various consequences such as the reduction of stomatal activities (Iljin, 1957); effects of photosynthesis due to the reduction of carbon uptake and cell growth (Avecedo et al, 1971); fruit set and ripening, etc.

Water stress also affects a number of biochemical processes within plants. Protein synthesis was reported reduced due to water stress (Fukutoku and Yamada, 1981); polyribosome content, regarded as an indicator of protein synthesis, was decreased up to

50%; chlorophyll and RNA were also reduced by water stress (Biswan and Choudhuri, 1984).

Some physical relationships are affected during water stress such as water uptake and transport being inhibited due to xylem cavitation. Milburn (1966), found the uptake of water by severely water stressed leaves deviated markedly from less stressed ones as a result of cavitation. The resistance to water flow through the soil to the plant roots would also increase due to the reduction of soil moisture; this is mainly due to the shrinkage of soil around the root surface (Cowan and Milthorpe, 1968). As a consequence the formation of a vapour gap between the two surfaces would occur, therefore reducing the effectiveness of the contact area between root surface and soil water (Osonubi, 1984).

2.3. *Osmotic adjustment*

2.3.1. *Definition, significance and measurement*

The development of water deficits is a regular pattern undergone by plants both in arid and humid regions. The degree of damage by water stress depends on the level of stress itself and the degree to which the plant possesses mechanisms to avoid or to tolerate the stress. If the plant is to remain metabolically active it must retain sufficient water in cells to maintain their metabolic processes while the stress is in progress. If growth is to continue, sufficient water must be retained to maintain turgor pressure, since turgor or positive cell wall pressure is necessary for growth (Hsiao et al, 1976; Morgan, 1980).

The volume of water in the cells depends on the osmotic potential of the cells themselves. Under water stress conditions, the volume of cell water is reduced due to the loss of water. In some plants "osmotic adjustment" may be employed as a useful way to retain cellular water volume and hence cell water.

Osmotic adjustment refers to a phenomenon which may take place in plant cells (and some microorganisms) when exposed to water and salt stresses. The process is defined as a decrease in osmotic potential greater than can be explained by solute concentration during dehydration (Kramer, 1983). This process is achieved either by uptake of external solutes into the cell, or the synthesis of new solutes within the cells (Hsiao et al, 1976; Morgan, 1980; Wyn-Jones and Gorham, 1983).

This process has been given a variety of different names and definitions by different authors: osmoregulation (Hellebust, 1976; Morgan, 1984), turgor regulation (Zimmermann, 1978), osmotic regulation (Kauss, 1977), osmotic adaptation (Turner and Jones, 1980), osmotic adjustment (Hsiao et al, 1976; Turner and Jones, 1980; Munns, 1988). Other definitions include the process of change in solute content after recovery from water stress (Morgan, 1984), or the regulation of osmotic potential within a cell by addition or removal of solutes until the intracellular osmotic potential is approximately equal to the potential of the medium surrounding the cell (Borowitzka, 1981). Thus all the definitions involve the ability of plant cells to regulate the total number of intracellular solute molecules (Wyn-Jones and Gorham, 1983; Steponkus, 1980).

Turgor pressure has been recognized to play an important role in the maintenance of growth and leaf expansion and contributes to the ability of the root apex to penetrate soil (Drew, 1987). Hence, plants with the ability to employ osmotic adjustment during drought to reduce osmotic potential will tend to retain their turgor pressure a few days longer than those without osmotic adjustment (see Morgan's diagram, 1984 as reproduced in Figure 2.1). This theoretical model has been confirmed in a recent study by Basnayake et al (1993), who found in inbred sorghum that lines with high osmotic adjustment would survive 10 days longer, finally dying at a higher RWC but lower water potential.

Figure 2.1

Typical response of leaf osmotic potential to changes of leaf water potential of a plant with osmotic adjustment (broken line) and without (dotted line). Note that plant with osmotic adjustment would retain its turgor pressure longer (2 days) than without, provided that the loss of water from medium is not rechargeable. Black and blank bars on the *x*-axis refer to night and day respectively (*Redrawn from Morgan, 1984*).

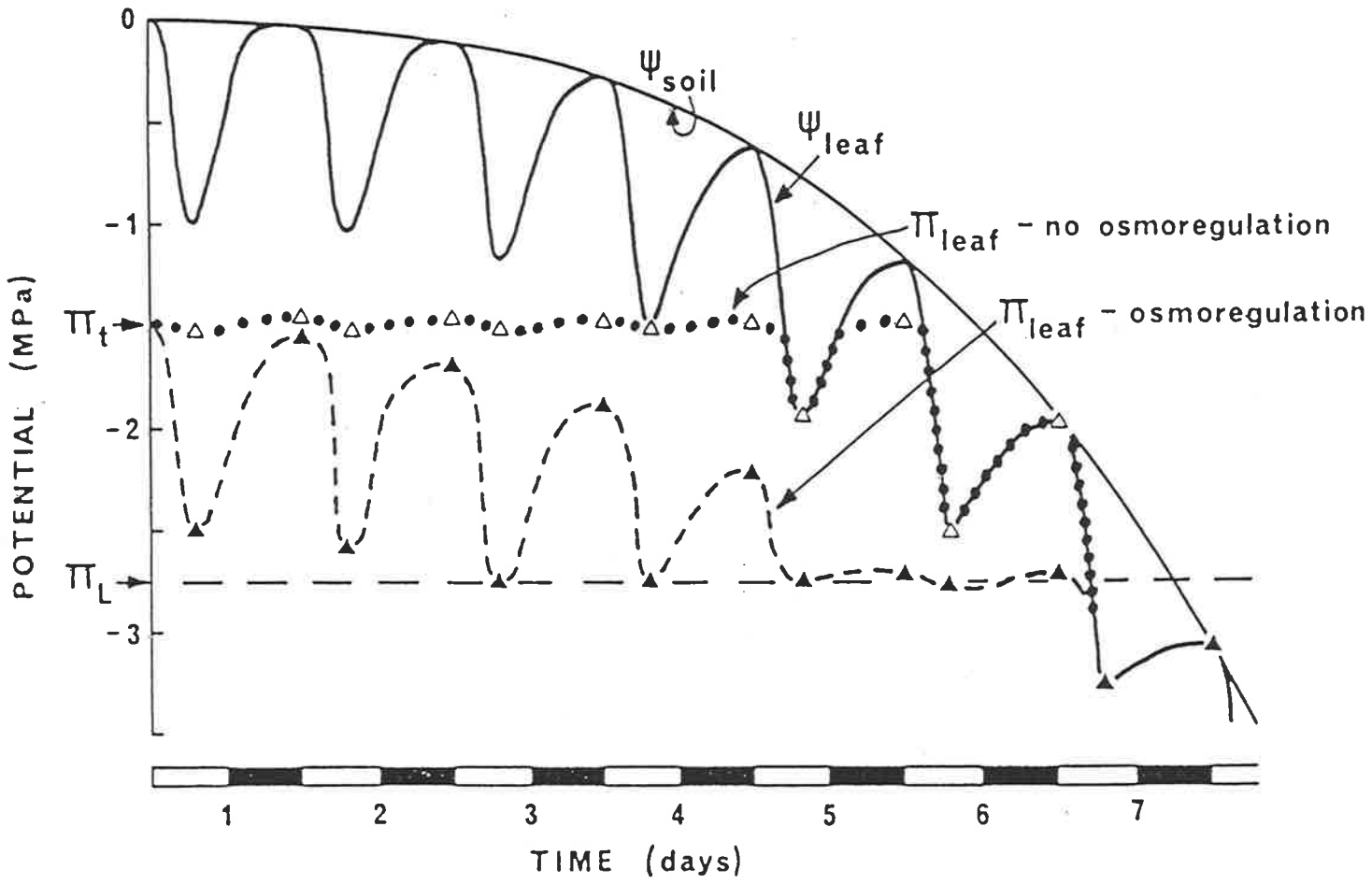
ψ_{leaf} : leaf water potential

ψ_{soil} : soil water potential

π_{leaf} : leaf osmotic potential

π_t : osmotic potential at full turgor

π_L : osmotic potential at the limit of solute accumulation


0
-1
-2
-3
POTENTIAL (MPa)
Ψ_{soil}
Ψ_{leaf}
π_{leaf} - no osmoregulation
π_{leaf} - osmoregulation
π_t
π_L
1
2
3
4
5
6
7
TIME (days)

However, in other plant groups, osmotic adjustment may not always be associated with the continuation of growth, but rather the extension of the survival period. Schultz and Matthews (1993) found that in grapes, although growth was completely inhibited, turgor could be retained by osmotic adjustment. Chimente and Hall (1994) also found in sunflower genotypes that osmotic adjustment was the only attribute contributing to turgor maintenance during drought but there was a negative association between osmotic adjustment and leaf expansion.

The osmotic adjustment process occurs in various parts of the plants e.g. leaves (Morgan, 1977, 1980; Avecedo et al, 1979; Osonubi and Davies, 1978; Ike and Thurtell, 1981) spikelet (Morgan, 1980), phloem (Smith and Milburn, 1980a, 1980b), root systems (Graecen and Oh, 1972; Osonubi and Davies, 1978; Sharp and Davies, 1979), and the fibre of cotton (Dhindsa et al, 1975).

Osmotic adjustment has been described and measured in various ways. Morgan (1977; 1980) demonstrated the existence of this phenomenon in wheat by plotting values of water potential against osmotic potential. The line drawn through the means of water potential and osmotic potential for each 0.5 MPa increment of water potential, showed the response of osmotic potential to the fluctuations of water potential, which varied between genotypes. In the absence of osmotic adjustment this line should approach the "line of equality" i.e. beyond a certain point osmotic potential and water potential should be equal when turgor pressure has decreased to zero. Morgan showed the differences between genotypes, by the deviation of the response line away from the line of equality, which is the magnitude of turgor pressure. By this method, the magnitude of turgor maintenance due to the occurrence of osmotic adjustment is seen clearly; however, Morgan did not calculate the magnitude of osmotic adjustment.

As evidence for osmotic adjustment, Myers and Neales (1986) measured the difference in osmotic potential at full turgor between well-watered and rewatered plants after they were subjected to one or two water stress treatments.

The fluctuation of osmotic adjustment depends on the level and rate of change of stress (Jones and Rawson, 1979; Steponkus et al, 1982). It also has limits (Turner and Jones, 1980). This is true for example for pot grown plants, and for field grown wheat (Hsiao et al, 1976; Turner et al, 1978). In field grown perennial plants which grow in arid conditions, the process of adjustment may take place over a season. During two years of intensive measurement in one single mature field grown tree of *Eucalyptus cladocalyx*, Sinclair (Pers. Comm.) found that a steady degree of osmotic adjustment allowed turgor maintenance of up to 3 MPa by the plant throughout the season.

2.3.2. *Solute accumulation*

To achieve more negative (lower) osmotic potential by osmotic adjustment, some low molecular weight-water soluble solutes have to be accumulated or generated within the cells, thus lowering the osmotic potential of all the cell compartments: vacuole, cytoplasm and organelles (Kauss, 1977; Zimmermann, 1978; Ford and Wilson, 1981; Tyree and Jarvis, 1982).

The accumulation of solutes in osmotic adjustment, has to be distinguished from the concentration of solutes due to water loss from the cell (Turner and Jones, 1980; Morgan, 1984). The change of osmotic potential caused solely by passive concentration of solute as a result of water loss is expressed as:

$$\Psi_\pi = \frac{\Psi_{\pi o}\ V_o}{V} \quad \ldots\ldots\ldots (1)$$

where $\Psi_{\pi o}$ and V_0 are the osmotic potential and osmotic volume at full turgor, Ψ_π and V are the final osmotic potential and volume. In this case the cells behave like a perfect osmometer (Ben-Amotz, 1974; Turner and Jones, 1980; Morgan, 1984), with no solute accumulation either by synthesis or transport; and volume regulation is terminated after the completion of water exchange (Zimmermann, 1978), thus no turgor maintenance.

However, if osmotic adjustment takes place due to solute accumulation by the cells, the osmotic potential would decrease further than its calculated value using (*equation 1*), following the loss of water. This will maintain turgor pressure down to more negative values of water potential than would otherwise be possible.

From the biochemical point of view, solutes (osmotic constituents) might be divided into two large groups, i.e.

a. *inorganic*, and

b. *organic compounds*

The main inorganic solute is usually potassium although sometimes sodium and chloride are involved as well (Ford and Wilson, 1981; Marschner, 1986; Kylin and Quatrano, 1975). Amino acids (especially proline) and sugars are considered the most important organic solutes; some organic acids may play a minor part. Table 2.1 summarizes a number of solutes which have been reported to accumulate in various plant species.

The term "compatible solutes" was introduced by Brown and Simpson (1972) and confirmed by Borowitzka and Brown (1974). It refers to low molecular weight compounds which accumulate in higher concentration in stressed plant cells during dehydration. These substances are believed to be very poor enzyme inhibitors, to be less toxic than others and protect and permit the activity of enzyme systems, hence they are compatible with metabolic functions. They include sugars, amino acids, organic acids, betaine, polyols such as sorbitol,

Table 2.1

List of solutes which accumulate within plants during water stress

ORGANIC

Name of solute	Plant species	Family	References
proline	rye grass	Poaceae	MacPherson, 1954
	turnip	Brassicaceae	Thompson & Morris, 1957
	bermuda grass	Poaceae	Barnett & Naylor, 1966
	soybean	Papilionaceae	Jager & Meyer, 1977
	barley	Poaceae	Singh et al, 1972
	green panic	Poaceae	Ford and Wilson, 1981
	buffel grass	Poaceae	*Idem*
	spear grass	Poaceae	*Idem*
	alfalfa	Papilionaceae	Parameshwara, 1984
	wheat	Poaceae	Naidu, 1987
	Melaleuca	Myrtaceae	Naidu et al, 1987
	oak	Fagaceae	Kim and Kim, 1994
	Phaseolus vulgaris	Papilionaceae	Raggi, 1994
	coffee	Rubiaceae	Maestri et al, 1995
betaine	green panic	Poaceae	Ford & Wilson, 1981
	buffel grass	Poaceae	*Idem*
amino acid (total)	sorghum	Poaceae	Jones et al, 1980
	sunflower	Asteraceae	*Idem*
sucrose	green panic	Poaceae	Ford & Wilson, 1981
	buffel grass	Poaceae	*Idem*
	spear grass	Poaceae	*Idem*
	soybean	Papilionaceae	Meyer & Boyer, 1981
	Populus deltoides	Salicaceae	Gebre et al, 1994
fructose	green panic	Poaceae	Ford & Wilson, 1981
	spear grass	Poaceae	*Idem*
	apple	Rosaceae	Wang and Stutte, 1992
	Populus deltoides	Salicaceae	Gebre et al, 1994
glucose	spear grass	Poaceae	Ford & Wilson, 1981
	apple	Rosaceae	Wang and Stutte, 1992
	Populus deltoides	Salicaceae	Gebre et al, 1994
glucose + fructose	soybean	Papilionaceae	Meyer & Boyer, 1981

Name of solute	Plant species	Family	References
soluble sugar	cotton	Bombacaceae	Cutler & Rains, 1978
	corn	Poaceae	Barlow et al, 1976
Organic acids			
malate	cotton	Bombacaceae	Cutler & Rains, 1978
	cotton fibre	Bombacaceae	Dhindsa et al, 1975
	green panic	Poaceae	Ford & Wilson, 1981
	spear grass	Poaceae	*Idem*
	Populus deltoides	Salicaceae	Gebre et al, 1994
aconitic	spear grass	Poaceae	*Idem*
sorbitol	apple	Rosaceae	Wang and Stutte, 1994
myoinositol	*Populus deltoides*	Salicaceae	Gebre et al, 1994
salicin	*Populus deltoides*	Salicaceae	Gebre et al, 1994
INORGANIC			
potassium			
	cotton fibre	Bombacaceae	Dhindsa et al, 1975; Cutler & Rains, 1978
	sorghum	Poaceae	Jones et al, 1980
	buffel grass	Poaceae	Ford & Wilson,1981
	spear grass	Poaceae	*Idem*
	Populus deltoides	Salicaceae	Tschaplinski and Tuskan, 1994
sodium			
	cotton	Bombacaceae	Cutler & Rains, 1978
	green panic	Poaceae	Ford & Wilson, 1981
chloride			
	sorghum	Poaceae	Jones et al, 1980
	green panic	Poaceae	Ford & Wilson, 1981
	buffel grass	Poaceae	*Idem*
	spear grass	Poaceae	*Idem*
	siratro	Papilionaceae	*Idem*

mannitol etc. (Brown and Simpson, 1972; Borowitzka and Brown, 1974; Wyn-Jones and Gorham, 1974; Tyree and Jarvis, 1982).

Solutes might originate from synthesis, breakdown or retransport. The evidence of new solute synthesis is expressed by significant differences in solute content between control and stressed samples. Proline was significantly increased in water stressed samples up to 20 to 100 times the concentration in well-watered treatments (Jones et al, 1986). Sucrose increased markedly in stressed treatment of four grass species (Ford and Wilson, 1981), in sorghum and sunflower (Jones et al, 1980). Sugars were three times more concentrated in stressed maize than in controls (Premachandra et al, 1989).

Solute may also be generated from the breakdown of more complex to simpler compounds. Munns and Weir (1981) found that the reduction in carbohydrate consumption in the growing leaf during water stress was equal to the increase of sugar for osmotic adjustment. Fukutoku and Yamada (1981) found some of the labelled N in protein was detected in proline after water stress.

The source of solute for accumulation may originate from import (Morgan, 1984) or retransport from other parts of the plants (Aspinall and Paleg, 1981). Meyer and Boyer (1981) reported that the removal of soybean seedling cotyledons reduced the degree of osmotic adjustment in the hypocotyls proving that solute transport from the cotyledon itself is essential for this osmotic adjustment.

The accumulation of potassium within the leaf in response to water stress (Ford and Wilson, 1981) indicated the transport of this osmotic constituent from other parts of the plant or soil.

2.3.3 *The role of bulk modulus of elasticity* ε *in determining changes in cell water relations as water potential is reduced during drought.*

Osmotic adjustment has an energy cost due to the requirement for synthesis of organic solutes such as proline, betaine etc, or the transport of solutes. Instead of such energy consuming steps, some plants face the stresses by developing cells with more elastic walls. Such cells may undergo less severe stress than those with more rigid walls, because they can shrink more as drought develops and so maintain higher turgor pressure. (Hellkvist *et al.* 1974, Dale and Sutcliffe 1986, in Fan *et al.*, 1994).

Cell wall elasticity may be determined by measurement of the modulus of elasticity, ε (Sinclair and Venables, 1983). This modulus is a function of cell turgor and water content, defined as:

$$\varepsilon = W \frac{d\Psi p}{dW}$$

where Ψp is pressure potential, and W is weight of cell water content.

When the value of ε is small, the cell elasticity is great (Hellkvist *et al.*, 1974; Fan *et al.*, 1994). When plants undergo water stress, those with elastic tissues may benefit by a greater ability to maintain turgor compared to plants with more rigid cell walls (Zimmermann and Steudle, 1979 in Fan *et al.*, 1994). Fan et al.,(1994) found that in each case examined turgor was greater following a decline in ε, when plants were stressed. This implies that elastic shrinkage promotes turgor during tissue dehydration.
Variation of ε is thus another possible response to water stress, but this was not studied in this project.

2.4. *The measurement of water potential and its components for the estimation of the degree of osmotic adjustment*

Osmotic adjustment is a result of the interactions of the water potential components within the living plant cells. Morgan (1980) demonstrated osmotic adjustment in wheat (*Triticum*) by plotting values of water potential against osmotic potential for six genotypes *T.spelta*, *T.dicoccum*, *T.dicoccoides*, *T.aestivum* cv Capella Desprez and cv Condor and *T.durum* respectively. He found that osmotic adjustment took place in spikelets of all genotypes, although only in leaves of some. He classified the genotypes into three groups on the basis of the different patterns of response.

Myers and Neales (1986) interpreted the difference between osmotic potential at full turgor of well-watered plants and plants rewatered after one and two periods of water stress as evidence for osmotic adjustment. They found measurable osmotic adjustment in three eucalypt species after one period of stress, which increased significantly after the second drought cycle, with a tendency for greater difference in one species (*E.polyanthemos*) than the others.

Others have seen the magnitude of osmotic adjustment through the differences of osmotic potential before and after exposure to stress or drying cycles, or in field grown plants, through the magnitude differences of osmotic potential or turgor pressure within a day or between wet and dry (seasonal) periods (Jones et al, 1980; Redman, 1976; Roberts et al, 1980; Monson and Smith, 1982; Nilsen et al, 1983; Bowman and Roberts, 1985; Meinzer et al, 1986).

Hence, the measurement of the fluctuations of water potential components is the foundation of the interpretation of the degree of osmotic adjustment.

There are a number of methods that have been used to measure water potential and its components (for examples, see Bennet-Clark, 1959; Knippling, 1967; Zimmermann et al,

1980; Kramer, 1983). However, in recent time it seems that only a few methods are still used by workers in this field (see review by Losch, 1984, 1986 and 1989).

Among these methods, the pressure chamber and the thermocouple psychrometer are often used. Some controversy still exists about which of these two methods is the more reliable. However, it is difficult, complicated and even impossible to decide which method is better or superior (Talbot et al, 1975; Tyree and Jarvis, 1982), since these two methods approach the problem from different angles (see explanations of these methods below).

In some reports the thermocouple psychrometer is used as a reference to correct values obtained from the pressure chamber (for example Boyer, 1967; Boyer and Potter, 1973). Conversely, psychrometer results have been compared with the pressure chamber as standard (see Wenkert, 1980; Turner et al, 1984). A review by Ritchie and Hinckley (1975) revealed that in over 40 reports only about 25 percent of cases showed a good correlation between results from these two methods.

Each method however, has its advantages and disadvantages (see Sections below).

2.4.1. *Pressure Chamber*

2.4.1.1. *The principle and technique in measuring plant water relations particularly water potential and osmotic potential*

It seems that the principle of using high pressure gas in a pressure chamber to study plant water relations was first established by Dixon (1914), but the purpose was not the same as it is today. This was not developed until Scholander et al (1965), established a modern pressure chamber for measuring negative hydrostatic pressure or xylem sap tension within plants. Later, Scholander and his co-workers developed this invention which is capable of studying a wide range of aspects of plant water status.

Nowadays, the pressure chamber is considered a reliable tool for studying plant-water relations. Water potential components and some derivations are produced simultaneously in a relatively short time (hours) with relatively high reliability.

To measure water potential, a single leaf or shoot is sealed into the chamber and gas pressure is applied. The applied (balance) pressure which allows the water just to wet the surface of the cut end of a sample inside the chamber is a function of the water potential of the cells (Scholander et al, 1964, 1965; Waring and Cleary, 1967; Boyer, 1967). This balance pressure is actually equal (but opposite in sign) to the xylem pressure potential (Hellkvist et al, 1974). The values of the balance pressure or xylem pressure potential may not always equal the cells' water potential for two reasons. Firstly, when transpiration is in progress, the mesophyll cell beyond the xylem possess a lower water potential than the xylem sap (it is this water potential gradient that maintains the transpiration stream). The leaf/shoot cell water potential will come to equilibrium with the xylem pressure potential when the transpiration is stopped (Milburn, 1979). Secondly, some solutes may be dissolved in the xylem sap thus lowering its osmotic potential. In this case, the pressure chamber reading will overestimate cell water potential; that is, cell water potential will be more negative than indicated by pressure chamber reading. Hellkvist et al (1974) investigated this correction by measuring the osmotic potential of the xylem sap in sitka spruce using a thermocouple psychrometer. They found that the values of the xylem sap were close to zero and did not fall below -0.2 bar. A number of published papers in fact have not made corrections for the presence of solute in xylem sap (Ford and Wilson, 1981; Monson and Smith, 1982; Meinzer et al, 1986; Meinzer et al, 1988).

The measurement of osmotic potential is obtained from the construction of pressure volume (P-V) curves by employing the pressure chamber. Besides water potential and

osmotic potential, several other parameters may be obtained from the P-V curve as explained in the next section (Tyree and Hammel, 1972; Sinclair and Venables, 1983).

2.4.1.2. *Pressure Volume (P-V) Curve*

A common usage of the pressure chamber is to construct a Pressure Volume (P-V) curve. Discussion of the pressure volume curve in this thesis covered two parts. The first part dealing with the general principles and theory is presented in this Section (2.4.1.2), while the details of how the P-V curves were measured and constructed in these *Acacia* experiments are given in Chapter 3 (Section 3.2.3).

A P-V curve is derived from the tissue water potential isotherm, the relationship between the change of total water potential and volume of the tissue or cells within a living sample (Richter et al, 1980; Tyree and Jarvis, 1982). Following its introduction, the P-V curve eventually attracted great attention. Various theoretical concepts and interpretation have contributed to establish its reliability (for example Scholander et al, 1965; Boyer 1967; Tyree and Hammel, 1972; Hellkvist et al, 1974; and Ritchie and Hinckley, 1975), including use of computer program to increase the accuracy of interpretation (Jane and Green, 1983; Sinclair and Venables, 1983).

To build a P-V curve, the sample may be rehydrated to allow the tissue to reach full turgor (usually overnight or more, depending on the severity of dehydration of the samples). The sample is weighed, then sealed into the chamber. A wet tissue paper is placed inside the chamber, to reduce the possible loss of water vapour from the sample. The pressure is increased gradually in steps. At each increase, the extruded sap is collected in a small preweighed plastic tube (0.5 cm in diameter and 8 to 10 cm in length) packed with tissue paper. The difference between two respective weighings is the amount of extruded sap for

that pressure increment. The values of the extruded sap are plotted against the reciprocal of the corresponding balance pressure to construct a P-V curve.

Figure 2.2 shows a complete standard P-V curve. To derive the line for determining osmotic potential at least three points are needed to generate the straight line segment CC'. The P-V curve is concave in shape, but when the pressure potential (Ψ_p) no longer contributes to the water potential (Ψ) of the cells then the applied pressure is equal to the osmotic potential (Sinclair and Venables, 1983). Thus when the C'-C line is extrapolated to point B on the y-axis (i.e. $W_e = 0$, where W_e is the weight of symplastic water obtained by applied pressure), then the y-coordinate at point B corresponds to the reciprocal of the initial osmotic potential of the initial symplastic water (Tyree and Hammel, 1972; Sinclair and Venables, 1983).

However, other researchers did not apply the rehydration step for the P-V curve construction (see Wenkert et al, 1978; Bowman and Roberts, 1985). Whether or not the tissue is rehydrated mainly depends on the purpose of the study. Bowman and Roberts (1985) produced an artificially high osmotic potential especially during a drought period, when the samples were rehydrated. In these *Acacia* experiments, the tissues were not rehydrated for constructing the P-V curves (see Section 3.2.3). Figure 2.3 shows an "incomplete" P-V curve generated without rehydration.

The similarities and differences between the two types of P-V curve, namely with or without *pre-rehydration* are discussed as follows, referring to Figure 2.2 and 2.3. The points made are those relevant to this study of *Acacia*, particularly Chapter 3 and Chapter 6.

(Note: **a:** *refers to fully hydrated*, **a_1:** *refers to non-rehydrated*, etc.):

(a). *Water potential at full turgor* (Ψ_0), corresponds to the value of balance pressure which allows water to wet the surface of the protruding sample in the pressure chamber (which is actually xylem pressure potential) when all the cells have been fully rehydrated,

Figure 2.2

Typical standard P-V curve, generated from Pressure Chamber (*redrawn from Sinclair and Venables, 1983)*. Note that the shape of the curve may be similar to the "incomplete" ones as in Figure 2.3. However, some values/components may be different and some, such as water potential at full turgor, cannot be generated from the later type. See text for more explanation.

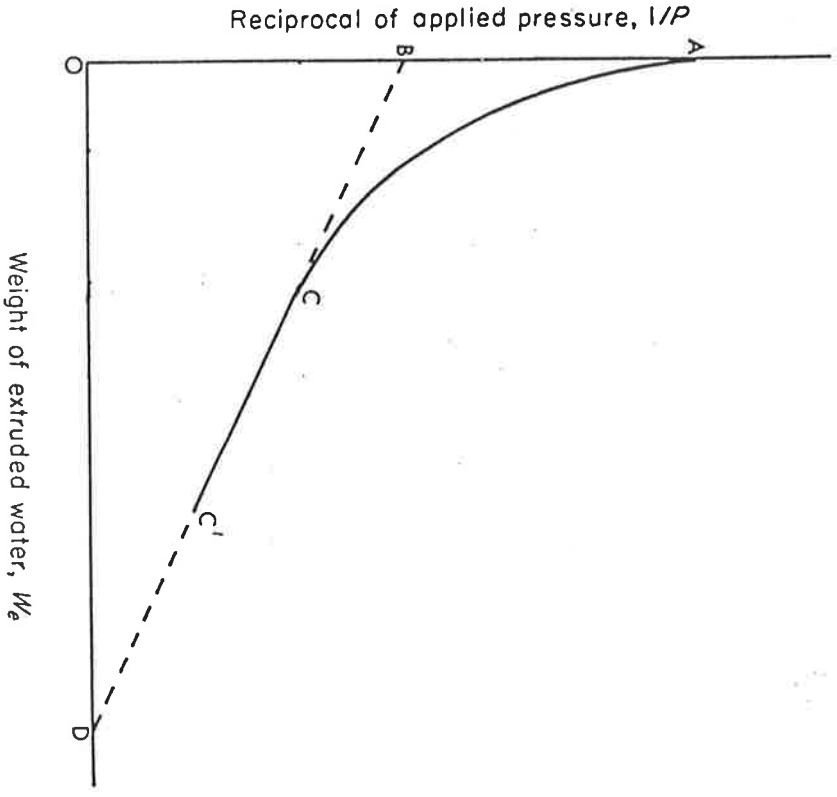
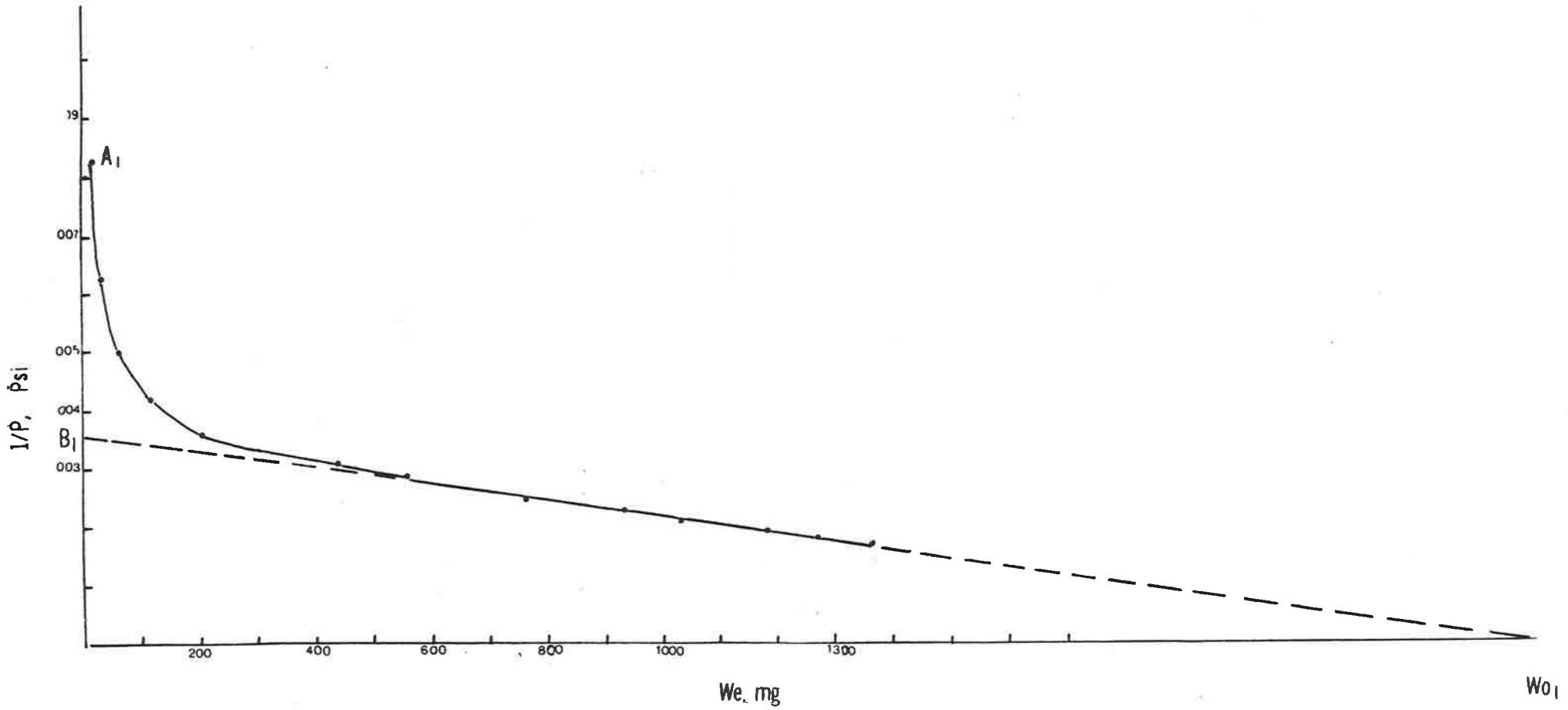

Reciprocal of applied pressure, 1/P
O
B
A
C
C'
D
Weight of extruded water, We

Figure 2.3

An example of an incomplete P-V curve. A_1 is reciprocal initial water potential, while B_1 is reciprocal initial osmotic potential. This Figure was taken from a well-watered *A.leiophylla* at November 30th, 1986. See explanation in text and Figure 2.2.

Note that since the tissue was not rehydrated before the P-V curve construction, the point (A_1) indicates the reciprocal of the initial non-zero water potential, a value less than infinity.


A1
B1
1/P, Psi
.009
.007
.005
.004
.003
200
400
600
800
1000
1300
We. mg
Wo1

thus the wall pressure is at maximum. This Ψ would ideally be zero. In Figure 2.2, point A represents the reciprocal of this Ψ_0 value.

(a_1). *Initial water potential* (Ψ_i), corresponds to the value at point A_1, but as the sample was not rehydrated previously, the value (particularly for field grown plants) usually does not reach zero, and may even be very negative depending on the level of stress and species.

(b). *Osmotic potential at full turgor* ($\Psi_{\pi 0}$), is the osmotic potential generated by solute concentration of cells after rehydration. This value is achieved by extrapolating the straight line part of the curve to meet the reciprocal pressure axis at B.

(b_1). *Initial osmotic potential* ($\Psi_{\pi 1}$) *of the initial symplastic water*, is the osmotic potential generated by the solute concentration of the symplastic water volume at the time when the sample was collected and sealed in to the chamber.

The value achieved at point B_1, represents the osmotic potential of tissues without rehydration. The point B_1 may be similar to B, however alterations in water relations occur when the sample is rehydrated. The rehydration will dilute the symplastic water, so that the osmotic potential of a rehydrated sample will be less negative.

(c). In Figure 2.2, extrapolating the C-C' line (which is experimentally determined) until it reaches the x-axis at D ($1/P = 0$), defines O-D (Wo), the total weight of symplastic water within the cells' semipermiable membranes (cytoplasm and vacuole) when the cells reached fully turgor. This value corresponds to the symplastic water volume at full turgor.

(c_1). In Figure 2.3, *the total initial symplastic water content* (W_{oi}) corresponds to W_o, but will be smaller, because in this case the tissue has not been rehydrated.

(d and d_1). The total water content is the possible amount of water that can be obtained from the whole tissue by weighing and drying. In this case, the value for the

rehydration tissue will be bigger than that for the non-rehydrated tissue for the same reason as for the symplastic water contents.

(e and e_1). *The volume of apoplastic (bound) water*. The apoplastic water content, considered as water which occupies the cell wall (Slavik, 1974; Kramer, 1983), or all water not in the symplastic volume including water in xylem vessel lumens, tracheids and fibres (Tyree and Jarvis, 1982), generated by 'intercept method' (Richter et al, 1980) is obtained using equation:

$$W_{ap} = (FW_{tissue} - DW_{tissue}) - W_o \quad \text{.....(2)}$$

where W_{ap} is the weight of apoplastic water and FW and DW are fresh weight and dry weight of the tissue respectively.

In this case, values of W_{ap} should be the same for rehydrated or non-rehydrated tissues since the apoplastic water is tightly bounded by cell wall structure. However, in some cases, the water content of the apoplasm may decrease, especially in very stressed samples to balance water stress in the symplasm (Tyree and Jarvis, 1982), therefore the value of apoplastic water may change depending on the course of water distribution between these two cell components.

However, it is still difficult to determine whether apoplastic and interspace water will or will not interfere and be mixed with symplastic water, if the applied pressure became very high. As pointed out by Bowling (1976), water occupies spaces within the cell wall, where the spaces are bounded by microfibrils. Whether or not high pressure will damage the microfibrils or cell membranes, thus allowing symplastic water to upset the apoplastic water readings, is not known yet. Richter et al (1980) emphasized the importance of membrane integrity when estimating the apoplastic water. (*Note: apoplastic water will be referred to in Chapter 6 as well*).

In very stressed non-hydrated samples it was found that the shape of the P-V curve became linear, indicating that there was no contribution of pressure potential to water potential, thus the turgor loss point had already been passed. Two examples are presented in Figure 2.4 in comparison with well-watered treatments.

The values of osmotic potential derived from P-V curves from non-rehydrated tissue will be more negative as the samples' water potential decreases during a drying cycle. This decrease may simply be due to the concentration of symplastic solution as the cells lose water, or it may have a component of osmotic adjustment as well. The way in which osmotic adjustment was deduced from such P-V curves is explained in Chapter 3.

2.4.1.3. *Limitation*

The pressure chamber technique nowadays is used in routine study of plant water relations (Losch, 1986). However, it has some limitations. As water may be cavitated within the xylem especially on stressed plants (Milburn, 1966, 1973a, 1973b), balance pressure may be overestimated when determining Ψ (West and Gaff, 1976; Hardegree, 1989; Tyree and Sperry, 1989), or during P-V curve construction.

In addition, P-V curve construction is a time consuming technique (Tyree and Jarvis, 1982). Thus in a comparative study which involves a large number of living samples variation may be introduced due to the time lapse between the first and the last samples. Also, the pressure chamber cannot be used with very young or small living samples such as seedlings.

However, pressure chamber acceptance is probably due to the fact that it is simple and robust and can generate various values in addition to plant water potential.

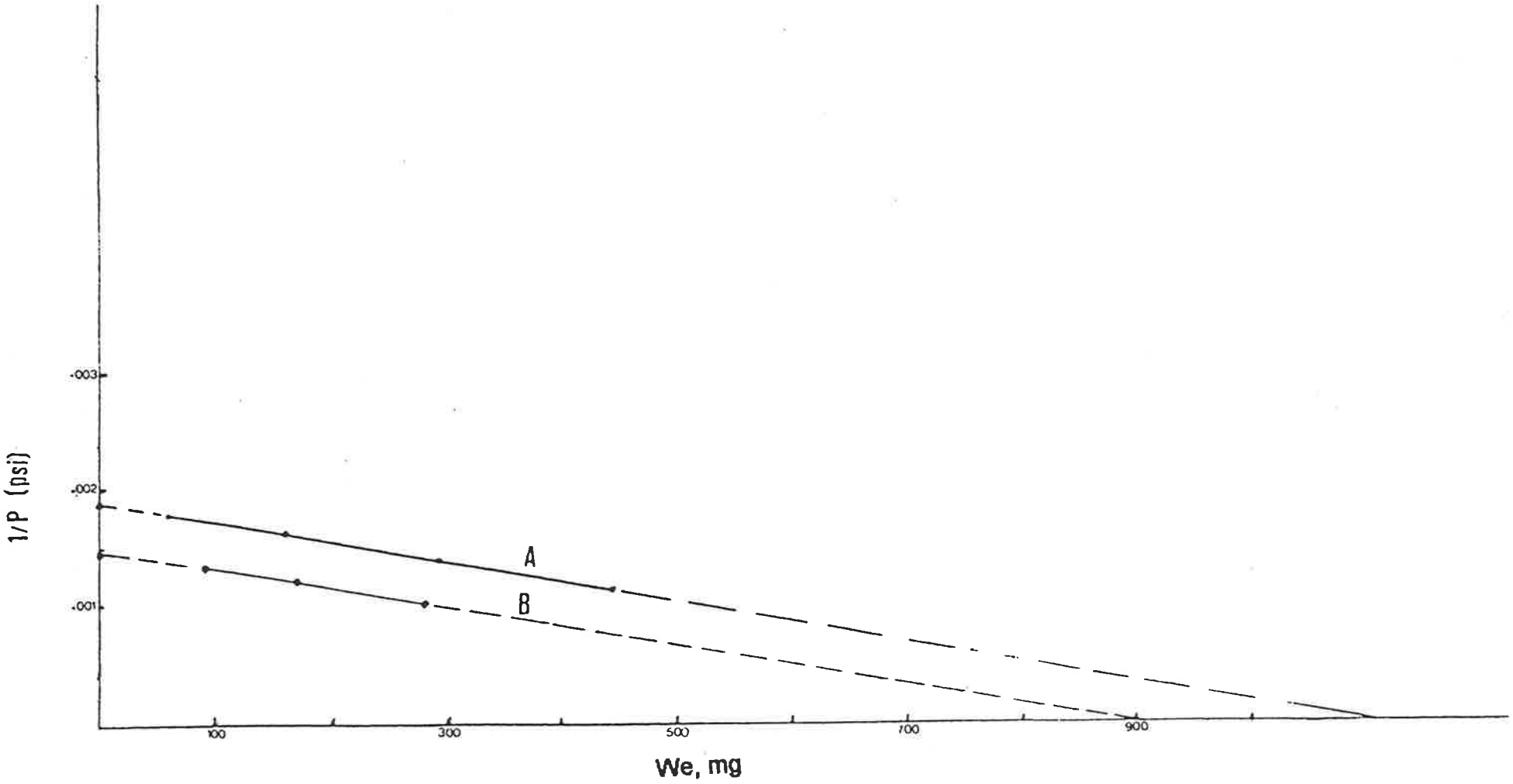
Figure 2.4.*a*.

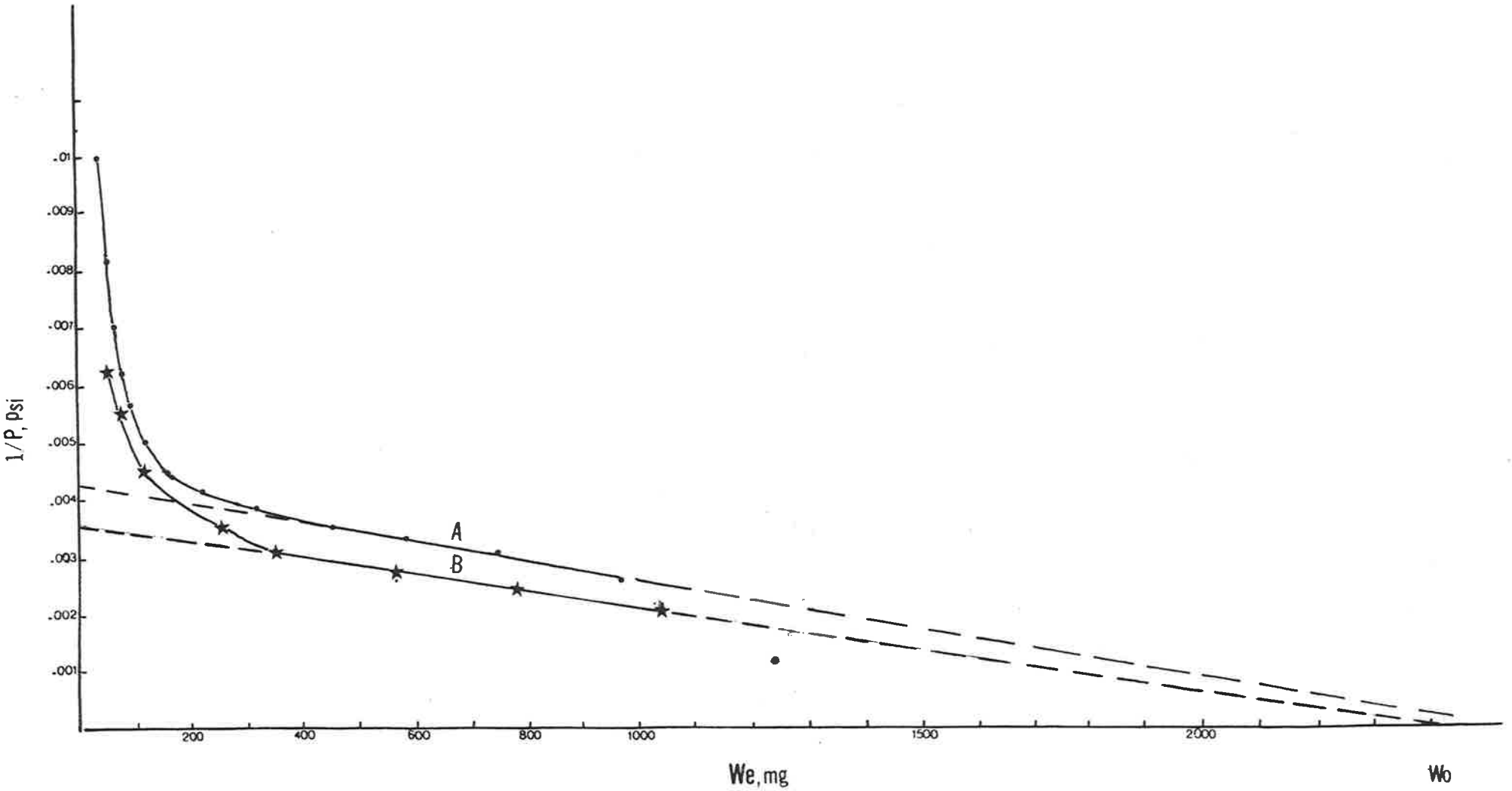
Two P-V curves generated with the pressure chamber on 20 November 1986 obtained from two replicates (A and B) of severely stressed samples of *A.gillii* during the drying cycle of pot grown seedlings. It is clear that since turgor loss had already been reached, the two lines obtained were without the non-linear parts.

Figure 2.4.*b*.

Two P-V curves generated from the well-watered treatment of a pot grown seedling of the same species (*A.gillii*) as in Figure 2.4*a*. The non-linear part show the effect of initial turgor pressure.

Note that all cases the phyllodes were not rehydrated


1/P (psi)
.003
.002
.001
A
B
100
300
500
700
900
We, mg


.01
.009
.008
.007
.006
.005
.004
.003
.002
.001
1/P, psi
A
B
200
400
600
800
1000
1500
2000
We, mg
Wo

2.4.2. *Thermocouple psychrometer*

2.4.2.1. *The principles and technique*

The theoretical background to the thermocouple psychrometer is given here. Experimental details are given in Section 4.3.2.

The thermocouple psychrometer may be used to measure water potential in plants or soil, and osmotic potential in plants or liquids. The principle (introduced by Spanner in 1951), is to measure the vapour phase relative humidity or relative vapour pressure in equilibrium with the sample, from which water potential can be obtained (Rawlins, 1972; Zanstra and Hagenzieker, 1977; Turner, 1981).

The relationship between vapour pressure and water potential at any given temperature is expressed by Kelvin equation:

$$\Psi = \frac{RT}{V} \ln \frac{e}{e_o} \quad \ldots\ldots\ldots\ldots \quad (3)$$

where V is the partial molar volume of water, e is the vapour pressure of the solution or sample and (e_o) is the vapour pressure of pure water at ambient (atmospheric) pressure.

In this *Acacia* study the Wescor HR33T Dewpoint Microvoltmeter was used with C52 sample chambers. The basic procedure begins with the sample being loaded into the sample holder and sample chamber. A certain length of time (depending on the level of stress, hence, the water potential of samples), is required to obtain sufficient thermal and vapour equilibration.

The sample chamber contains a thermocouple junction, made from chromel and constantan wire. Current is passed through the thermocouple to cool (by Peltier effect) the measuring junction to a temperature below dew point. Pure water vapour which has accumulated around the chamber is condensed upon the junction (Spanner, 1951).

After the liquid water film builds up on the junction, the current is switched off and on intermittently, maintaining the junction at dew point as the water film evaporates back to the surrounding chamber. This dew point temperature is governed by the surrounding relative humidity (RH) and temperature. The difference between the junction temperature before and after the cooling process is a function of the relative humidity which in turn is a function of water potential of the sample.

To generate values from samples, a calibration curve is needed. Usually, KCl or NaCl solutions of known osmotic potential are used as a calibration solution, i.e. 0.1, 0.3, 0.5, 0.8 and 1.0 molal (Wiebe et al, 1971).

The value of osmotic potential equals water potential, according to equation:

$$\Psi = \Psi_\pi + \Psi_p \quad \ldots\ldots\ldots\ldots \quad (4)$$

where Ψ is water potential, Ψ_π is osmotic potential and Ψ_p is turgor pressure (Sutcliffe, 1979; Kramer, 1983), if turgor pressure has been brought to zero by freezing (killing) the tissues or cells (Brown, 1972; Grange, 1983). Assuming that the freezing or killing does not change the osmotic potential of the sample, freezing is a better technique for eliminating turgor pressure than others such as ether vapor, heating or immersing enclosed tissues in boiling water (Ehlig, 1962; Gavande and Taylor, 1967; Brown, 1972).

2.4.2.2. *Limitation*

The advantages of using the thermocouple psychrometer for plant samples is that the result is obtained almost directly. Samples can be measured in the form of liquid or solid. For example leaf samples can be loaded as discs or cuttings of leaf blades or ground to obtain sap.

However, in this approach a dilution factor is involved, and as a result, apparent negative turgor pressure may be generated (Tyree, 1976b; Wenkert, 1980; Grange, 1983; Kramer, 1983). (See Section 4.4.1).

Another disadvantage comes from the fact that the thermocouple junction is sensitive to changing temperature (Wiebe et al, 1971; Turner, 1981; Turner et al, 1984), since it is a temperature sensing instrument. Hence, ambient temperature stability is the main critical factor for this instrument. For plant tissues, heat may also be generated from the respiration of the living cells during the equilibration period (Barrs, 1968).

A small temperature fluctuation might interfere with the result since the apparatus should measure the temperature differences down to 0.001^0 C.

Errors also can arise as a result of high resistance of samples to water vapour exchange caused by humidity depletion by sample in the chamber (Shackel, 1984). Abrasion of the surface of a solid sample such as a leaf disc is suggested to maximize water vapour exchange.

The production of solutes might be affected by freezing and thawing. Grange (1983) found that the osmotic potential of frozen and thawed samples, was more negative than for non treated samples measured with the pressure chamber. He suggested that this discrepancy is probably related to starch hydrolysis. As stated by Richter et al (1980), some simplifying assumptions are involved in the thermocouple psychrometer approach when used for plant samples, such as ignoring the presence of solutes in the apoplastic water, changes in vacuolar solute content and enzymatic degradation when the cell wall is mixed with the protoplast.

A detailed explanation of the calibration of the Wescor HR33T Microvoltmeter, precautions taken to minimize the effect of ambient temperature fluctuations, and the problem of the dilution factor when measuring tissue osmotic potential are presented in Chapter 4.

CHAPTER 3

OSMOTIC ADJUSTMENT IN POT GROWN SEEDLINGS

3.1. *Introduction*

Starting from the question, "why do some Australian *Acacia* species have the ability to withstand very harsh arid zone conditions?", an experiment was set up to test whether osmotic adjustment plays a role in their successful tolerance of water stress.

As a consequence of their wide distribution in Australia, the Australian acacias as xeromorphic plants may have a big range in the degree of osmotic adjustment they can produce. Some previous and recent studies have supported this prediction in other taxa, even at intraspecific level. For example genotypic study in wheat by Morgan (1977; 1980), rice (Steponkus et al, 1982), eucalypt (Myers and Neales, 1986; Lemcoff et al, 1994), sorghum (Basnayake et al, 1993), sorghum and millet (Blum and Sullivan 1986), *Brassica* (Kumar et al, 1984; Kumar and Elston, 1992) and maize (Premachandra et al, 1992), sunflower (Chimenti and Hall, 1994), oak (Kim and Kim, 1994) and pine (Anderson and Helms, 1994).

The experiment described in this chapter was carried out in a glasshouse, using potted seedlings of ten *Acacia* species. The purpose of this pot experiment was to study osmotic adjustment within the *Acacia* species. There is little available information regarding this subject in Australian *Acacia* especially native South Australia species. The points of interest were:

1. *Are there different degrees of osmotic adjustment in different species?*

2. *Are these differences related to distribution patterns of the species?*

The pot experiment results were used as initial information which led to a wider and deeper study of water relations under field conditions.

3.2. *Materials and methods*

3.2.1. *Samples preparation and design*

In order to limit the very wide possible choice of plant material to be used in this experiment, the seedlings were selected only from South Australian species except one, *A.saligna*. Ten species were selected as shown in Table 3.1 (data from Whibley, 1980). Species were chosen to represent wet, medium and dry areas of distribution.

About eight month old seedlings were used, which had been grown in tubes (approx. 10 cm high, 5 cm in diameter) by The Black Hill Native Flora Centre (BHNFC), owned by the South Australian State Government. A mixed native sand and peat (3:1) was used as growth medium by the BHNFC. The seedlings were transplanted to larger pots (approx. 15 cm in height, 15 cm in diameter at the upper end and 10 cm at the lower end) with holes in the bottom. The same medium, obtained from the BHNFC nursery was used. *See Plate 1.*

The experiment was carried out in an unregulated glasshouse with temperature fluctuations of 28.7^0 maximum and 8.6^0 C minimum. The relative humidity reached 83 and 25.6 percent maximum and minimum respectively.

Some time after transplanting, six pots for each species were chosen by selecting from the original 10 pots for a uniform group of six. Using a table of random permutation (Cochran and Cox, 1960), three pots were randomly chosen to be well-watered and the three others to be the stressed treatment, i.e. to be subjected to a drying cycle. The experiment was set up as a 2x10 factorial design in 3 randomized blocks, that is, 10 species x 2 treatments x 3 blocks (replicates). Pots were arranged randomly on the glasshouse bench. All the sixty pots were watered during the first three months from the randomization date (July 23^{rd}, 1986). to allow acclimatization to glasshouse conditions.

After that, the treatments were applied. The well-watered pots were kept watered, while water was withheld from the stressed group, thus beginning the drying cycle. All

Table 3.1

The selected 10 *Acacia* species used in the experiment: general site of distribution, and the average rainfall of the area of distribution.*

No. Name	Area of distribution	Average rainfall	Status
1. *A. anceps*	South Aust.: Nullarbor Eyre and Yorke Peninsula	200-500 mm	endemic to SA
2. *A.aneura*	wide distrib. S.A, Qld,NSW, N.T	150-250	"pan australia"
3. *A.cyclops*	South Aust.: Eyre Penin N.Lofty, S.E Kangaroo I	200-500	endemic to S.A
4. *A.gillii*	South Aust.: Eyre Penin.	500	endemic to S.A
5. *A.iteaphylla*	South Aust.: Gairdner-Torrens, Flinders Ranges, Eyre Pen., N.Lofty	200-500	endemic to S.A
6. *A.leiophylla*	South Aust.: Yorke Pen.,S.E Kangaroo I.	500-800	endemic to S.A
7. *A.longifolia*	S.A,Qld,NSW, Vic., Tas.	500-1000	
8. *A.myrtifolia*	all Australia except N.T	500-1200	
9. *A.rivalis*	South Aust.: Flinders Ranges	150-300	endemic to S.A
10. *A.saligna*	W.A, introduced to other states	300-700	endemic to W.A

* From Whibley (1980)

Plate 1. Potted acacias used in the first glasshouse study.

species were not stressed simultaneously, as there would not have been time to take all the readings required including water potential, osmotic potential and soil water content. Instead, drying cycles were begun for one or two species at a time, while the others were kept watered. The reading of these parameters from each species, was usually done every one or two days, say the first, third and fifth day of the week, while another species was measured at the second, fourth and sixth day. The frequency and dates (1986) of harvests for water potential and osmotic potential are presented in Table 3.2.

Readings were taken on 3 pots every day, starting at about 7 a.m. While measurements were made on the first pot, the two other pots that would be recorded on the same day were placed underneath the tables (bench) in shade to avoid big changes in their water status. To complete a drying cycle for each species took about 5 to 10 days. By the end of the stress cycle, the soil water content had declined to between 2 and 4%.

The well-watered (control) plant readings for the same parameters were done at the end of stress treatment for each species.

3.2.2. *Measurement of water potential*

Water potentials were measured with the pressure chamber. A single phyllode or shoot sample was taken from the area two thirds of the distance down the stem from the apex. It was suspected that in this region, the phyllodes would be adequately mature (fully expanded but not senescent). Also in this seedling stage, the vertical gradient of water relations would not be great, as the height of seedlings used ranged between 30 and 80 cm.

To derive water potential, a single shoot or phyllode was selected and sealed into the pressure chamber. Tissue paper, saturated with water, was placed in the chamber to reduce the loss of water via transpiration during the P-V curve construction (Sinclair and Venables, 1983).

Table 3.2

The frequency and dates (1986) of harvesting for P-V curves of the pot grown seedlings of the ten *Acacia* species during drying cycles.

OCTOBER

A.cyclops:	8, 9, 10, 11, 12, 13,
A.longifolia:	15, 16, 17, 18, 19
A.anceps:	21, 23, 25, 27, 29, 31
A.aneura:	22, 24, 26, 28, 30

NOVEMBER

A.saligna:	3, 4, 5, 6, 7
A.iteaphylla:	8, 10,12
A.gillii:	15, 17, 19, 20
A.myrtifolia:	9, 11, 13, 14
A.rivalis:	21, 23, 25, 27
A.leiophylla:	24, 26, 28, 30

The pressure was then applied slowly until the sap appeared on the cut end of the sample. Water potential was recorded i.e. the value of gas pressure at which the sap first appeared on the surface of the sample's cut end (Boyer, 1967; Hellkvist et al, 1974; Ritchie and Hinckley, 1975). This was observed by a hand lens.

No measurements were made of the osmotic potential of the xylem sap, hence the effect of the presence of solute in the xylem sap could not be calculated. It was assumed that the pressure chamber values which are really the measurement of negative pressure potential of the xylem sap, were equal to water potential of the tissue (please refer to Section 2.4.1.1).

3.2.3. *Measurement of osmotic potential*

The same leaf or shoot sample which was used for water potential determination, was also used to build up a P-V curve. The procedure to build this curve followed steps as explained in Section 2.4.1.2.

The line to generate reciprocal osmotic potential was drawn by eye. This may generate errors due to the "subjective" decision as to which of the points should be included in one straight line.

To search the range of possible errors from those P-V curve constructions, 10 curves were chosen from the whole packet of P-V data, which represent both well-watered and stress treatments.The actual line shown was plotted by eye through the points considered to lie on the straight line (see Appendix 4a, Figure 1 to 10). Linear regressions were also calculated for the same points in each of the ten P-V curves. Good agreement was found between the two methods. (See Appendix 4b for Results and Explanation).

The P-V curves were derived from fresh tissue without previous rehydration (cf. Wenkert et al, 1977; Roberts et al, 1980; Nilsen et al, 1983; Meinzer et al, 1988) as the actual osmotic potential at the time of harvesting was required, not the osmotic potential at

full turgor. The samples ranged from well-watered to extremely stressed; it was thought that perhaps a number of cells might have died due to the very stressed treatment. As a consequence, the uptake of water during rehydration of this stressed tissue may have differed from uptake solely by the living cells, which may have distorted the P-V curves. Also, the complete rehydration of a sample, especially a very stressed one might have needed two to three days. Thus lack of time was another reason for not rehydrating phyllodes.

The validity of a non-rehydrated P-V curve depends on the purpose of the measurement. In this pot experiment, the values needed from the pressure chamber were initial water potential and initial osmotic potential. In fact, the method used to construct P-V curves from *non-rehydrated* tissues to generate initial osmotic potential values produced good results for the purpose of this study. As will be shown in Section 3.3, these measurements of water potential and osmotic potential in non-rehydrated tissue can be analyzed to show whether or not osmotic adjustment had occurred.

Each day, three standard P-V curveswere built up. At least three hours was needed to complete all of these, or longer depending on the level of stress. The whole P-V curves construction took about one and a half months.

3.2.4. *Measurement of soil water content*

Soil water content was measured following each water potential and osmotic potential reading. The soil sample was collected with a cork borer, weighed, placed in an oven at approximately 80^0 C for 48 hours, then reweighed. This length of time was tested before-hand by weighing and drying until the samples reached constant weight. It was found that this time lapse was suitable for the achievement the constant weight. Water content was calculated as:

$$WC_{soil} = \frac{FW_{soil} - DW_{soil}}{DW_{soil}} \times 100\% \quad \text{.....(5)}$$

where FW and DW refer to fresh weight and dry weight of soil respectively. The soil which had been collected for water content estimation, was replaced in the pots after weighing.

3.3. *Analysis of data*

At the end of stress treatment (where soil water content had reached about 2 to 4 %), the values of water potential and osmotic potential were analyzed by comparing with the same variables measured on the well-watered (control) treatment. This comparison showed the differences between species in developing their osmotic potential following the fall of water potential. The relationship between the two variables (water potential and osmotic potential) during the course of the drying treatment can reveal the status of osmotic adjustment among species.

When osmotic adjustment occurs, it is expected that the osmotic potential decreases more rapidly than water potential and remains slightly below water potential in order to maintain positive turgor (Morgan, 1977; Avecedo et al, 1979; Turner and Jones, 1980).

If water potential is plotted against osmotic potential as stress increases, the two will draw closer together as turgor pressure decreases. In the absence of osmotic adjustment, a point will be reached when water potential equals osmotic potential, and beyond that point the two will always be the same, hence the plotted points will lie on the 1:1 line (See Figure 3.1, Curve a). However, if significant osmotic adjustment takes place, values of osmotic potential will be more negative than in the first case, and the curve will resemble Curve b in Figure 3.1, where points remain significantly below the 1:1 line.

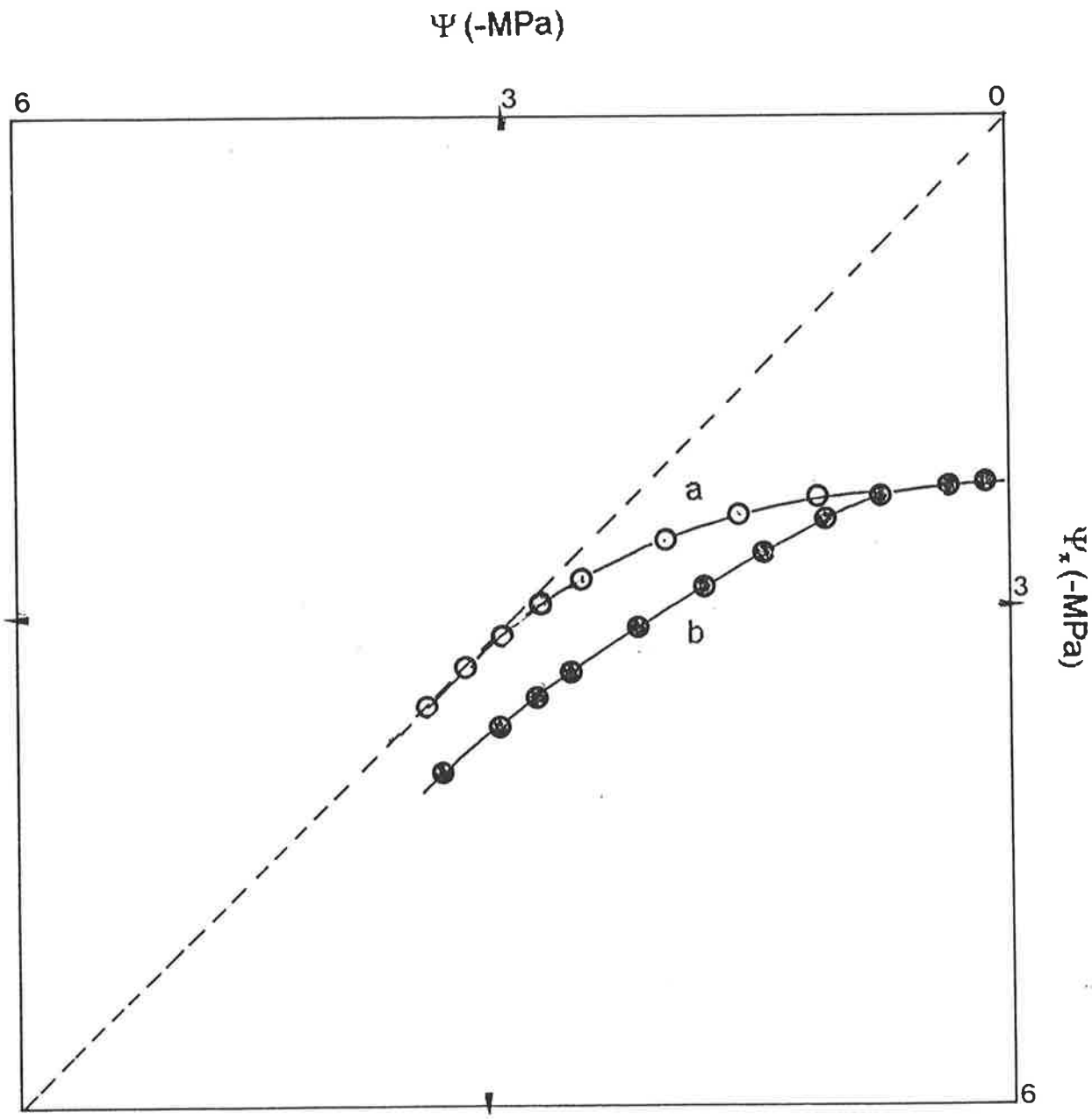
If a suitable empirical curve can be fitted to the experimental points, then it is possible to test whether this curve is statistically significantly different from the 1:1 line. If it is , then it can be said that significant osmotic adjustment has occurred. This treatment assumes that there has been no change in the bulk modulus of elasticity of the cells during stress. Such a change would be a complicating factor, which was not considered in this study. See comment, p13a. The method is a development from that used by Morgan (1980).

Figure 3.1

Schematic form of the relationship between water potential and osmotic potential, when a plant is exposed to water stress conditions and osmotic adjustment occurs.

Curve a, --- --- : non-adjustment line i.e. when osmotic adjustment is absent

Curve b, --- --- : adjustment line, i.e. when osmotic adjustment has occurred.


Ψ (-MPa)
6
3
0
3
6
a
b
Ψ_t (-MPa)

In this study, an exponential function was used to fit the data points. Although this did not give a realistic fit to the data at high water potential (well-watered conditions), it fitted the linear part at low water potential well (Which is the main area of interest) and could be simply used to test whether, in this region, the curve differed significantly from the 1:1 line.

The assumed model is:

$$\Psi_p = \beta_0 + \beta_1 . e^{\gamma\Psi} \quad \text{.....(6)}$$

where Ψ_p refers to turgor pressure, Ψ is water potential, β_0 is the level where Ψ_p reached steady state, $\beta_0 + \beta_1$ is the initial turgor pressure and γ is a constant, whose value is determined by the regression.

However, in order to express the relationship between water potential and osmotic potential (cf. Figure 3.3), some rearrangement has been done.

Since

$$\Psi = \Psi_\pi + \Psi_p, \quad \text{(see Equation 4),}$$

then $\Psi_p = \Psi - \Psi_\pi,$

thus Equation (6) is rewritten as

$$\Psi - \Psi_\pi = \beta_0 + \beta_1 e^{\gamma\Psi},$$

therefore

$$\Psi_\pi = \Psi - \beta_0 - \beta_1 e^{\gamma\Psi}$$

The approximately linear part represents the values of Ψ_π under stress. Osmotic adjustment (i.e. the process of maintaining turgor pressure) is taking place if the linear part of the curve remain separated from the 1:1 line. For such a condition to be true, β_0 has to be greater than zero. For the linear part to be statistically different from 1:1 line β_0 must be at least twice its standard error.

This curve fitting was done by Mr.Ian Lundy, Department of Statistics, The University of Adelaide.

3.4. *Results*

3.4.1. *Water potential and osmotic potential*

Table 3.3 provides the original individual values of water potential and osmotic potential from the 10 *Acacia* species during the time course of the drying cycles.

The mean initial water potential value of the ten species was -0.54 MPa and osmotic potential was -1.83 MPa; after stress, the average water potential was -4.34 MPa and osmotic potential registered at -5.24 MPa. The average values of water potential and osmotic potential of well-watered plants and those stressed plants at the end of a drying cycle (i.e. when the soil water content had declined to between 2 and 4%) are presented in Table 3.4.

The summary of these values is also expressed as a histogram in Figure 3.2. The water potential of the well-watered treatment (left hand side) shows that the highest (least negative) was *A.cyclops* and the lowest was *A. rivalis*, i.e. -0.36 MPa and -0.83 MPa respectively. However, after stress *A.saligna* had the highest water potential (-2.61 MPa) and *A. rivalis* the lowest (-6.44 MPa). This pattern was similar in the osmotic potential values (right hand side). Within well-watered treatments there was little variation. They ranged between -1.54 MPa (*A.iteaphylla*) and the most negative, -1.98 MPa (*A.anceps*, *A.cyclops* and *A.leiophylla*). After stress, the values scattered as shown, where *A.saligna* was the highest -3.63 MPa, while *A.rivalis* was the lowest, -6.38 MPa.

At the end of the stress period, large differences in water potential and osmotic potential had developed between species (see Table 3.4). Table 3.5.a shows the anova for the ten species before and after stress. For water potential, there were very significant differences

Table 3.3

The original data of water potential (Ψ, -MPa) and osmotic potential (Ψ_π, -MPa) generated from the 10 species of *Acacia* during the drying cycles in the glass house. Right hand side column shows the well-watered (control) values.

R = Replication, TRT= treatments, WW = Well-Watered (control)

Species/ TRT	R	Values from drying cycles						WW (control)
A.anceps								
	1	1.89	1.31	1.55	1.76	2.03	4.14	0.72
Ψ	2	1.93	1.45	1.55	1.93	1.93	3.10	0.62
	3	1.03	0.90	1.59	2.90	2.62	3.62	0.62
	1	2.26	1.95	2.08	2.45	3.38	5.39	1.68
Ψ_π	2	2.21	2.50	2.13	2.61	3.57	5.15	2.03
	3	2.48	2.33	2.50	3.38	4.15	5.75	2.22
A. aneura								
	1	1.24	1.86	1.45	1.83	3.69		0.41
Ψ	2	1.31	1.69	1.76	1.21	3.45		0.69
	3	1.45	1.93	1.52	2.00	2.34		1.07
	1	1.98	2.09	2.09	2.45	4.93		1.88
Ψ_π	2	1.94	1.98	1.98	2.80	5.47		1.89
	3	2.09	1.86	2.09	3.36	4.86		1.93
A. cyclops								
	1	1.41	1.79	1.83	1.10	1.79	1.72	3.62	0.31
Ψ	2	1.24	1.65	1.69	1.03	1.07	1.83	3.79	0.35
	3	1.31	1.10	1.76	0.93	1.79	1.45	4.21	0.41
	1	2.10	2.11	2.39	2.59	2.99	3.08	4.79	2.03
Ψ_π	2	1.93	1.98	2.53	2.04	2.65	3.13	4.18	1.91
	3	2.10	2.10	2.53	2.15	2.68	2.77	4.06	1.99
A. gillii								
	1	1.34	1.34	2.97	4.65				0.41
Ψ	2	1.28	1.79	3.87	5.16				0.38
	3	1.31	1.41	2.93	3.59				0.45
	1	1.68	1.73	3.13	4.63				1.80
Ψ_π	2	1.78	2.17	4.08	6.16				1.67
	3	1.96	1.85	3.65	3.65				1.94
A. iteaphylla								
	1	0.97	2.34	4.83					1.07
Ψ	2	1.00	2.21	5.17					0.41
	3	0.97	2.21	5.69					0.45
	1	2.03	2.87	5.56					1.65
Ψ_π	2	1.93	2.45	4.99					1.48
	3	1.95	2.59	5.70					1.49

continued.....

A. leiophylla							
		1	1.48	1.52	3.14	4.48	0.48
	Ψ	2	0.34	0.96	2.48	4.48	0.48
		3	0.55	2.17	3.14	4.65	0.38
		1	1.69	1.89	3.81	5.61	1.96
	Ψ_π	2	1.89	1.72	2.52	4.31	1.97
		3	1.98	3.11	3.80	3.80	2.00
A. longifolia							
		1	1.38	1.72	2.10	2.76	0.31
	Ψ	2	1.55	1.90	2.31	3.76	0.48
		3	1.17	1.59	1.86	3.69	0.48
		1	1.85	2.18	2.83	3.92	1.81
	Ψ_π	2	1.86	2.24	2.92	4.34	1.64
		3	1.86	1.95	2.56	4.54	1.60
A. myrtifolia							
		1	1.34	2.14	4.65		0.46
	Ψ	2	1.52	2.24	5.10		0.55
		3	0.76	2.03	3.34		0.38
		1	2.03	2.52	5.07		0.88
	Ψ_π	2	2.10	2.65	5.39		1.83
		3	1.93	2.43	3.48		1.99
A. rivalis							
		1	1.00	2.07	3.23	5.69	0.72
	Ψ	2	1.07	0.72	2.17	7.24	0.48
		3	1.72	1.77	2.41	6.38	1.28
		1	1.97	2.28	3.67	5.39	1.93
	Ψ_π	2	2.03	2.22	2.65	7.49	1.79
		3	2.19	4.20	2.89	6.27	1.60
A.saligna							
		1	0.79	1.48	1.38	1.79	2.76	0.34
	Ψ	2	1.52	1.55	1.72	1.52	2.38	0.49
		3	0.93	1.24	1.45	1.93	2.69	0.34
		1	1.48	1.79	1.52	2.02	4.57	1.79
	Ψ_π	2	1.88	1.79	1.85	1.88	3.01	1.80
		3	1.67	1.44	1.65	2.13	3.32	1.71

Table 3.4

The average (n = 3) water potential and osmotic potential of the ten *Acacia* species grown in the glass house.

WW = Well-watered control plants
STR = stressed plants at the end of the drying cycle

Species	Water potential (-MPa)		Osmotic potential (-MPa)	
	WW	*STR*	*WW*	*STR*
A.anceps	0.65 ± 0.05	3.62 ± 0.42	1.98 ± 0.21	5.43 ± 0.25
A.aneura	0.72 ± 0.27	3.16 ± 0.59	1.90 ± 0.03	5.09 ± 0.27
A.cyclops	0.36 ± 0.05	3.87 ± 0.25	1.98 ± 0.06	4.34 ± 0.32
A.gillii	0.41 ± 0.03	4.47 ± 0.65	1.80 ± 0.13	4.81 ± 1.03
A.iteaphylla	0.64 ± 0.37	5.23 ± 0.35	1.54 ± 0.10	5.42 ± 0.31
A.leiophylla	0.45 ± 0.06	4.54 ± 0.08	1.98 ± 0.02	4.57 ± 0.76
A.longifolia	0.42 ± 0.09	3.40 ± 0.46	1.68 ± 0.11	4.27 ± 0.26
A.myrtifolia	0.46 ± 0.07	4.36 ± 0.75	1.90 ± 0.08	4.65 ± 0.84
A.rivalis	0.83 ± 0.34	6.44 ± 0.63	1.77 ± 0.16	6.38 ± 0.86
A.saligna	0.39 ± 0.06	2.61 ± 0.17	1.77 ± 0.05	3.63 ± 0.67

Figure 3.2

The mean values of water potential (left) and osmotic potential (right). Values under well-watered conditions are lightly shaded, the darker shading shows values at the end of the stress period, when soil water content had declined to 2 to 4 %.

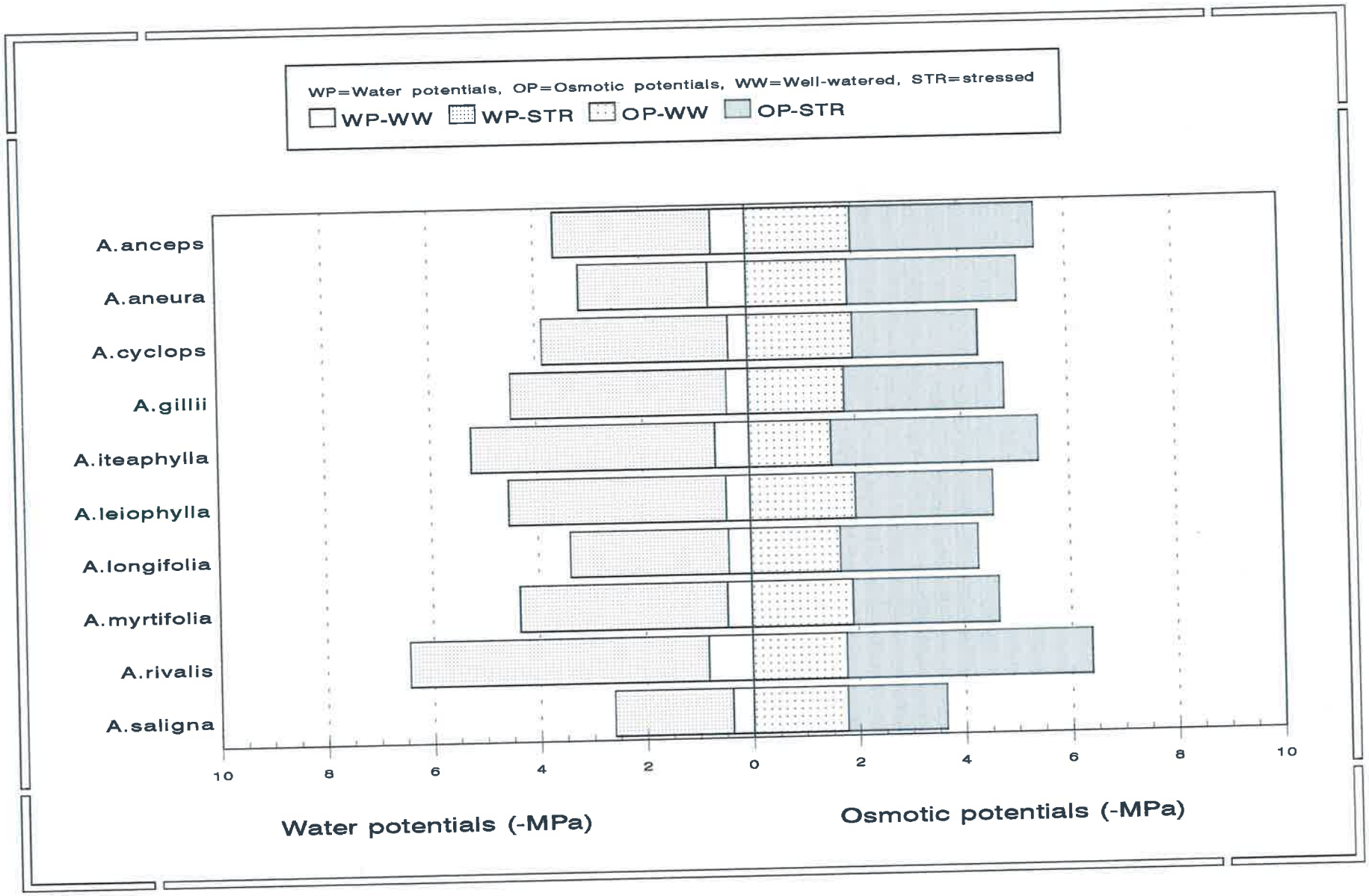

WP=Water potentials, OP=Osmotic potentials, WW=Well-watered, STR=stressed
WP-WW
WP-STR
OP-WW
OP-STR
A.anceps
A.aneura
A.cyclops
A.gillii
A.iteaphylla
A.leiophylla
A.longifolia
A.myrtifolia
A.rivalis
A.saligna
10
8
6
4
2
0
2
4
6
8
10
Water potentials (-MPa)
Osmotic potentials (-MPa)

Table 3.5.*a*

ANALYSIS OF VARIANCE TABLE
FOR WATER POTENTIAL

A = species, B = water treatment

Code	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
1	Rep	2	0.18	0.089	0.44	
2	A	9	19.20	2.133	10.47	.000
4	B	1	198.31	198.307	972.96	.000
6	AB	9	14.38	1.598	7.84	.000
-7	Error	38	7.75	0.204		

Table 3.5.*b*

ANALYSIS OF VARIANCE TABLE
FOR OSMOTIC POTENTIAL

A = species, B = water treatment

Code	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
1	Rep	2	0.67	0.336	1.11	.338
2	A	9	7.83	0.870	2.88	.010
4	B	1	137.68	137.683	456.57	.000
6	AB	9	8.52	0.946	3.14	.006
-7	Error	38	11.46	0.302		

between the control and stressed treatments, and less significant differences between species. The *LSD* test at the 5% significance level showed no significant interspecific differences of water potential in the well-watered controls, but at the end of the stress period there was an overlapping gradation of differences between species (Table 3.6.a).

The anova for osmotic potential (Table 3.5.b) revealed a significant interaction term between species and treatment. Hence no statements can be made about the significance of the two factors separately from the anova. However, the *LSD* test at 5% showed no significant differences between the osmotic potential of the well-watered plants whereas there was again an overlapping gradation of differences between species at the end of the stress periods (Table 3.6.b).

The fact that there were no significant differences among the well-watered plants confirmed that the time lapse of about two months between the first and the last of the stress treatments did not cause significant differences in water relations between species unless they were treated to drought.

As discussed in Section 3.3, water potential was plotted against osmotic potential (Figure 3.3) to show evidence for osmotic adjustment. All data taken during the drying cycles, and also the well-watered data are included. The 1:1 line indicates zero turgor. Points lying below the 1:1 line indicate positive turgor pressure, and a continuing trend of points below this line is evidence for osmotic adjustment. The significance of the trend was tested using the exponential regression in Section 3.4.3.

3.4.2. *Soil water content*

Table 3.7a contains the original data of soil water content of individual pots during the drying cycles. Table 3.7b, summarizes the average values of soil water content of well-watered and stressed pots at the end of drying cycles from the 10 *Acacia* species.

Table 3.6.*a*

The result of *lsd* test against the mean (n=3) values of *water potential* (-MPa) of pot grown seedlings of A: Well Watered, B: Stress treatment. Values with the same symbol (letters) are not significantly different.

A		B	
A.rivalis	0.83 *a*	***A.rivalis***	6.44 *a*
A.aneura	0.72 *a*	*A.* ***iteaphylla***	5.23 *b*
A.anceps	0.65 *a*	*A.* ***leiophylla***	4.54 *bc*
A.iteaphylla	0.64 *a*	***A.gillii***	4.47 *c*
A.myrtifolia	0.46 *a*	***A.myrtifolia***	4.36 *cd*
A.leiophylla	0.45 *a*	***A.cyclops***	3.87 *cde*
A.longifolia	0.42 *a*	***A.anceps***	3.62 *de*
A.gillii	0.41 *a*	***A.longifolia***	3.40 e
A.saligna	0.39 *a*	***A.aneura***	3.16 *ef*
A.cyclops	0.36 *a*	***A.saligna***	2.61 *f*

Table 3.6.*b*

The result of *lsd* test against the mean (n=3) values of *osmotic potential* (-MPa) of pot grown seedlings of A: Well Watered, B: Stress treatment of 10 *Acacia* species. Values with the same symbol (letters) are not significantly different.

A		B	
A.anceps	1.98 *a*	***A. rivalis***	6.38 *a*
A.leiophylla	1.98 *a*	***A. anceps***	5.43 *b*
A.cyclops	1.98 *a*	***A.iteaphylla***	5.42 *b*
A.myrtifolia	1.90 *a*	***A.aneura***	5.09 *bc*
A.aneura	1.90 *a*	***A.gillii***	4.81 *bc*
A.gillii	1.80 *a*	***A.myrtifolia***	4.65 *bc*
A.rivalis	1.77 *a*	***A.leiophylla***	4.57 *bc*
A.saligna	1.77 *a*	***A.cyclops***	4.34 *cd*
A.longifolia	1.68 *a*	***A.longifolia***	4.27 *cd*
A.iteaphylla	1.54 *a*	***A.saligna***	3.63 *d*

Figure 3.3

The correlation between water potential and osmotic potential during a drying cycle for the 10 *Acacia* species in the glass-house pot experiment.

The dashed line indicates a 1:1 correspondence. Units are (-MPa).

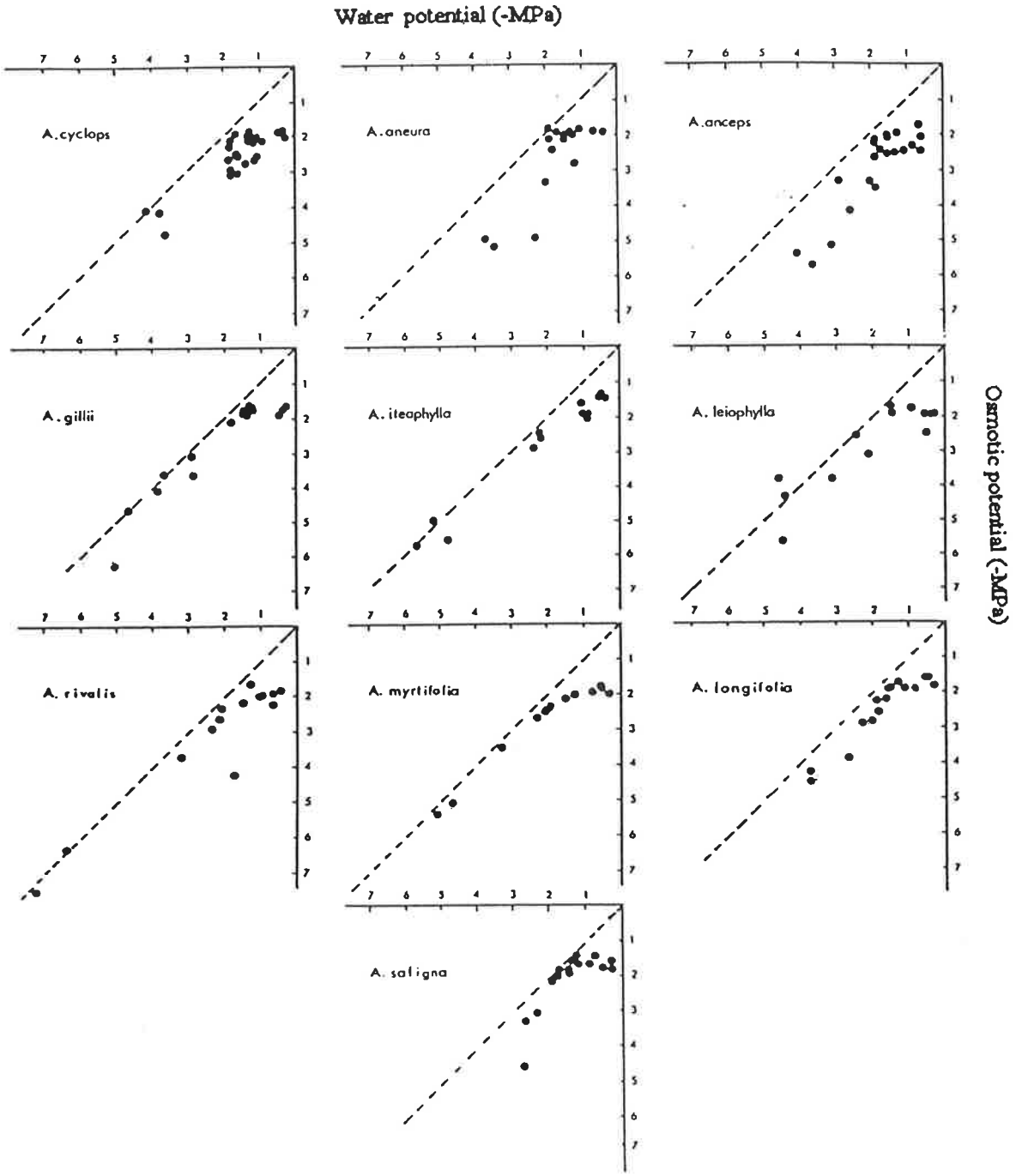

Water potential (-MPa)
Osmotic potential (-MPa)
A. cyclops
A. aneura
A. anceps
A. gillii
A. iteaphylla
A. leiophylla
A. rivalis
A. myrtifolia
A. longifolia
A. saligna

Table 3.7.*a*

The dates (1986) and values of soil water content (% DW) measurements during PV curve construction for the pot grown seedlings of ten *Acacia* species during drying cycles. Letters *a*, *b* and *c* show the replicates.

A.anceps

Date	21-10	23-10	25-10	27-10	29-10	31-10
a.	*26.52*	*15.65*	*10.11*	*5.10*	*3.12*	*2.72*
b	*20.22*	*12.86*	*10.53*	*5.40*	*3.94*	*2.64*
c.	*24.15*	*11.07*	*5.27*	*3.79*	*2.88*	*2.71*

A.aneura

Date	22-10	24-10	26-10	28-10	30-10
a.	*25.22*	*17.40*	*6.89*	*3.95*	*2.14*
b.	*24.27*	*11.64*	*6.50*	*3.91*	*2.30*
c.	*21.62*	*15.55*	*5.92*	*4.45*	*2.31*

A.cyclops

Date	8-10	9-10	10-10	11-10	12-10	13-10
a.	*23.73*	*12.22*	*5.22*	*3.57*	*3.44*	*2.28*
b.	*29.00*	*17.14*	*6.14*	*5.51*	*3.99*	*3.58*
c.	*27.06*	*16.53*	*11.44*	*5.69*	*4.02*	*3.15*

A.gillii

Date	15-11	17-11	19-11	20-11
a.	*22.65*	*14.28*	*4.74*	*3.32*
b.	*21.24*	*14.39*	*3.28*	*2.88*
c.	*22.58*	*12.95*	*4.19*	*3.40*

A.iteaphylla

Date	8-11	10-11	12-11
a.	*21.19*	*15.12*	*3.14*
b.	*23.59*	*15.30*	*3.35*
c.	*22.13*	*14.75*	*3.45*

continued

A.leiophylla

Date	24-11	26-11	28-11	30-11
a.	*25.38*	*15.44*	*3.18*	*2.13*
b.	*21.84*	*19.87*	*4.19*	*2.50*
c.	*27.84*	*13.88*	*3.03*	*2.20*

A.longifolia

Date	15-10	16-10	17-10	18-10	19-10
a.	*25.90*	*14.81*	*4.51*	*2.81*	*2.55*
b.	*25.46*	*14.98*	*6.05*	*3.82*	*2.44*
c.	*20.98*	*16.47*	*5.41*	*3.51*	*2.30*

A.myrtifolia

Date	9-11	11-11	13-11	14-11
a.	*22.00*	*15.81*	*3.86*	*2.92*
b.	*20.26*	*14.80*	*3.09*	*2.99*
c.	*20.99*	*17.38*	*4.04*	*3.67*

A.rivalis

Date	21-11	23-11	25-11	27-11
a.	*23.26*	*14.57*	*3.05*	*2.23*
b.	*21.11*	*17.47*	*3.45*	*2.19*
c.	*16.09*	*11.54*	*2.09*	*2.19*

A.saligna

Date	3-11	4-11	5-11	6-11	7-11
a.	*25.65*	*14.62*	*3.80*	*3.62*	*3.62*
b.	*22.18*	*13.78*	*3.75*	*3.43*	*3.39*
c.	*27.65*	*13.99*	*3.96*	*3.26*	*2.90*

Table 3.7.*b*

The average (n=3) values of soil water content (% DW) in the pots of ten *Acacia* species grown in glasshouse. Values are for the well-watered controls, and for the stressed plants at the end of their drying cycles.

Species	Well-watered	After stress
A. anceps	30.77 ± 1.18	2.69 ± 0.04
A. aneura	30.83 ± 1.00	2.25 ± 0.09
A. cyclops	30.04 ± 0.28	3.00 ± 0.66
A. gillii	30.32 ± 1.16	3.20 ± 0.28
A. iteaphylla	29.40 ± 0.89	3.31 ± 0.16
A. leiophylla	30.67 ± 1.40	2.30 ± 0.24
A. longifolia	28.54 ± 0.68	2.43 ± 0.13
A. myrtifolia	30.36 ± 1.27	3.19 ± 0.41
A. rivalis	30.42 ± 0.67	2.20 ± 0.02
A. saligna	30.28 ± 0.83	3.30 ± 0.37

Table 3.8.a shows the anova for soil water content at 5% confidence level. There was no significant difference between species in their mean soil water content; however, a significant difference was detected between treatments (well-watered and stressed) and significant interaction between soil water treatment and species.

The further *LSD* test at 5% level (Table 3.8b), showed a small significant difference between species in well-watered treatment, perhaps due to different sizes of leaves which affected the water lose due to transpiration. However, there were no significant differences between pots at the end of the stress periods. Hence, at the same level of soil water content at the end of stress, each species had developed its osmotic potential differently to the others as a response to the falling water potential, thus there was an opportunity to observe the differences in the species water potential and osmotic potential.

3.4.3. *Exponential regression*

Figure 3.4 shows the plots of osmotic potential against water potential for each species separately, together with the curves fitted to the data points by the exponential regressions. Table 3.9 shows the coefficients of the exponential regressions. In fact, the Table shows that only six species have fulfilled the requirement of having β_0 values at least twice their standard error. Hence, there is significant osmotic adjustment within the six species (*A.anceps*, *A.aneura*, *A.gillii*, *A.longifolia*, *A.myrtifolia* and *A.saligna*). The remaining four species revealed no significant osmotic adjustment (*A.cyclops*, *A.iteaphylla*, *A.leiophylla* and *A.rivalis*). These species are listed in Table 3.10.

3.5. *Discussion*

Plants living in dry regions where water shortages occur regularly must develop mechanisms to cope with these water shortages. Osmotic adjustment may be one of these.

Table 3.8.*a*

ANALYSIS OF VARIANCE TABLE
FOR SOIL WATER CONTENT
A = species, B = water treatment

Code	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
1	Rep	2	3.64	1.822	3.90	.028
2	A	9	8.15	0.906	1.94	.074
4	B	1	11240.31	11240.312	24090.81	.000
6	AB	9	10.98	1.220	2.61	.018
-7	Error	38	18.00	0.474		

Table 3.8.*b*

The result of *lsd* test against the mean (n=3) values of soil water content (%) of A: Well-Watered, B: stress (at the end of drying cycles) in pot grown seedlings of 10 *Acacia* species. Values with the same symbols (letters) are not significantly different.

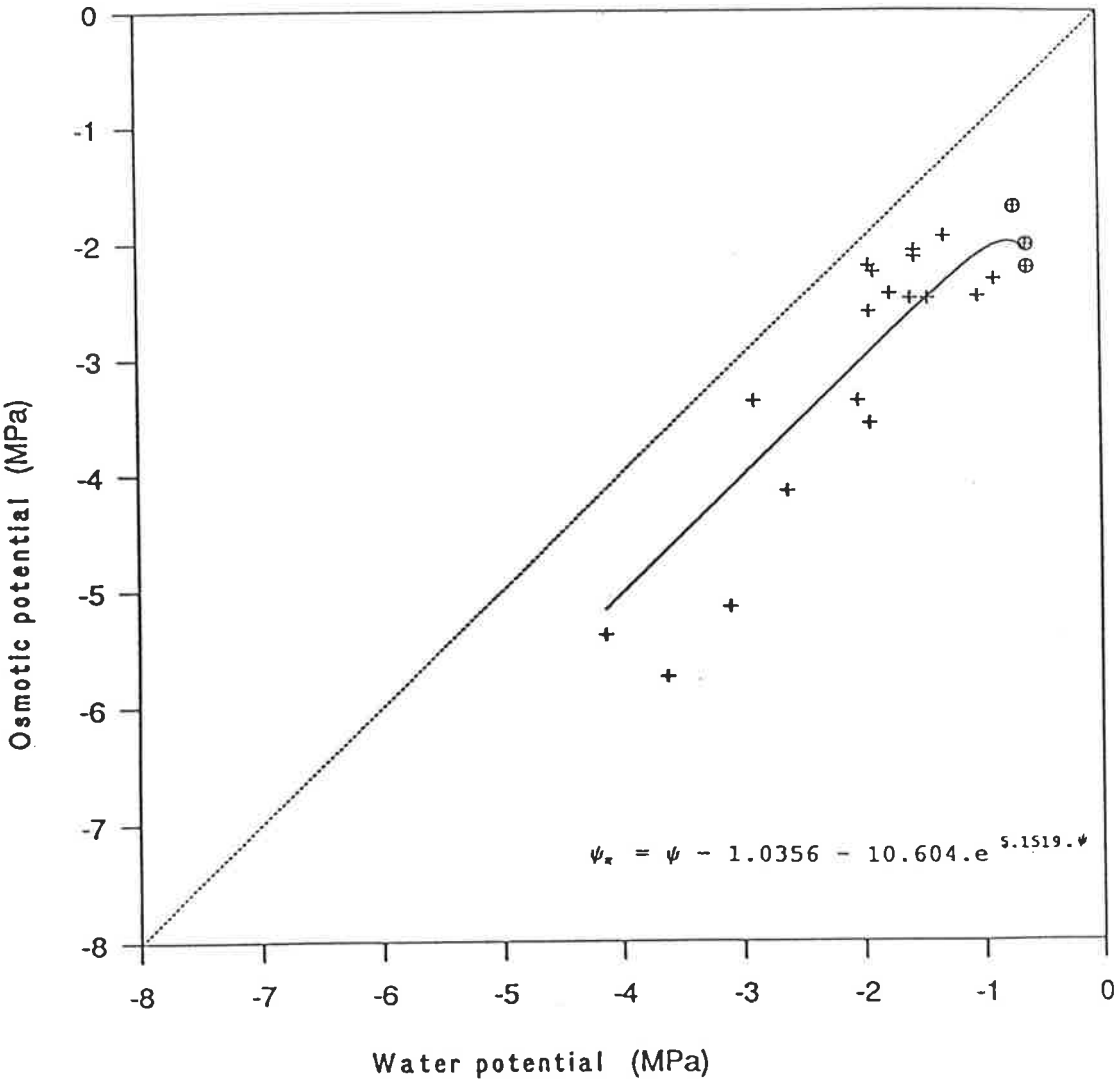
Species	A	B
A.anceps	30.77 a	2.69 a
A.aneura	30.83 a	2.25 a
A.cyclops	30.04 ab	3.00 a
A.gillii	30.32 ab	3.20 a
A.iteaphylla	29.40 bc	3.31 a
A.leiophylla	30.67 a	2.28 a
A.longifolia	28.54 c	2.43 a
A.myrtifolia	30.36 ab	3.19 a
A.rivalis	30.42 ab	2.20 a
A.saligna	30.28 ab	3.30 a

Figure 3.4
The exponential relationship between the two variables water potential and osmotic potential during drying treatment of pot grown seedlings of ten *Acacia* species.

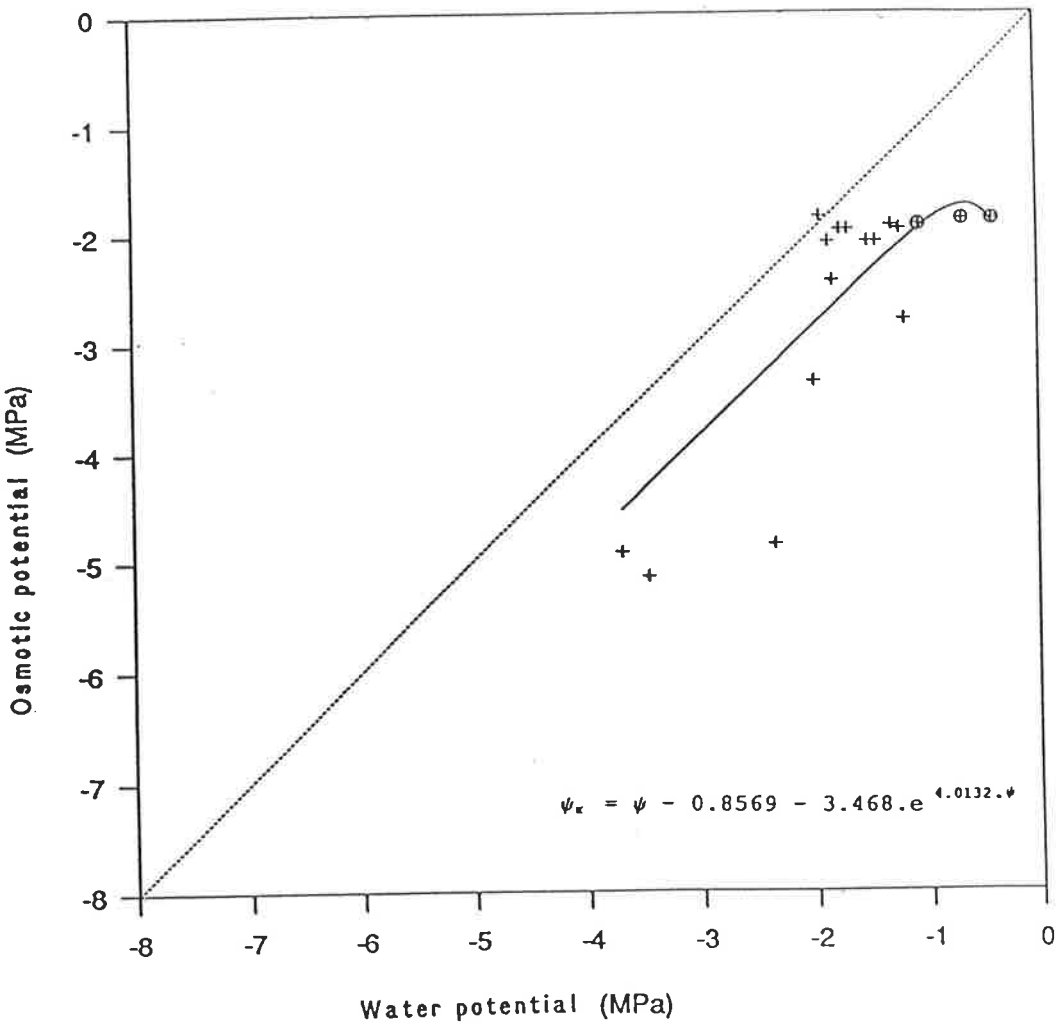
⊕ : well-watered

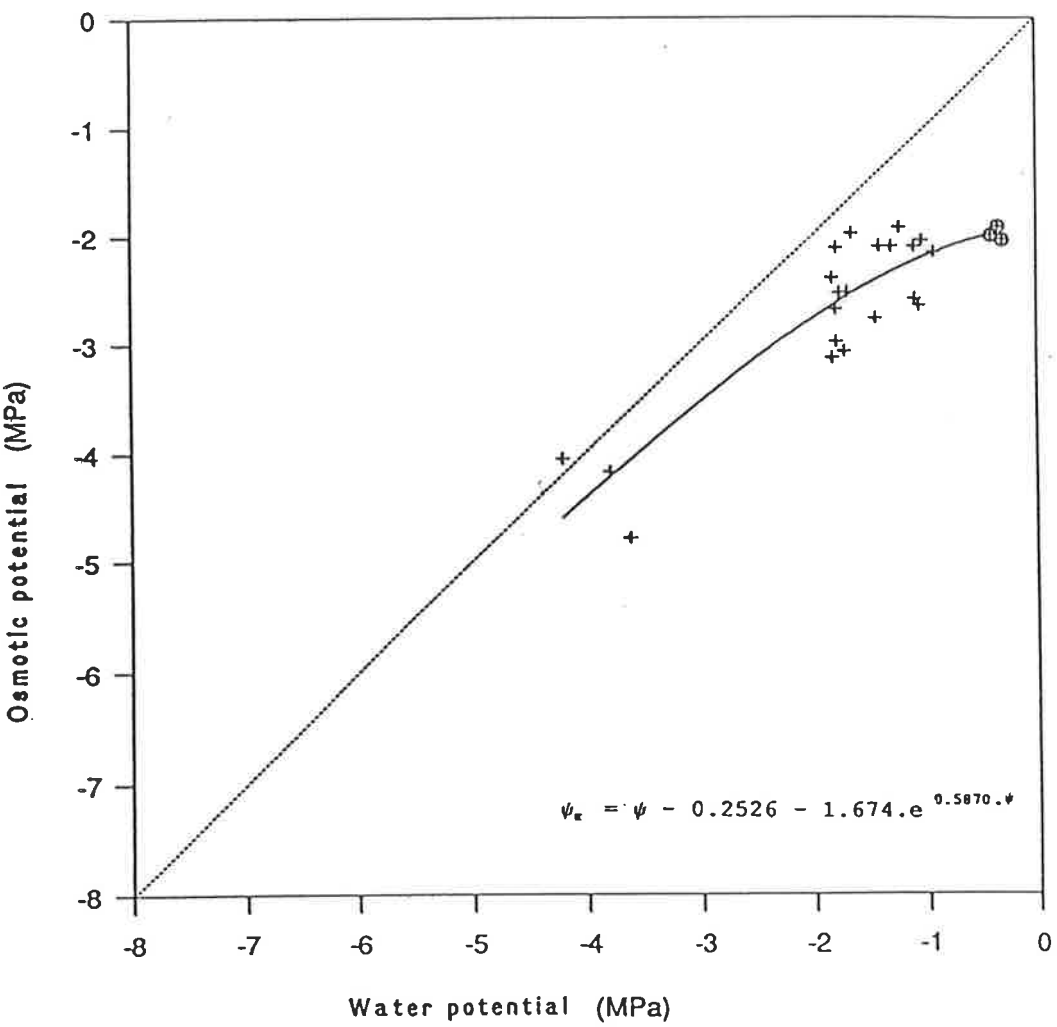
\+ : stressed

Acacia anceps

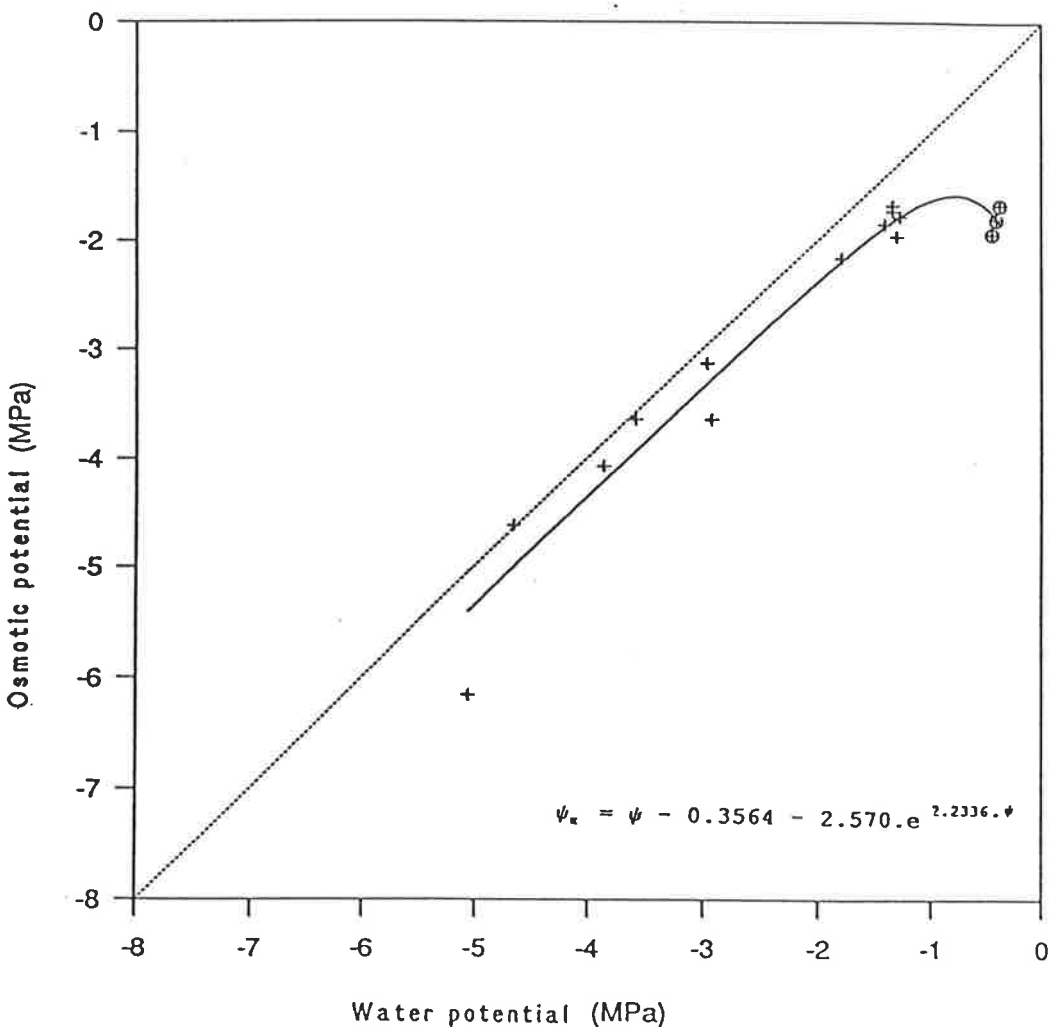


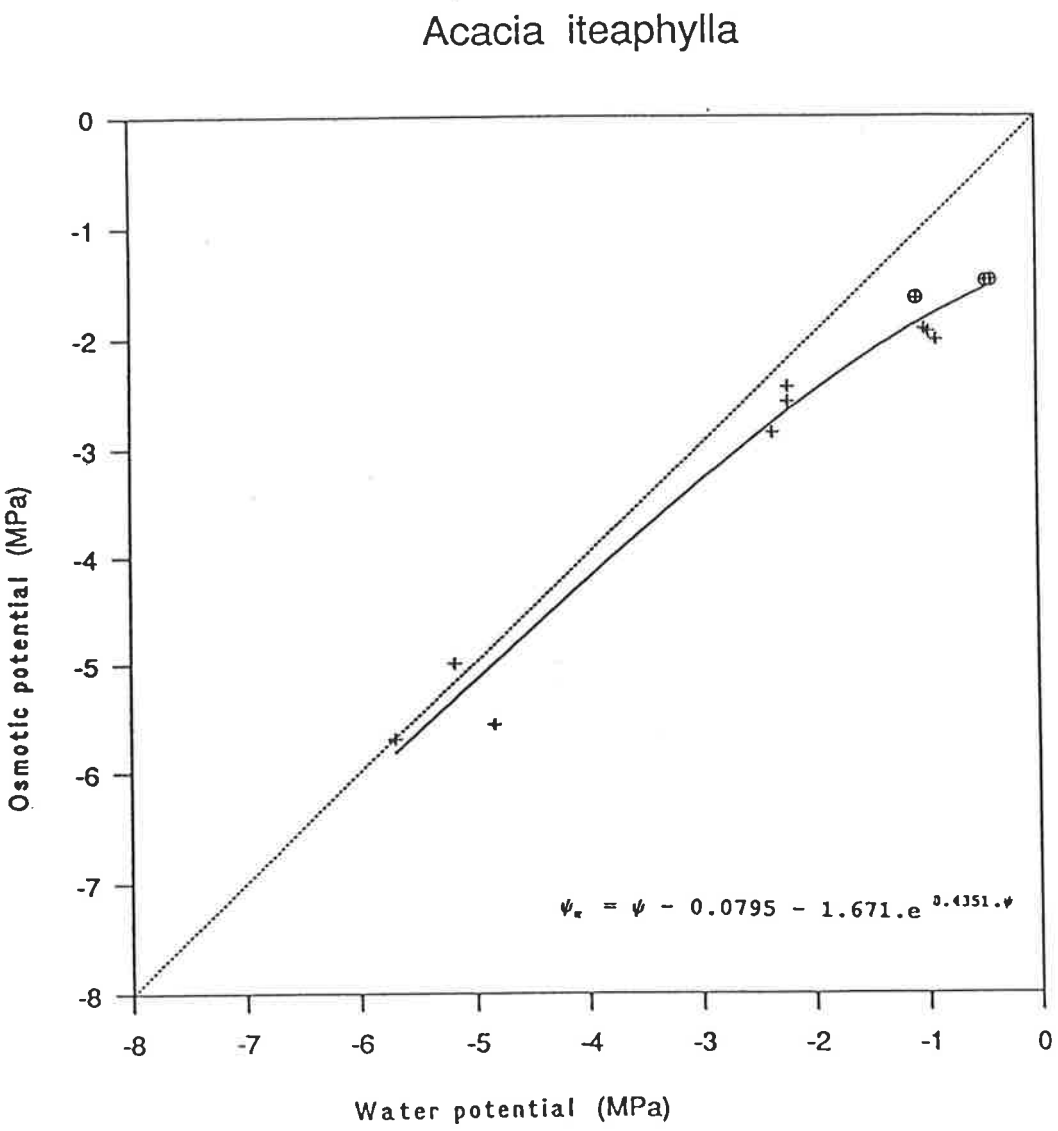
Acacia aneura

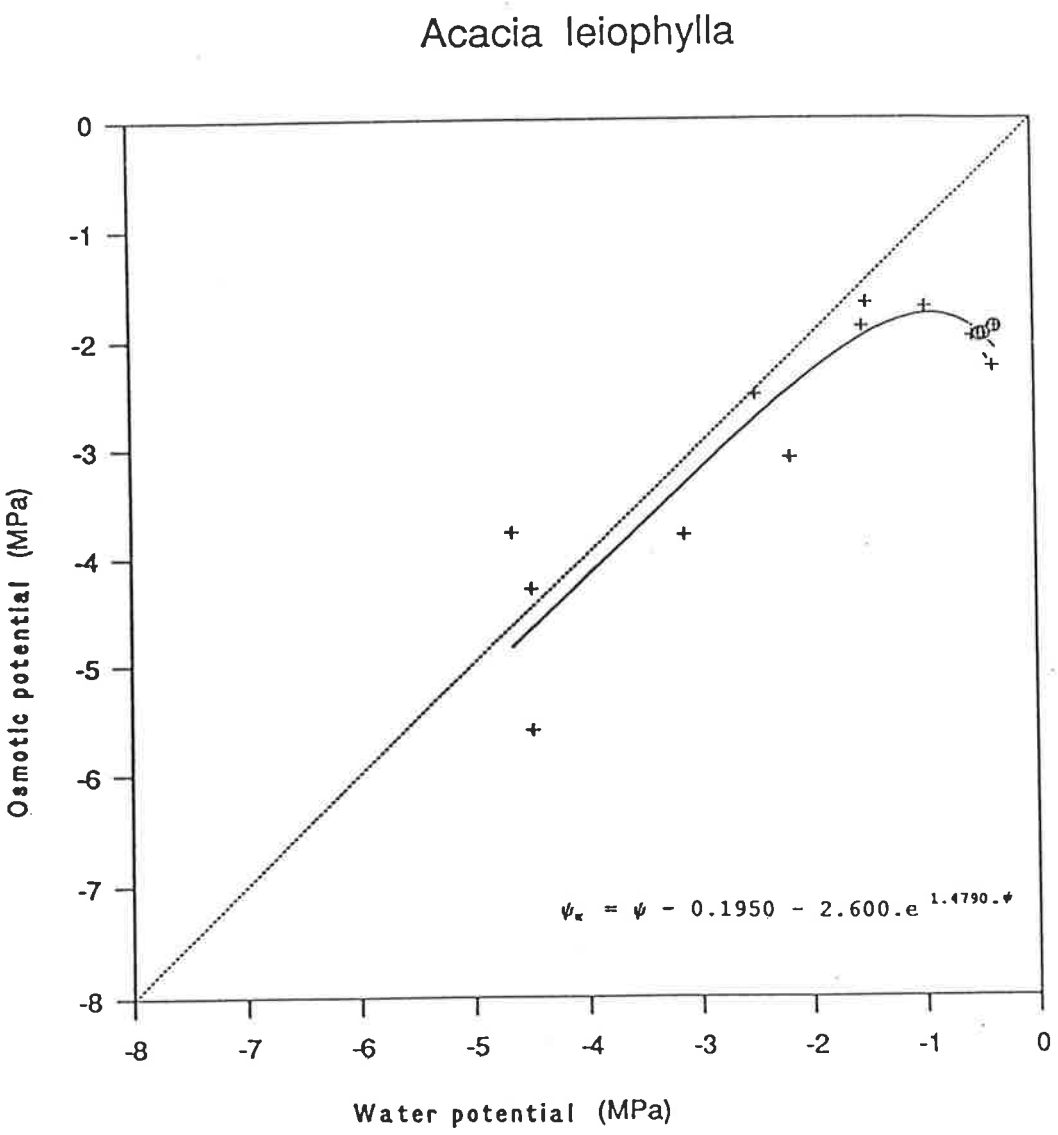



Acacia cyclops
0
-1
-2
-3
-4
-5
-6
-7
-8
Osmotic potential (MPa)
-8
-7
-6
-5
-4
-3
-2
-1
0
Water potential (MPa)
$\psi_\pi = \psi - 0.2526 - 1.674.e^{0.5870.\psi}$

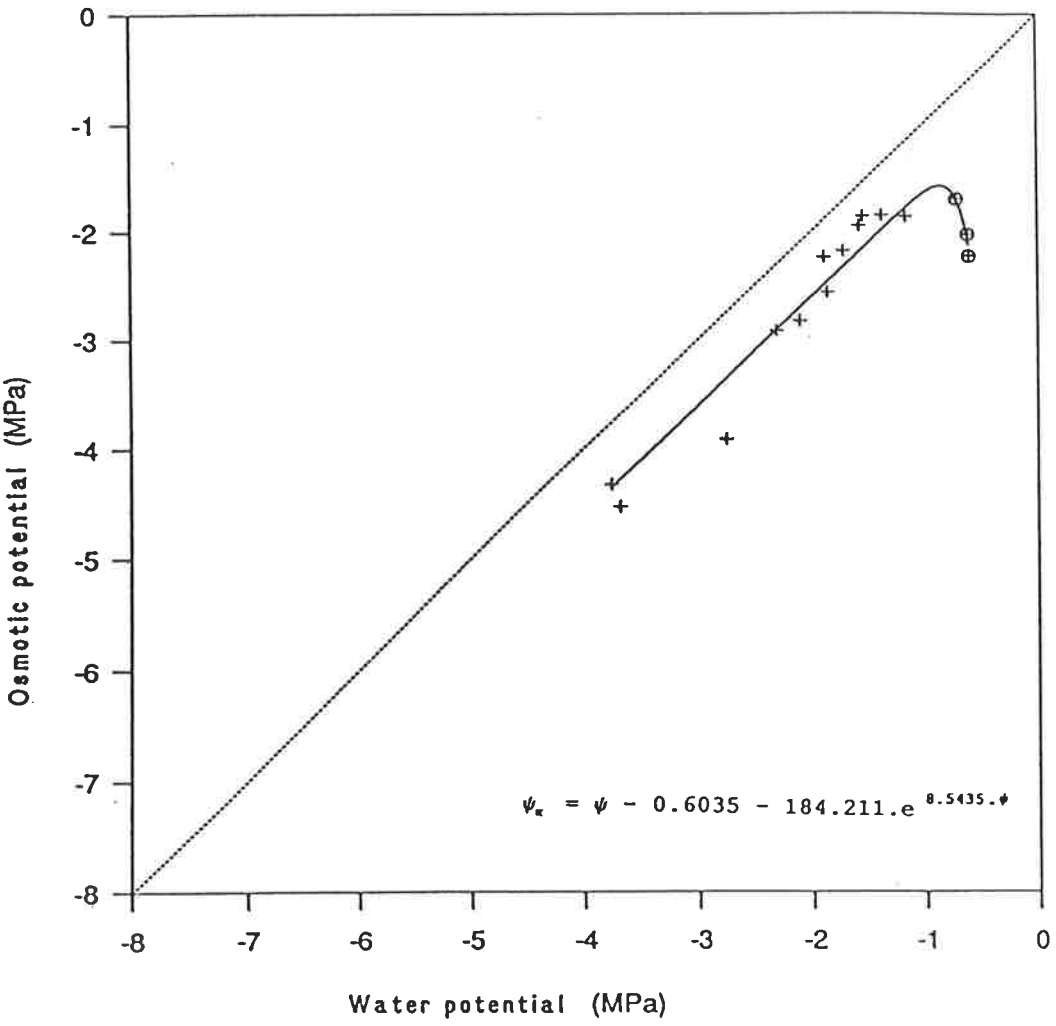
Acacia gillii



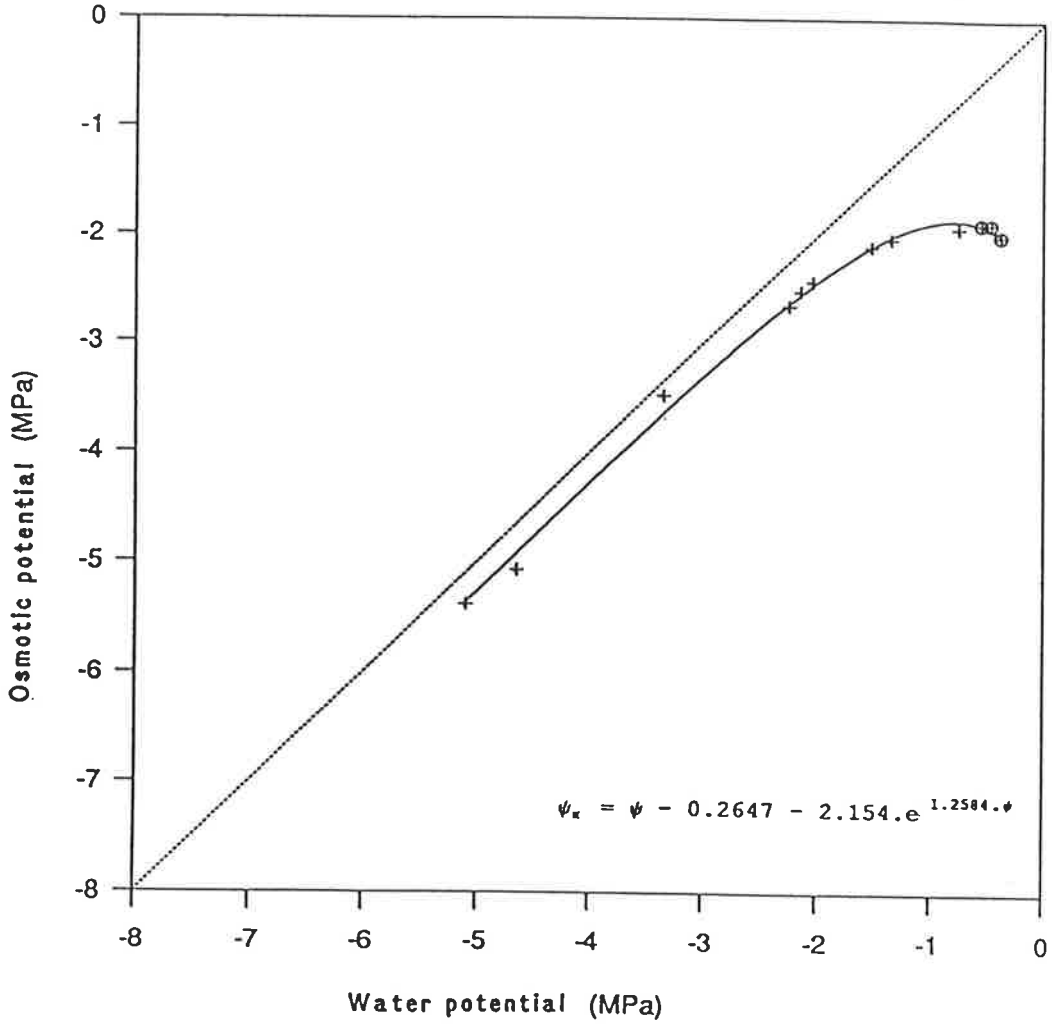

Acacia iteaphylla
0
-1
-2
-3
-4
-5
-6
-7
-8
-8
-7
-6
-5
-4
-3
-2
-1
0
Osmotic potential (MPa)
Water potential (MPa)
$\psi_\pi = \psi - 0.0795 - 1.671.e^{0.4351.\psi}$


Acacia leiophylla
0
-1
-2
-3
-4
-5
-6
-7
-8
Osmotic potential (MPa)
-8
-7
-6
-5
-4
-3
-2
-1
0
Water potential (MPa)
$\psi_\pi = \psi - 0.1950 - 2.600.e^{1.4790.\psi}$

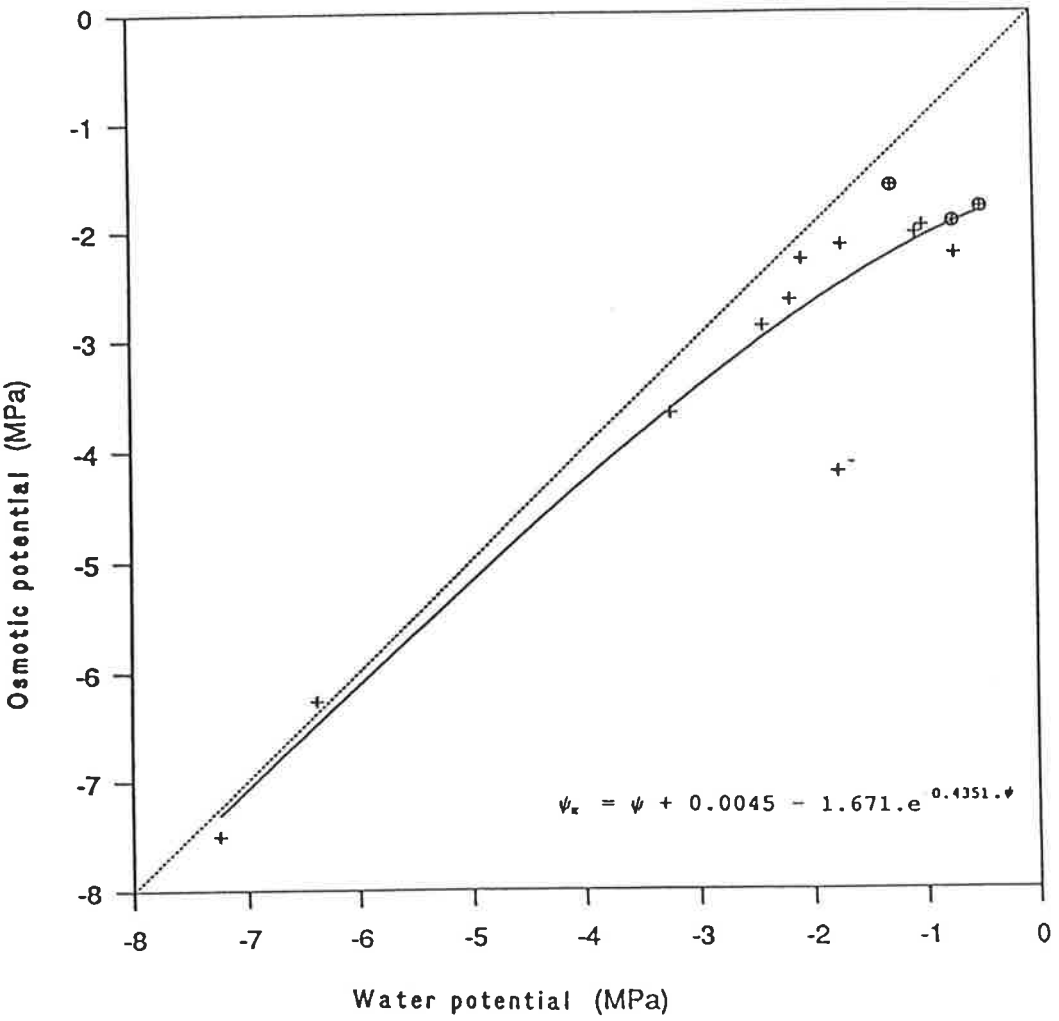
Acacia longifolia



Acacia myrtifolia



Acacia rivalis



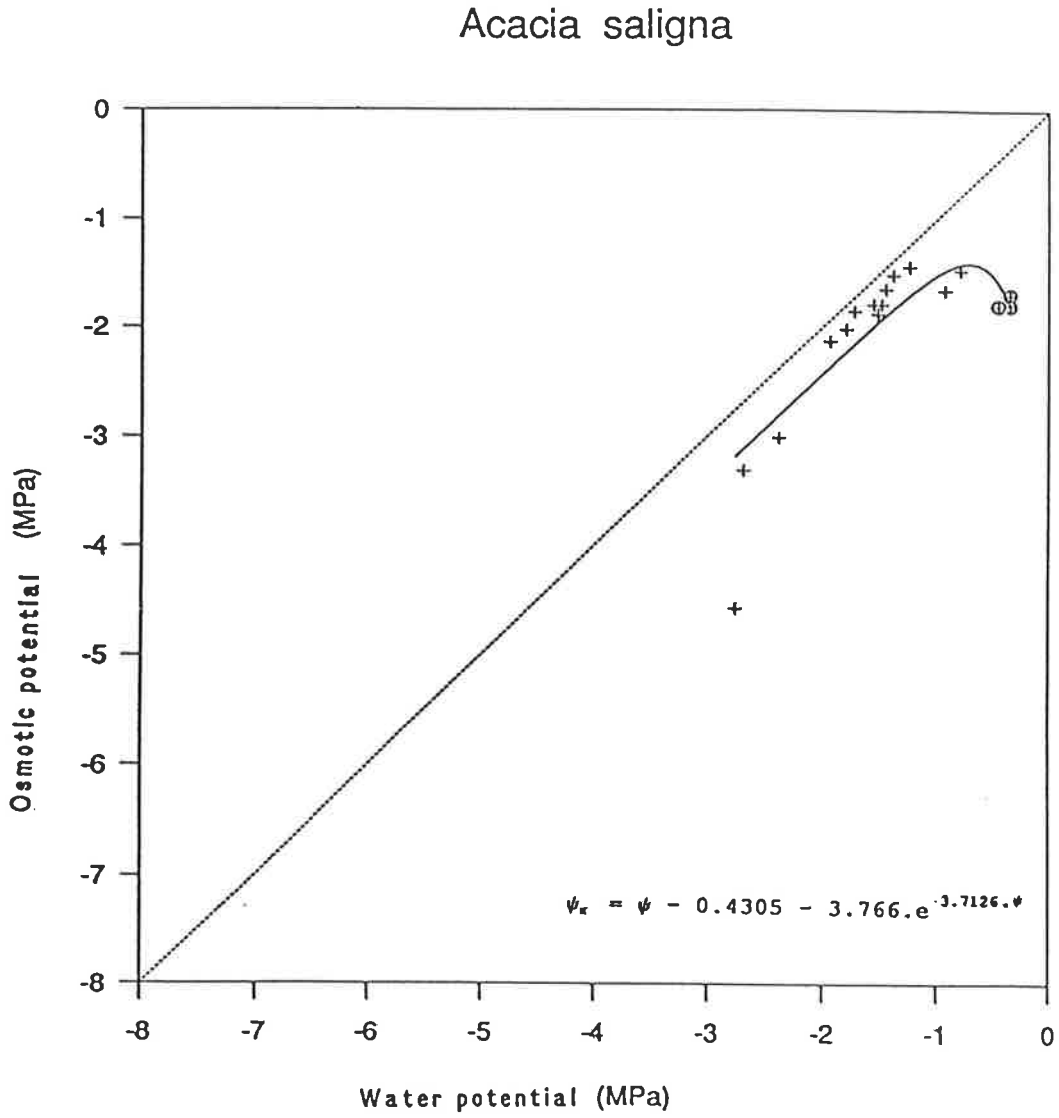

Acacia saligna
0
-1
-2
-3
-4
-5
-6
-7
-8
Osmotic potential (MPa)
-8
-7
-6
-5
-4
-3
-2
-1
0
Water potential (MPa)
$\psi_\pi = \psi - 0.4305 - 3.766.e^{-3.7126.\psi}$

Table 3.9

The coefficients of the exponential regression describing the relationship between water potential and osmotic potential of ***pot grown*** plants of 10 *Acacia* species. The two variables were measured during a drying cycle.
The equation of the regression has the form:

$$\Psi_\pi = \Psi - \beta_0 - \beta_1 e^{\gamma\Psi}$$

where Ψ is water potential, Ψ_π is osmotic potential and β_0, β_1 and γ are constants. Units are MPa. The asymptote of the exponential curve is significantly different from the 1:1 line if β_0 is at least twice its standard error.

Species	β_0	β_1	γ	SE(β_0)	SE(β_1)	SE(γ)	r^2
A.anceps	1.0356	10.604	5.1519	0.14591	98.1621	62.4737	0.8065
A.aneura	0.8569	3.468	4.0132	0.20103	16.9223	30.6308	0.6990
A.cyclops	0.2526	1.674	0.5870	0.46196	0.4196	0.3781	0.7906
A.gillii	0.3564	2.570	2.2336	0.12303	1.4744	2.6939	0.9587
A.iteaphylla	0.0795	1.671	0.4351	0.27475	1.31149	0.5503	0.9764
A.leiophylla	0.1950	2.600	1.4790	0.25375	1.1501	1.6943	0.8410
A.longifolia	0.6035	184.211	8.5435	0.06894	753.5836	41.4669	0.9495
A.myrtifolia	0.2647	2.154	1.2548	0.04871	0.1690	0.2631	0.9965
A.rivalis	-0.1671	1.671	0.4351	0.64001	0.6777	0.5510	0.9094
A.saligna	0.4305	3.766	3.7126	0.13464	4.9452	8.4952	0.7580

Table 3.10

The groups of *Acacia* species which did or did not perform significant osmotic adjustment during pot drying treatment

Adjustment	
significant	non-significant
A. anceps	*A. cyclops*
A. aneura	*A. iteaphylla*
A. gillii	*A. leiophylla*
A. longifolia	*A. rivalis*
A. myrtifolia	
A. saligna	

Osmotic adjustment occurs to balance a hydrostatic disequilibrium between the cells and their surroundings, which may be through a change of volume, a change of solutes or both volume and solutes. The increase of solutes is needed to retain cell volume i.e. turgor maintenance (Morgan, 1983, 1984). Osmotic adjustment may be inferred from the relationship between water potential and osmotic potential during the course of stress.

In this glasshouse experiment, under well-water conditions both water potential and osmotic potential of all species were similar (not significantly different). By the end of the stress period, however, large and significant differences were detected between species in their water potential and osmotic potential (Table 3.6a and 3.6b).

As shown in Table 3.6a, for water potential, an *LSD* test split the 10 *Acacia* species, with *A.rivalis* the most severely stressed and *A.saligna* the least stressed, where the difference reached -3.83 MPa. The pattern appeared in osmotic potential as well. As shown in Table 3.6b, the *LSD* test produced a range of significant differences between species in their osmotic potential after stress. *A.saligna* was the least negative while *A.rivalis* was the most negative, where the differences was -2.75 MPa.

Apparently, these differences were not correlated with soil water content at the end of stress, as Table 3.8b shows that the soil water content at the end of stress i.e. between 2 to 4%, was not significantly different between species.

The results provided evidence that osmotic adjustment did take place in some of these *Acacia* species. As discussed previously, a species is qualified as having a statistically significant osmotic adjustment, when its β_0 value in the exponential regression is at least twice its standard error. The values in Table 3.9 revealed that only six species fulfilled this condition. These are considered as having a significant osmotic adjustment. The other four species are grouped as having non-significant adjustment (Table 3.10).

Some of these results of the non linear analysis seem inconclusive and some were unexpected. Take as an example, *A.cyclops*, where in early days of the drying cycle, the points were scattered around a small area. When the stress increased, there were only a few points left around the 1:1 line, not enough for a good fitting. *A.aneura* occurs in arid areas (average rainfall 150 to 250 mm y^{-1}) and *A.anceps* in coastal environments with the average rainfall of 200 to 500 mm y^{-1}. Thus it was expected that these two species should display strong osmotic adjustment. The other species in the significant adjustment group were not expected to do so strongly considering their area of distribution. *A.gillii* is endemic to a small area of Eyre Peninsula (South Australia), with the average rainfall of about 500 mm y^{-1}. *A.longifolia* is found grown over 500 to 1000 mm rainfall areas in open forest or low open forest or woodland, while *A.myrtifolia* occurs in many high rainfall areas of Australia, with annual average rainfall of 500 to 1200 mm (Whibley, 1980). *A.saligna* is found in 300 to 700 mm rainfall areas.

There are species in the "non-significant" group which were expected to have strong evidence of osmotic adjustment. For example *A.iteaphylla* is native to low rainfall areas (200 to 500 mm) in Flinders Ranges (South Australia), and might be expected to show more strongly developed osmotic adjustment. *A.cyclops* is a coastal area species, found in open shrubland in areas with 200 to 500 mm rainfall. The other species, *A.leiophylla* is a relatively high rainfall species (500 to 800 mm) in coastal regions, while *A.rivalis* has a status as an arid zone species. This phenomenon is similar to the findings of Dibble and Spomer (1987) in wheatgrass (*Pseudoroegneria spicata*). When three populations were grown in glasshouse, osmoregulation occurred, but the differences did not correlate with their original habitats. However for closely related ecotypes, differences that correlate with origin may appear in the glasshouse. Weng (1993) was able to show in 4 clones of *Mischantus* sp. that clonal

differences in osmotic adjustment did associate with the rainfall characteristics of the sample's region.

Several factors may have contributed to the inconclusive results in *Acacia*. The soil type used was a sandy potting mixture, in which the pattern of moisture release leads to small differences in water content causing large differences in soil water potential at the dry end of the moisture release curve (cf. Slatyer, 1967a; Kramer, 1983). This combined with the limited soil content in the pots may have resulted in the plants not being at equilibrium with the soil in their pot during the rapid dehydration. Also, this rapid drying may not have allowed the plants time to adjust.

Turner and Jones (1980) warned that osmotic adjustment may not appear in a rapid drying cycle.

Other possible factors may be differences in leaf area and different weather conditions, as the drying cycles were done at different times. There were relatively big variations in leaf area in the *Acacia* species used in this experiment as the plants grew at different rates. Moreover, the species were exposed to drought treatments at different times, thus perhaps leading to different rates of dehydration.

The drying cycle in the glasshouse with small pots of sandy soil may be very rapid. Plant responses to more realistic field conditions of stress may be different. Hence, the result may not show what typically happens in the field, so this experiment was used as a trial run to be followed up by a field trial (Chapter 4). The field experiment results may vary from the glasshouse. The osmotic adjusting species may possibly become "non-adjuster", or conversely a "non-adjuster" may show stronger adjustment. The magnitude of adjustment could be different as well, where bigger osmotic adjustment may be found in the field grown plants. The evidence for osmotic adjustment in pot grown seedlings while not conclusive, is suggestive.

CHAPTER 4

SEASONAL FLUCTUATIONS OF OSMOTIC ADJUSTMENT IN FIELD GROWN PLANTS

4.1. *Introduction*

It is a common understanding that pot or glasshouse-grown plants do not always behave like the mature plants grown in the field. As stated by Kramer (1983) in the case of plant water relations, water stress sometimes develops too rapidly under experimental conditions which give results that are not comparable to the field experiment, where water stress develops more gradually.

As shown in Chapter 3, species used in the glasshouse experiment did not always show the osmotic adjustment that might have been expected from their distribution in the wild. Hence a field experiment was carried out as an extension of the glasshouse treatment.

The aims of this field experiment were:

a. *To study and compare in detail the osmotic adjustment processes in five of the Acacia species used in the glasshouse study, under more realistic field conditions.*

b. *To study the seasonal osmotic adjustment of these 5 Acacia species with respect to their adaptational characteristics to drought*

c. *To investigate both inorganic and organic compounds which might be responsible for the decrease of osmotic potential in these species.*

There have been very few field studies of water relations of Australian acacia species (for example Slatyer, 1960; Tunstall and Connor, 1975). These studies extracted data mainly

from the comparison of natural field grown plants, but the samples had a wide range of ages so may vary from the values of plants of the same age.

This project was a comparative study of five *Acacia* species of similar ages under similar environmental conditions. It is true that the species used in this extended field experiment originated from different ecological backgrounds; however they were selected to be compatible as nearly as possible with the field experimental site conditions.

4.2. *Study location*

The field experiment was carried out in the BHNFC site, Maryvale Road, Athelstone, South Australia. The area is situated at the western base of Black Hill which is part of the chain of the Adelaide Hills, the southern part of the Mount Lofty Ranges. The recorded average annual rainfall for 5 years commencing in 1979 was 691mm (Commonwealth Bureau of Meteorology, 1988).

The soil of the field experiment area is described as varying from hard pedal to reddish friable, red duplex combined with loams with rough-ped fabric (Laut et al, 1977).

4.3. *Materials and Methods*

4.3.1. *Field Plot Design*

At the end of the glasshouse experiment (Chapter 3) the seedlings were moved to an open site in North Adelaide belonging to the Botany Department. During the "storage" period the pots were kept well-watered and received sufficient sunlight; they were thus not subjected to any stress. The seedlings produced new shoots and phyllodes during the storage time. Ten pots of each species were available from which to select plants for transplanting to the field.

Hence some of the same plants which had been used for the glass house were used again for the field experiment. This was done mainly to reduce the variability which may arise from using plants of different ages and different sources. The number of species was reduced to five for reasons of the availability of materials, space, manpower and time. Some difficult decisions were involved in selecting five species. The selected samples had to be of similar age and size at the time the experiment started. Sufficient samples were necessary for replications. Not less important was that the samples should be in reasonable health to avoid false readings during measurement, or death during the course of the experiment.

Species selected were *A.anceps, A.aneura, A.gillii, A.iteaphylla* and *A.myrtifolia*. Due to the difficulties mentioned, only one of these came from the group that had showed no significant osmotic adjustment (Table 3.10), namely *A.iteaphylla*. However, it was felt that these 5 would provide a reasonably good range of possible adaptations, including one widely distributed (*A.aneura*), one from higher rainfall area (*A.myrtifolia*) and three endemic to South Australia i.e. coastal (*A.anceps*) and inland (*A.gillii* and *A.iteaphylla*) (see Whibley, 1980, for available information on the eco-distribution of South Australian acacia species).

A plot, 30 m x 15 m in area was fenced with Boral Cyclone chainwire 2 m high security fence to protect the experiment from vandalism. Within the enclosure four blocks were laid out, each consisting of two rows of five plants. The ten plants in each block were made up of two of each species, one to be well-watered, the other to be stressed. They were planted in holes 40 cm deep, 25 cm dia, spaced 2.5 m apart. the distance between blocks was 3.3 m. Plants were placed randomly within each block using a Table of Random Permutation (Cochran and Cox, 1960). The experiment thus was a factorial design, consisting of 5 species x 4 replicates which were sampled 7 times.

The seedlings were planted on 20 July 1987 (*see Plate 2*). During the first four and half months from the field planting date, all seedlings received water to stabilize their growth

Plate 2 Black Hill Flora Centre field experiment site for growing five of the ten acacias under investigation.

and wash out the possible solute accumulation during their storage period. All plants were well-watered prior to the application of the new treatment, as shown by their water potential values on the first reading (see Figure 4.3 and Table 4.2.a and 4.2.b) where the total water potential values were very high mostly ranging between -0.3 and -0.5 MPa. indicating well-watered conditions.

The watering system for the well-watered treatments was installed throughout the experimental area using 1.5 cm black PVC irrigation hose. A programmable automatic water regulator was used. At the base of each well-watered treatment plant, a spinning nozzle was inserted into the hose. The automatic water regulator was programmed to turn on every second day at 6.00 a.m. for 22 minutes. This delivered approximately 15 litres of water to each of well-watered treatment plants. *See Plate 3.*

December 1st is considered as the first day of summer in South Australia, so the application of treatments was started at that date. From this date water was withheld from the stressed treatment plants, while the watering system was activated for the well-watered plants.

Data gathered included dawn water potential, osmotic potential (hence, turgor pressure) and samples for later determination of solute content. Readings were taken at seven harvests within four months during the summer. The solute determinations were done only for the last four harvests.

The first harvest was on 2 December 1987, followed by the others on 22 December 1987, 8 January 1988, 1 March 1988, 17 March 1988 and 26 March 1988 respectively.

4.3.2. *Water potential, osmotic potential and turgor pressure determination: Osmotic adjustment estimation*

The pressure chamber was used to determine dawn water potential values. The reading during the seven harvests were normally started at 02.30 to 03.00 a.m.,

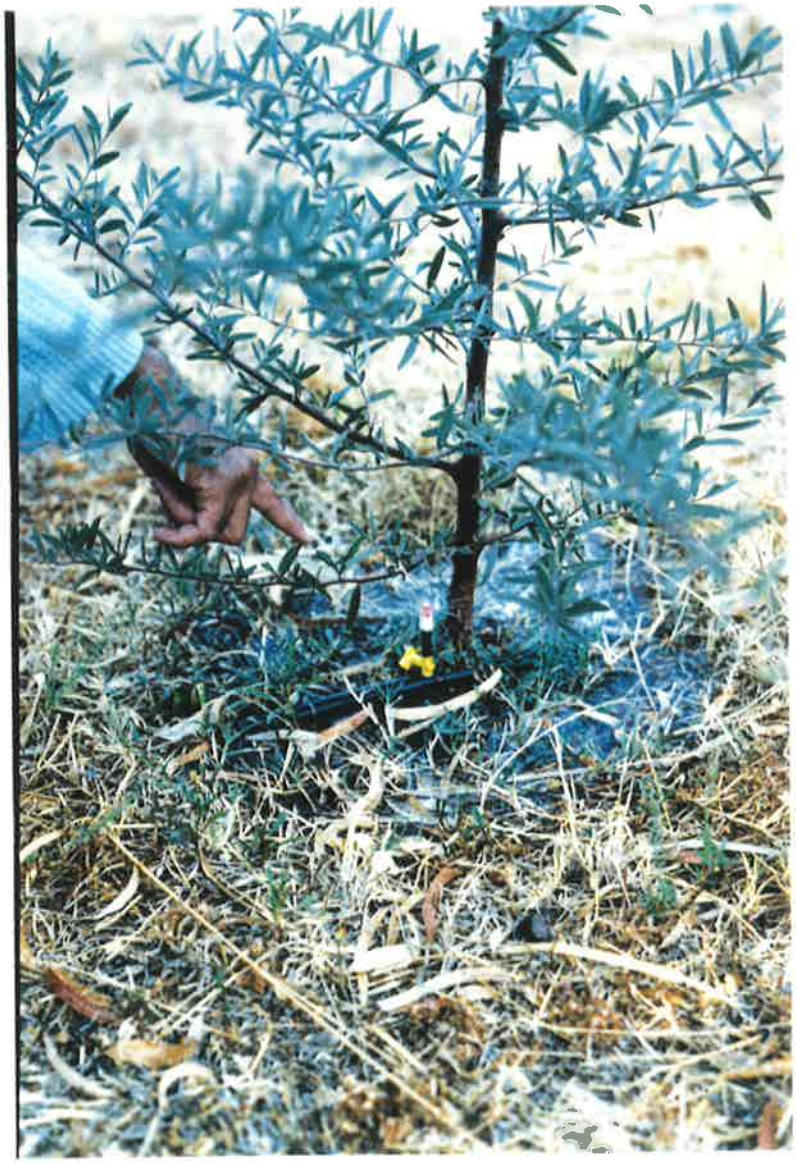


Plate 3. The well watered treatment field experiment plants use an automatic sprinkler at the plant base. Mulga pictured above.

and the reading of the last sample usually finished by 05.00 to 05.30 a.m. At most times the last sample's reading was *applauded* by one or two kookaburras laughing from the top of the beautiful gum trees naturally growing around the site.

A single phyllode or shoot was collected and quickly sealed into the chamber. Pressure was applied and the balance pressure (i.e. total water potential) was recorded. The sample was then wrapped with marked/ numbered aluminum foil, placed in a small tube and plunged into liquid nitrogen for several minutes, as required for the breakdown of cell membranes for the eventual osmotic potential determination by thermocouple psychrometer. The sample was then stored in dry ice for osmotic potential readings in the laboratory.

The reason for using the psychrometer for measuring osmotic potential, rather than the P-V curve method, was that in this study dawn water potentials were required, and the P-V curve method was too time consuming to be used in the field. As phyllodes could be frozen immediately after the pressure chamber reading and as the handling was done in cool, humid conditions it was assumed that water loss from phyllodes was minimal and hence osmotic potentials would have been little affected by the initial pressure chamber reading.

A Wescor HR-33 Dew Point Microvoltmeter, with C-52 sample chambers, was used to measure osmotic potential of the samples (see Section 2.4.2.1). The measurements were carried out in a constant temperature room. To increase temperature stability sample chambers were placed in a box approximately 75 cm x 40 cm x 50 cm made from 0.5 cm thick perspex; the outer wall of the box was covered with 1 cm thick polystyrene plates. Only two holes (15 cm each in diameter) were provided to allow sample loading, hence the temperature was constant enough for good reproducibility. The sample chamber was wrapped with polystyrene, in order to reduce the possible temperature changes during sample loading.

To measure the osmotic potential, the samples were taken out from the container with dry ice, the surface was quickly dried as required with tissue paper, wrapped with transparent plastic and crushed with a mortar. The sap was quickly collected with a small Whatman filter paper disc produced by a paper punch. This disc was placed in the sample holder, which was loaded into the sample chamber. The remaining extra tissue was quickly wrapped back and stored in the freezer for later solute determinations.

The equilibrium time (i.e. when the reading had stabilized) was between 20 and 40 minutes. The psychrometers were calibrated with a series of KCl solutions (Wiebe et al, 1971), at 20, 25 or 30° C. Figure 4.1 is an example of a calibration curve. Not less than 40 samples were measured for osmotic potential at each harvest. Four sample chambers were available. Thus each was employed to read 10 samples. It was assumed that this number of samples would not affect the accuracy of the thermocouples, so they were only calibrated before the reading of samples at each harvest.

The magnitude of seasonal osmotic adjustment was interpreted by looking at the fluctuation of osmotic potential of stressed plants compared to the well-watered plants over time. However, this fluctuation has to be linked with the water potential as well since osmotic adjustment is manifested as the lowering of osmotic potential beyond that due to the drop in water potential, if turgor maintenance is to be extended.

4.3.3. *Rainfall record*

The daily rainfall data at the experimental site (Black Hill) was recorded from Meteorological Office, Adelaide. However, due to vandalism of the Blackhill Meteorological Station, after March 1988 figures were replaced by the recorded data from Hope Valley, the closest station to the Black Hill experimental site.

Figure 4.1

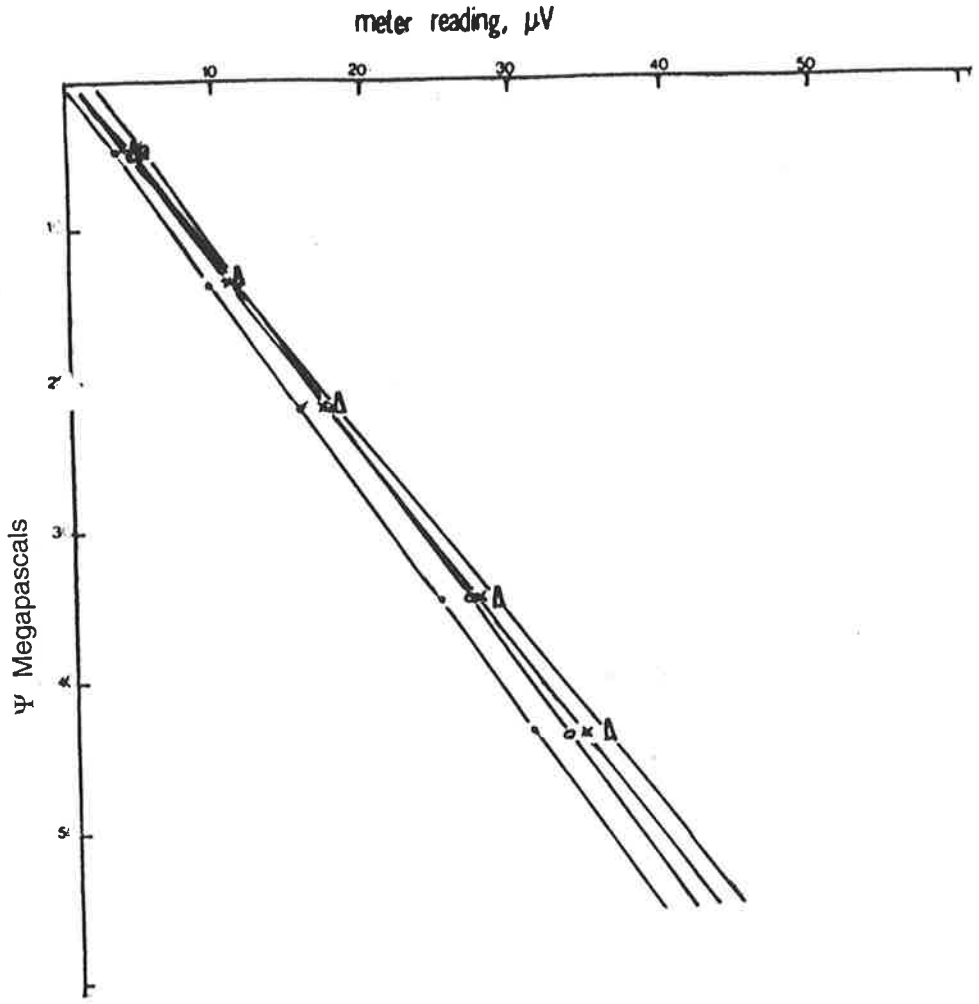
Examples of calibration curves for 4 thermocouple psychrometer chambers at 20^{0}C. The calibration solution was KCl with 0.1, 0.3, 0.5 0.8 and 1.0 molal concentrations.

Δ : Chamber 1

✚ : Chamber 2

○ : Chamber 3

● : Chamber 4


meter reading, μV
10
20
30
40
50
Ψ Megapascals
1
2
3
4
5

4.3.4. *Statistical analysis*

It should be noted that the model in this section considered the two water treatments (well-watered and stressed) as two different levels of stress, say less stress and more stress.

Since the data were obtained from multiple observations on the same plants, it is necessary to take into account the correlation between observations in order to increase the accuracy of estimation of the means, and the estimation of variation between and within treatment groups correctly.

The water potential, osmotic potential and turgor pressure data were subjected to the split-plot repeated measures method of analysis.

Details of the repeated measured analysis are given in Appendix 4. This analysis was done by Dr. A.P. Verbyla and Mr. K. McNamara, Department of Statistics, the University of Adelaide. A complete report of the statistical treatments is given in McNamara (1990), available from the author or Department of Statistics or Botany, The University of Adelaide.

The original purpose of the analysis was to determine whether or not the seasonal fluctuations of variates over time (seven harvests) depended upon the species, and whether or not they were a response to water treatment (well-watered or stressed). The magnitude of the differences between species is also a point of interest. Although the plants were originally set out in randomized blocks this block design was ignored in the final statistical treatment, as it was considered that the blocks were close enough together not to be treated as independent of each other. All tests were done at the 5% significance level.

Evidence for seasonal osmotic adjustment was also sought, using the exponential curve-fitting procedure described in Chapter 3.

In the following statistical tables, unless otherwise indicated, the units of water potential components are *bars*.

4.4. *Results*

4.4.1. *"Negative turgor pressure"*

One fundamental problem that arose in a number of samples was that the osmotic potential of the expressed sap, measured by the thermocouple psychrometer, was less negative than water potential, measured by the pressure chamber. As a consequence, the calculation of turgor pressure would produce apparently "negative turgor". This phenomenon may be the result of generating two parameters by using two different pieces of equipment.

The dilution factor is thought to be responsible for the discrepancy. Water which occupies the cell wall (apoplastic water) may be up to 30% (Boyer, 1967; Tyree and Hammel, 1972) and more (cf. Table 4.1 in this experiment) of the total water content. This apoplastic water would dilute the cell sap during sample preparation for psychrometric measurement leading to underestimates of osmotic potential. The dilution factor is a problem for the method used by Myers and Neales (1984)

There is some evidence that negative turgor may occur in field grown plants or during salinity stress. A number of authors noted that the negative turgor phenomenon might exist as an adaptation to soil salinity (Bennet-Clark, 1959; Slatyer, 1967b; Noy-Meir and Ginzburg, 1969; Tunstall and Connor, 1975; Kramer, 1983; Mandzhavidze, 1986, as quoted by Losch, 1989). Oertli (1986) maintained the concept by noting that the phenomenon could exist by applying a non-penetrating cell wall solute into the tissues or cells. However, other authors did not agree with this idea and considered it a fallacy (see for example Tyree, 1976; Markhart III et al, 1981).

4.4.2. *Calibration of psychrometer measurement of osmotic potential*

The problem of the dilution effect had to be taken into account in this experiment, and so the original field values of osmotic potential (generated from the

thermocouple psychrometer) were calibrated against the osmotic potential generated by pressure chamber.

The calibration curve was constructed by plotting the measured values of osmotic potential obtaining from both the pressure chamber and the psychrometer using samples from another series of pot grown seedlings of *A.iteaphylla* which were subjected to a drying cycle. Before a shoot was sealed into the pressure chamber for the P-V construction (Section 2.4.1.2), a few phyllodes were collected from that shoot for the estimation of osmotic potential using the thermocouple psychrometer method (Section 4.3.2). Twenty seven points were used for the calibration line. A linear regression was fitted to describe the relationship between the two values:

$$\mathbf{Ps = 0.6069Pc - 0.5043} \quad \ldots\ldots\ (7)$$

where Ps = psychrometer measurement, Pc is pressure chamber measurement with r^2 = 0.9227, indicating a good relationship (Figure 4.2). The two readings were reasonably close together when the plants were well-watered; but the line gradually moved away from the 1:1 relationship as osmotic potential decreased due to increasing stress. This shows that the thermocouple psychrometer becomes less sensitive when osmotic potential becomes more negative. this phenomenon has been reported when measuring water potential (see review by Ritchie and Hinckley, 1975). Turner et al (1984) from a comparative study of these two methods found that water potential values generated from the thermocouple psychrometer could underestimate or overestimate at low water potentials depending on the water potential gradient across the leaves or low epidermal conductance. So far, no other data regarding the comparison of osmotic potentials have been reported.

The calibration curve was obtained only for *A.iteaphylla*. Apoplastic water volume is the main contributor to the dilution which leads to apparent negative turgor. Therefore to

Table 4.1

A: The apoplastic water volumes (%) of pot plants of *A.iteaphylla* (n = 27) generated from the same samples as for the calibration curve in Figure 4.2. The values below, ranged from 25 to 33% are arranged in ascending order. Mean value was 28.30 ± 2.66.

B: The apoplastic water volumes (%) of the 5 *Acacia* species grown in the field. The readings (n=3) were taken within about two weeks.

A

24.60	26.88	28.95
24.73	27.35	29.55
24.92	27.49	30.21
25.53	28.15	30.29
25.61	28.28	30.88
25.69	28.68	32.32
25.80	28.82	32.77
25.90	28.93	33.29
26.18	28.93	33.41

B

	Reading	Mean
A.anceps	26.73, 24.24, 25.94	25.64 ± 1.27
A.aneura	35.22, 32.25, 34.12	35.56 ± 4.13
A.gillii	35.23, 32.35, 31.11	31.58 ± 3.43
A.iteaphylla	14.32, 37.73, 25.12	25.72 ± 11.71
A.myrtifolia	33.74, 22.22, 24.65	26.87 ± 6.07

Figure 4.2

Linear regression describing the relationship between two values of osmotic potential generated from pressure chamber (x-axis) and thermocouple psychrometer (y-axis) using the same phyllode samples, taken from pot-grown *Acacia iteaphylla*. Units are MPa.

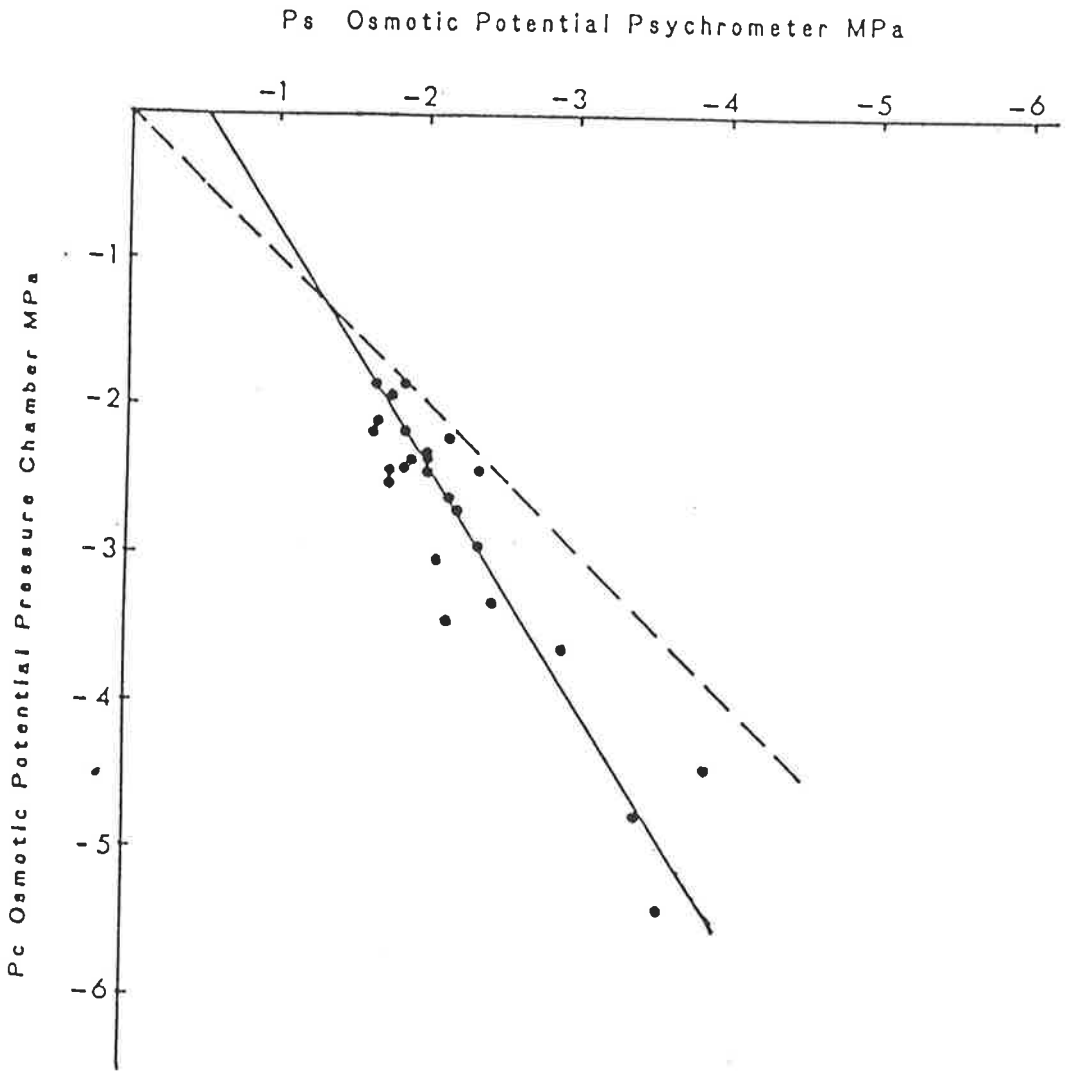
The form of equation is:

$$Ps = 0.6069\ Pc - 0.5043$$

where Ps refers to thermocouple psychrometer and Pc to pressure chamber.

Full line - regression

Dashed line - 1:1 relationship


Ps Osmotic Potential Psychrometer MPa
-1
-2
-3
-4
-5
-6
Pc Osmotic Potential Preasure Chamber MPa
-1
-2
-3
-4
-5
-6

determine whether or not this curve could be applied to other species, a series of pressure volume curves were obtained from all species grown in the field experimental site, from which apoplastic water volumes were calculated. These were compared with apoplastic water volumes previously measured i.e. the same way for the *A.iteaphylla* pot grown seedlings, to see if there were big differences between these and the field grown species. It was found that the mean apoplastic water volumes of all field grown plant species ranged between 26 and 36%, while in pot grown *A.iteaphylla* the mean was 28% with a range between 25 and 33% (Table 4.1). Thus it was considered valid to use the calibration curve for *A.iteaphylla* as the standard curve for the other species.

All measurements of osmotic potential discussed in the rest of this chapter were made with psychrometer and adjusted using this regression as seen in Figure 4.2.

4.4.3. *The fluctuations of water relations components in well-watered and stressed plants*

The original data of seasonal fluctuations of water potential, osmotic potential (calibrated data) and turgor pressure in both well-watered and stressed individual plants are presented in Table 4.2.a. There are 280 original points for water potential, 279 points for both osmotic potential and turgor pressure, since one of *A.myrtifolia*'s osmotic potential (hence, turgor pressure) is missing. The data, from 5 species, 2 treatments, each with 4 replications, were gathered in 7 harvests. Table 4.2.b displays the average values of water potential components from each species during 7 harvests. {*NB: for further statistical analysis, the missing values were replaced while fitting the split-plot design using the default method procedure (Genstat 5 anova)*}.

All plants were well-watered at the first harvest due to the 30 mm rainfall on the previous day (1 December 1987). This provided a good chance for the first harvest while all plants were equally hydrated.

Figure 4.3.a presents the mean water potentials, Figure 4.3.b the osmotic potentials and Figure 4.3.c the turgor pressure for well-watered and stressed plants of all species combined for the seven harvests, plotted against time. The harvest dates were attached at each harvest point on the graph. At the bottom of these Figures are displayed the daily rainfall at and around the experimental site.

The water relations parameters of the *well-watered* plants did change to some extent with the environmental fluctuations. Water potentials were relatively steady. The highest individual value was -0.21 MPa, the lowest was -1.38 MPa. The slightly more negative values of a few individual plants at certain times (up to -1.38 MPa), may be due to high evaporation on hot days or probably temporary failure of watering systems. For osmotic potential, the highest individual value was -1.02 MPa, the lowest -3.78 MPa, while turgor pressure ranged between 0.01 and 3.57 MPa (Table 4.2.a).

Three periods of stress were detected during the experiment (Figure 4.3.a). The first period was detected at the third harvest (five weeks after the first measurement) by which time the water potentials of all unwatered plants had declined sharply compared with the controls. The difference in mean water potential between stressed and control plants was 1.73 MPa. A 33 mm rainfall was more than enough for all stressed plants of all species to recharge their internal water content (as shown in the fourth harvest) thus returning their water potential to values similar to the control plants.

The second period of stress developed due to lack of significant rainfall in the month after the fourth harvest. Three rainfall events equal to or below 10 mm about three weeks prior to the fifth harvest (Figure 4.3, bottom), probably had a minor effect but were not strong enough to return the plants to the well-watered condition of harvest IV. Water potential of all species had fallen drastically by the fifth harvest. The difference in mean water potentials was now 2.31 MPa.

Table 4.2.a.

Water potential, Ψ (-MPa), osmotic potential, Ψ_π (-MPa) and pressure potential (turgor pressure), Ψ_p (MPa), for five *Acacia* species, well-watered (WW) and stressed (STR) from seven harvests over summer, Dec. 1987 - Jan. 1988. Individual values for the 4 replicates of each treatment.

Species, Treatment, Parameter		TIME OF HARVESTING																											
		I				II				III				IV				V				VI				VII			
Repl.		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
anceps	Ψ	0.21	0.89	0.28	0.34	0.93	0.79	0.62	0.52	0.38	1.03	1.10	0.34	0.62	0.89	0.55	0.31	1.14	1.31	0.86	0.96	0.59	0.96	0.72	1.00	1.10	1.03	1.07	0.45
WW	$\Psi\pi$	2.05	2.55	2.13	2.71	2.55	2.55	1.23	1.39	1.64	1.77	1.39	1.05	2.05	2.55	2.05	1.17	2.30	3.04	2.13	2.13	1.25	3.53	2.55	1.72	1.64	2.79	2.88	2.01
	Ψp	1.84	1.66	1.85	1.37	1.62	1.76	0.61	0.87	0.26	1.64	0.29	0.71	1.43	1.66	1.50	0.86	1.16	1.73	1.27	1.17	0.66	2.57	1.83	0.72	0.54	1.76	1.81	1.56
	Ψ	0.41	0.28	0.34	0.79	2.48	1.21	1.65	3.31	2.45	1.79	2.48	1.93	0.45	0.65	0.38	0.45	3.72	2.62	4.48	5.17	2.41	1.93	4.14	3.72	4.21	3.79	7.07	7.07
STR	$\Psi\pi$	2.63	2.88	3.53	2.88	3.12	2.71	2.55	3.95	3.37	2.88	3.42	2.96	3.53	3.37	3.41	3.37	5.26	4.41	5.96	6.55	3.67	4.24	6.01	5.45	4.94	3.82	7.65	7.85
	Ψp	2.22	2.60	3.19	2.50	0.64	1.50	0.90	0.64	0.92	1.09	0.94	1.03	3.08	2.72	3.03	3.92	2.54	1.79	1.48	1.38	1.26	2.31	1.87	1.73	0.73	0.03	0.58	0.78
aneura	Ψ	0.28	0.48	0.21	0.24	0.65	0.65	0.62	0.62	0.28	0.31	0.38	0.34	0.45	0.52	0.38	0.52	0.96	0.83	0.69	0.83	0.45	0.62	0.72	0.69	0.45	0.59	0.79	1.17
WW	$\Psi\pi$	3.77	1.80	3.78	3.38	3.12	2.38	2.55	2.38	2.22	1.48	2.05	2.63	2.55	2.01	2.59	2.13	2.30	3.04	3.21	2.13	2.55	1.89	2.05	2.38	1.64	2.38	1.60	2.59
	Ψp	3.49	1.32	3.57	3.14	2.47	1.73	1.93	1.76	1.94	1.17	1.67	2.29	2.10	1.49	2.21	1.61	1.34	2.21	2.52	1.30	2.10	1.27	1.33	1.69	1.19	1.79	0.81	1.42
	Ψ	0.28	0.48	0.34	0.21	0.69	1.07	1.31	2.00	1.10	2.28	2.96	3.24	0.34	0.48	0.31	0.52	1.24	2.76	3.86	3.38	1.17	1.59	2.00	3.17	2.00	2.69	5.38	4.65
STR	$\Psi\pi$	3.21	3.28	3.53	3.03	3.25	3.21	3.04	3.62	2.55	3.53	3.53	3.70	2.88	3.62	3.04	3.86	3.45	4.03	4.36	6.01	1.89	2.74	3.29	4.05	2.79	3.04	6.65	5.59
	Ψp	2.93	2.80	3.19	2.82	2.56	2.04	1.73	1.62	1.45	1.25	0.57	0.46	2.54	3.14	2.73	3.34	2.21	1.27	0.50	2.63	0.72	1.15	1.29	0.88	0.79	0.35	1.27	0.94
gilli	Ψ	0.41	0.48	0.31	0.34	0.65	0.62	0.69	0.59	0.96	0.83	0.34	0.55	0.62	0.48	0.34	0.48	0.62	0.55	0.52	0.55	0.59	0.72	0.48	0.38	0.72	0.62	0.59	0.65
WW	$\Psi\pi$	2.79	2.46	2.22	2.17	2.55	1.97	2.17	1.94	2.10	1.20	1.72	1.97	2.42	1.89	1.56	2.05	2.60	2.05	1.89	1.97	1.64	2.30	1.72	1.64	1.97	1.64	1.31	1.48
	Ψp	2.38	1.98	1.91	1.83	1.90	1.35	1.48	1.35	1.14	0.37	1.38	1.42	1.80	1.41	1.22	1.57	1.98	1.50	1.37	1.42	1.05	1.58	1.24	1.26	1.25	1.02	0.72	0.83
	Ψ	0.62	0.21	0.28	0.34	2.48	1.14	2.03	0.96	3.45	1.96	3.31	1.72	1.14	0.83	0.59	0.83	3.72	2.34	3.86	1.93	3.38	2.38	2.28	1.59	5.69	3.86	5.10	2.48
STR	$\Psi\pi$	3.04	2.71	3.12	2.38	3.86	2.55	3.29	2.05	4.44	2.71	4.36	2.13	3.78	3.21	2.96	2.18	4.65	3.62	5.43	2.38	4.03	3.75	3.12	2.67	5.76	3.87	5.51	3.09
	Ψp	2.42	2.50	2.94	2.04	1.38	1.41	1.26	1.09	0.99	0.75	1.05	0.41	2.64	2.38	2.37	1.35	0.93	1.28	1.57	0.45	0.65	1.37	0.84	1.08	0.07	0.01	0.41	0.61
iteaphyl	Ψ	0.90	0.41	0.62	0.62	0.52	0.65	1.00	0.86	0.90	0.48	0.76	0.55	0.62	0.76	0.55	0.65	0.96	1.38	1.10	1.00	0.76	0.69	0.59	0.65	0.62	0.65	0.48	0.83
WW	$\Psi\pi$	2.38	3.12	2.46	2.13	1.92	2.79	2.22	1.97	2.04	1.56	2.18	1.89	2.01	2.13	2.71	1.93	1.89	2.05	2.55	2.05	1.56	2.13	1.1	2.13	1.85	2.13	2.22	1.93
	Ψp	1.48	2.71	1.84	1.51	1.40	2.14	1.22	1.11	1.14	1.08	1.42	1.34	1.39	1.37	2.16	1.28	90.9	0.67	1.45	1.05	0.80	1.44	0.59	1.48	1.23	1.48	1.74	1.10
	Ψ	0.55	0.62	0.48	0.48	1.34	1.17	3.72	0.65	1.10	2.00	4.48	0.96	0.76	0.83	1.45	0.59	1.38	2.90	4.14	2.07	1.31	2.00	2.00	1.62	0.96	3.72	5.45	2.90
STR	$\Psi\pi$	2.60	3.53	2.05	3.57	2.30	2.71	6.25	1.97	2.27	3.12	5.84	1.89	2.13	2.50	2.01	1.80	2.13	3.42	5.35	2.79	2.71	3.12	3.04	3.12	2.55	3.73	6.62	3.70
	Ψp	2.05	2.91	1.57	3.09	0.96	1.54	2.53	1.32	1.17	1.12	1.36	0.93	1.37	1.67	0.56	1.21	0.75	0.52	1.21	0.72	1.40	1.12	1.04	1.50	1.59	0.01	1.17	0.80
myrtifoli	Ψ	0.28	0.48	0.41	0.21	0.41	0.62	0.72	0.55	0.69	0.69	0.55	0.48	0.45	0.34	0.28	0.41	0.48	0.55	0.62	0.41	0.31	0.45	0.48	0.38	0.48	0.65	0.59	0.45
WW	$\Psi\pi$	1.34	2.00	3.37	2.13	1.15	2.10	3.37	2.22	1.48	2.13	1.28	2.05	-*	1.89	1.89	2.13	1.89	2.13	2.30	2.38	2.10	1.77	1.97	1.25	1.20	1.76	1.68	1.02
	Ψp	1.06	1.52	2.96	1.92	0.74	1.48	2.65	1.67	0.79	1.44	0.73	1.57	-*	1.55	1.61	1.72	1.41	1.58	1.68	1.97	1.79	1.32	1.49	0.87	0.72	1.11	1.09	0.57
	Ψ	0.76	0.62	0.55	0.34	1.17	2.62	3.10	0.96	1.65	2.69	3.03	1.93	0.45	0.62	0.41	0.38	2.76	3.79	3.59	2.83	1.93	2.52	2.34	2.07	4.10	5.17	5.52	3.79
STR	$\Psi\pi$	2.84	2.55	2.22	2.46	2.55	3.45	4.28	1.89	2.46	3.95	6.34	2.63	2.79	3.04	2.71	1.72	3.70	5.68	5.07	3.78	2.88	3.24	2.96	2.61	5.64	6.09	5.77	4.55
	Ψp	2.08	1.93	1.67	2.12	1.38	0.83	1.18	0.93	0.81	1.26	3.31	0.70	2.34	2.42	2.30	1.34	0.94	1.89	1.48	0.95	0.95	0.72	0.62	0.54	1.54	0.92	0.25	0.76

*NB: * The original value was missing, but was replaced while fitting the split-plot using the default method of Genstat 5 anova procedure.*

Table 4.2.b

The average values (n=4) of *original* data points of water potentials (Ψ, -MPa), *calibrated* data points of osmotic potentials (Ψ_π, -MPa) and turgor pressure (Ψ_p, -MPa) from 7 harvests in the field experiment of 5 *Acacia* species.

TR = treatments, PRMT = parameters, X = the mean values over 7 harvests.

SP/TR/PRMT		TIME OF HARVESTING							
		I	II	III	IV	V	VI	VII	X (Mean)
A. anceps									
	Ψ	0.43	0.71	0.71	0. 59	1.07	0.82	0.91	0.75 ± 0.19
WW	Ψ_π	2.36	1.93	1.46	1.96	2.40	2.26	2.33	2.10 ± 0.31
	Ψ_p	1.93	1.21	0.75	1.36	1.33	1.44	1.42	1.35 ± 0.32
	Ψ	0.35	2.16	2.16	0.48	4.00	3.05	5.53	2.53 ± 1.72
STR	Ψ_π	2.98	3.08	3.16	3.42	5.54	4.84	6.06	4.16 ± 1.20
	Ψ_p	2.62	0.92	0.99	2.94	1.54	1.79	0.53	1.62 ± 0.82
A. aneura									
	Ψ	0.30	0.64	0.33	0.46	0.83	0.62	0.75	0.56 ± 0.19
WW	Ψ_π	3.17	2.61	2.09	2.32	2.67	2.22	2.05	2.45 ± 0.37
	Ψ_p	2.87	1.97	1.77	1.85	1.84	1.60	1.30	1.89 ± 0.45
	Ψ	0.33	1.27	2.39	0.41	2.81	1.98	3.68	1.84 ± 1.15
STR	Ψ_π	3.26	3.28	3.33	3.35	4.46	2.99	4.52	3.60 ± 0.57
	Ψ_p	2.93	2.01	0.93	2.94	1.65	1.01	0.84	1.76 ± 0.84
A. gillii									
	Ψ	0 .39	0.64	0.67	0.48	0.56	0.54	0.65	0.56 ± 0.09
WW	$\Psi\pi$	2.41	2.16	1.75	1.98	2.13	1.82	1.60	1.98 ± 0.26
	Ψ	2.02	1.52	1.08	1.50	1.57	1.28	0.95	1.42 ± 0.33
	Ψ	0.36	1.65	2.61	0.84	2.96	2.40	4.28	2.16 ± 1.23
STR	$\Psi\pi$	2.81	2.94	3.41	3.03	4.02	3.39	4.56	3.45 ± 0.59
	Ψp	2.45	1.28	0.80	2.18	1.05	0.99	0.27	1.29 ± 0.71
A. iteaphylla									
	Ψ	0.64	0.76	0.67	0.65	1.11	0.67	0.65	0.74 ± 0.16
WW	Ψ_π	2.53	2.22	1.92	2.20	2.13	1.75	2.03	2.11 ± 0.23
	Ψ_p	1.89	1.47	1.24	1.55	1.02	1.08	1.38	1.38 ± 0.28
	Ψ	0.53	1.72	2.14	0.91	2.62	1.73	3.26	1.84 ± 0.87
STR	Ψ_π	2.94	3.31	3.28	2.11	3.42	3.00	4.15	3.17 ± 0.57
	Ψ_p	2.40	1.58	1.14	1.21	0.80	1.28	0.90	1.33 ± 0.50
A. myrtifolia									
	Ψ	0.34	0.58	0.60	0.37	0.52	0.40	0.54	0.48 ± 0.10
WW	Ψ_π	2.21	2.21	1.74	1.84	2.18	1.77	1.42	1.91 ± 0.28
	Ψ_p	1.87	1.63	1.13	1.51	1.66	1.37	0.87	1.43 ± 0.32
	Ψ	0.57	1.96	2.33	0.46	3.24	2.22	4.65	2.20 ± 1.35
STR	Ψ_π	2.52	3.04	3.84	2.57	4.56	2.92	5.51	3.57 ± 1.04
	Ψ_p	1.95	1.08	1.52	2.10	1.32	0.70	0.86	1.36 ± 0.49

Figure 4.3.a

The seasonal fluctuations of ***water potential*** (MPa) in the field grown plants of five species measured on well watered and stressed plants. These data are plotted against harvest and time.

Significant rainfalls are plotted on the bottom (x-axis). Only high rainfall days (more than 10 mm) are dated. For legends, see Figure.

Note that on the x-axis the harvesting dates are attached, and ticks indicate weeks approximately.

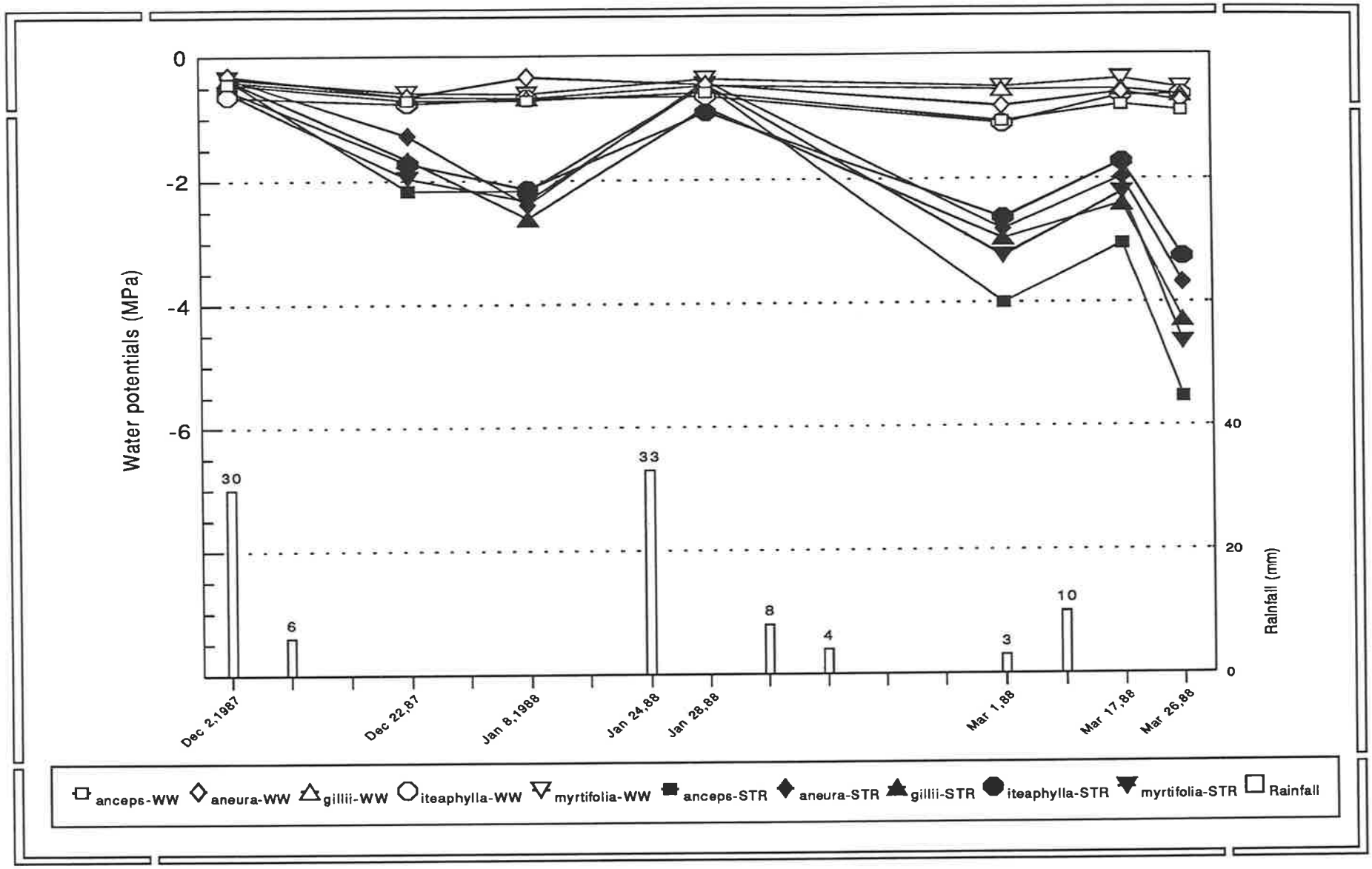

0
-2
-4
-6
Water potentials (MPa)
40
20
0
Rainfall (mm)
30
6
33
8
4
3
10
Dec 2,1987
Dec 22,87
Jan 8,1988
Jan 24,88
Jan 28,88
Mar 1,88
Mar 17,88
Mar 26,88
anceps-WW
aneura-WW
gillii-WW
iteaphylla-WW
myrtifolia-WW
anceps-STR
aneura-STR
gillii-STR
iteaphylla-STR
myrtifolia-STR
Rainfall

Figure 4.3.b

The seasonal fluctuations of ***osmotic potential*** (MPa) in the field grown plants of five species measured on well watered and stressed plants. These data are plotted against harvest and time.

Significant rainfalls are plotted on the bottom (x-axis). Only high rainfall days (more than 10 mm) are dated. For legends, see Figure.

Note that on the x-axis the harvesting dates are attached, and ticks indicate weeks approximately.

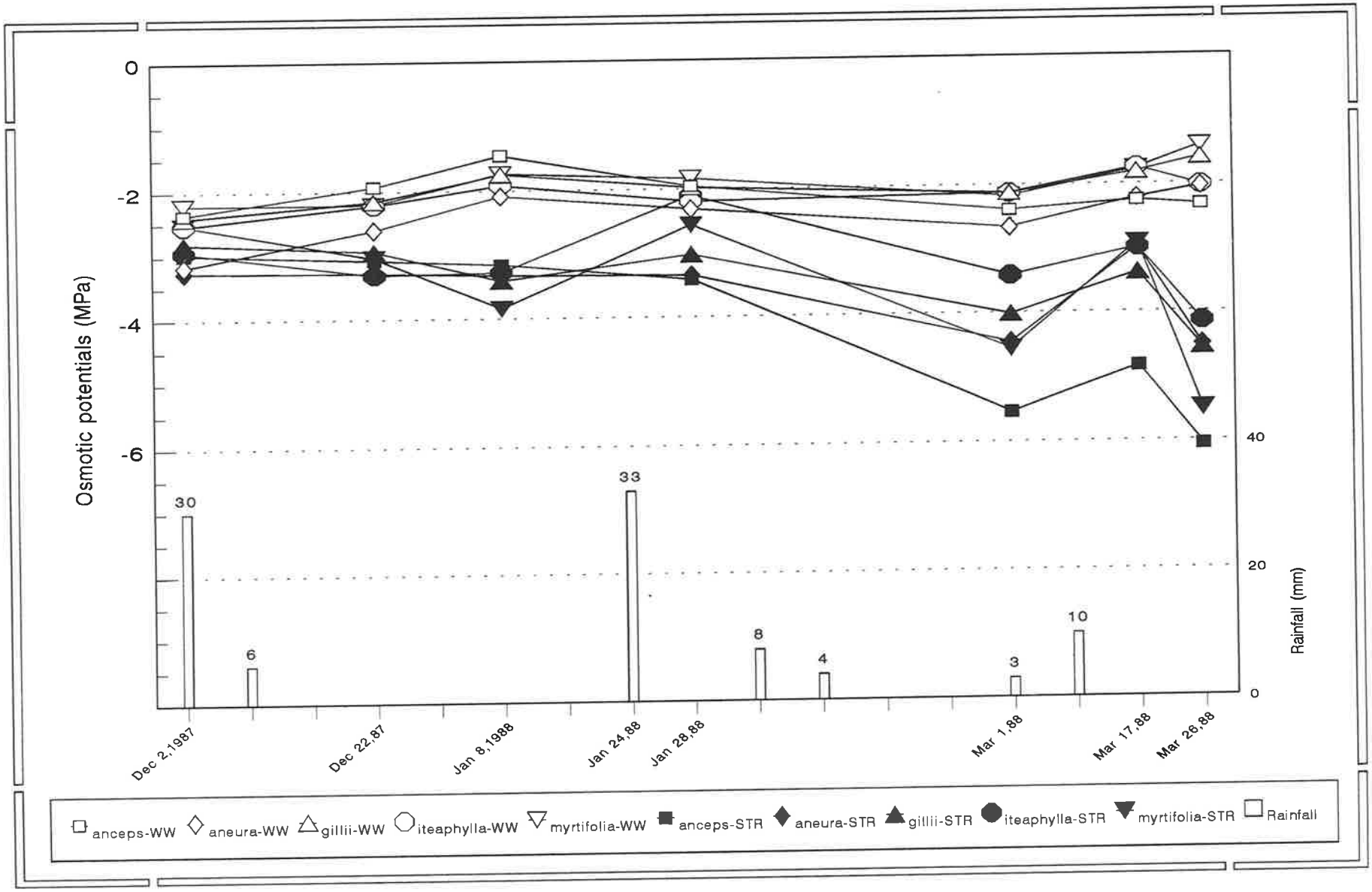
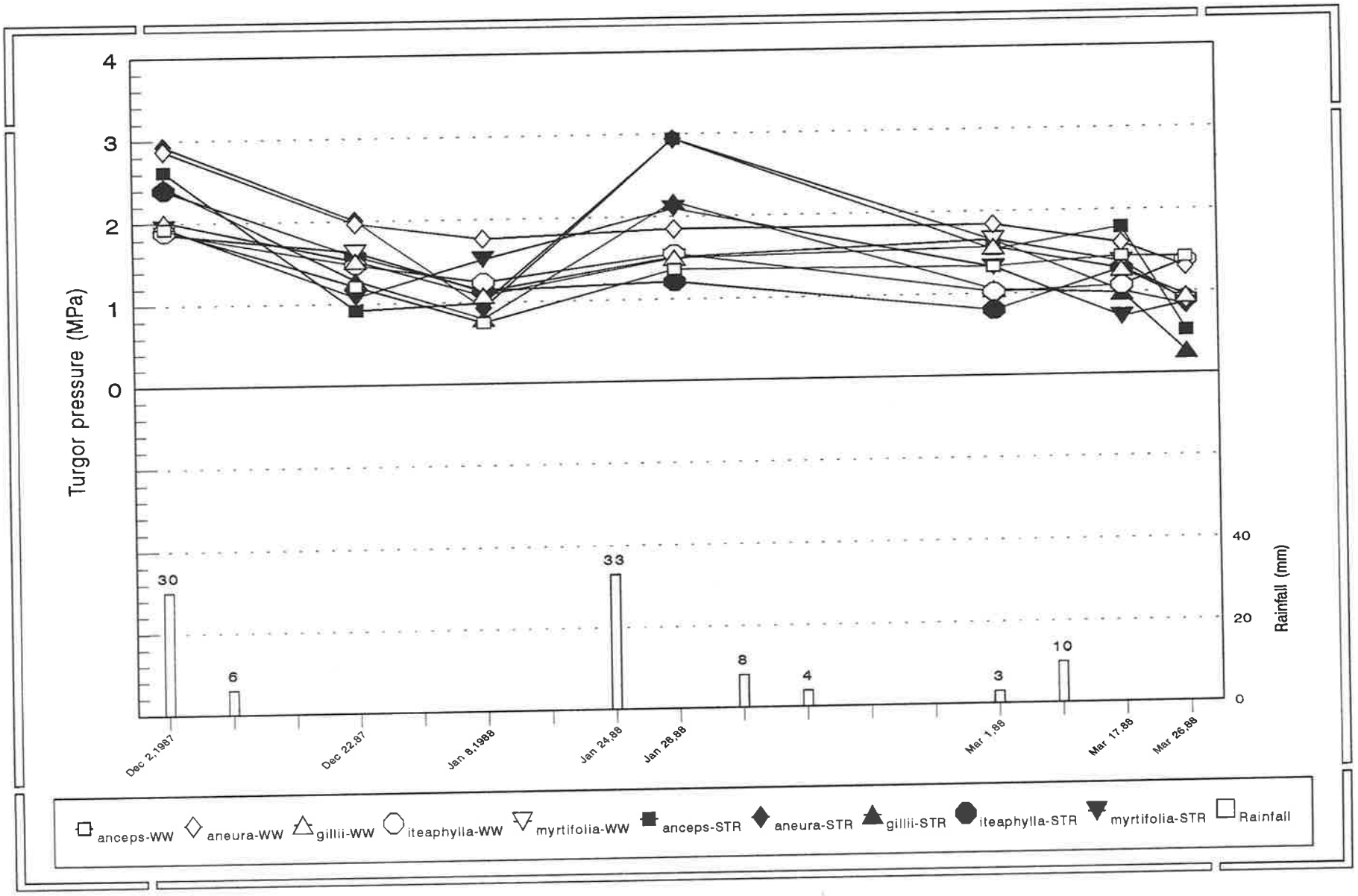

Osmotic potentials (MPa)
0
-2
-4
-6
Rainfall (mm)
40
20
0
30
6
33
8
4
3
10
Dec 2,1987
Dec 22,87
Jan 8,1988
Jan 24,88
Jan 28,88
Mar 1,88
Mar 17,88
Mar 26,88
anceps-WW
aneura-WW
gillii-WW
iteaphylla-WW
myrtifolia-WW
anceps-STR
aneura-STR
gillii-STR
iteaphylla-STR
myrtifolia-STR
Rainfall

Figure 4.3.c

The seasonal fluctuations of ***turgor pressure*** (MPa) in the field grown plants of five species measured on well watered and stressed plants. These data are plotted against harvest and time.

Significant rainfalls are plotted on the bottom (x-axis). Only high rainfall days (more than 10 mm) are dated. For legends, see Figure.

Note that on the x-axis the harvesting dates are attached, and ticks indicate weeks approximately.


Turgor pressure (MPa)
4
3
2
1
0
40
20
0
Rainfall (mm)
30
6
33
8
4
3
10
Dec 2,1987
Dec 22,87
Jan 8,1988
Jan 24,88
Jan 28,88
Mar 1,88
Mar 17,88
Mar 26,88
anceps-WW
aneura-WW
gillii-WW
iteaphylla-WW
myrtifolia-WW
anceps-STR
aneura-STR
gillii-STR
iteaphylla-STR
myrtifolia-STR
Rainfall

A 10 mm precipitation (after the fifth harvest) clearly did not restore sufficient cellular water to face the coming (third) stress period. Only two weeks after the sixth, the seventh harvest showed drastic loss of water as indicated by the steeper slope of water potential curves downward. These drops brought water potential of all stressed plants to the lowest level during the experiment, with the difference in mean water potentials between stressed and control plants at this last harvest being 3.58 MPa.

The seventh harvest was the time when all plants underwent the most stress during the experiment as seen from their water potential values. However, even at these lowest water potentials, none of the species had reached zero turgor.

If there were enough soil water available due to rainfall, water potential of unwatered plants would quickly increase to equal the well-watered treatment values. Conversely, these values would drastically decline if there was lack of soil water (due to lack of rain). Hence, large fluctuations were shown by all species, but the development of water stress was slower than in the pot experiments described in Chapter 3.

4.4.3.a.. *Statistical considerations*

These fluctuation phenomena were interpreted by the split-plot statistical analysis. The results of statistical considerations are presented in Tables below. Table 4.3.a presents the Anova and Table 4.3.b the mean estimates of water potential. Tables 4.4.a and 4.4.b show the same aspects for osmotic potential, and Tables 4.5.a and 4.5.b, for turgor pressure. *Note*: the units in these Tables are bars, not MPa.

The statistical analysis shows that:

a. There was variation in water potential and osmotic potential over time, but an additive model was retained. This means that at any time, a watered plant differs from a stressed plant, of the same species, but these differences are the same for each species.

Table 4.3.a

Analysis of variance for ***water potential*** data of 5 field grown *Acacia* species.

Variate: water potential (bars)

Source of variation	d.f	ss	m.s	v.r	F.pr
plant stratum					
species	4	619.08	154.77	0.91	0.469
stress	1	15734.85	15734.85	92.92	<.001
species.stress	4	479.84	119.96	0.71	0.593
Residual	30	5080.05	169.34		
plant.time stratum					
time	6	12778.77	2129.79	93.96	<.001
time.species	24	953.45	39.73	1.75	0.021
time stress	6	9390.63	1565.10	69.05	<.001
time.species.stress	24	692.56	28.86	1.27	0.188
Residual	180	4080.00	22.67		
Total	279	49809.32			

Table 4.3.b.

The mean estimate values for ***water potential*** of 5 field grown *Acacia* species.

Variate: water potential (bars)

Grand mean: 13.68

time	1	2	3	4	5	6	7
	4.25	12.10	14.63	5.67	19.72	14.45	24.90

species	anceps	aneura	gillii	iteaphylla	myrtifolia
	16.43	12.01	13.61	12.90	13.42

stress	ww	str
	6.18	21.17

time	species	anceps	aneura	gillii	iteaphylla	myrtifolia
1		3.92	3.15	3.75	5.86	4.57
2		14.40	9.52	11.46	12.41	12.72
3		14.39	13.62	16.42	14.05	14.66
4		5.39	4.39	6.64	7.76	4.18
5		25.34	18.19	17.63	18.66	18.79
6		19.35	13.02	14.74	12.03	13.10
7		32.24	22.15	24.66	19.52	25.95

time	stress	ww	str
1		4.21	4.29
2		6.66	17.55
3		5.98	23.27
4		5.12	6.23
5		8.17	31.27
6		6.12	22.78
7		7.00	42.81

species	stress	ww	str
anceps		7.51	25.36
aneura		5.62	18.40
gillii		5.62	21.61
iteaphylla		7.35	18.45
myrtifolia		4.80	22.04

Table 4.4.a

Analysis of variance for ***osmotic potential*** data of 5 field grown *Acacia* species.

Variate: osmotic potential (bars)

Source of variation	df	ss	m.s	v.r	F.pr
plant stratum					
species	4	1016.45	254.11	1.18	0.338
stress	1	15325.14	15325.14	71.45	<.001
species.stress	4	901.96	225.49	1.05	0.398
Residual	30	6434.27	214.48		
plant.time stratum					
time	6	3412.02	568.67	13.60	<.001
time.species	24	1913.80	79.74	1.91	0.009
time stress	6	4931.06	821.84	19.66	<.001
time.species.stress	24	839.70	34.99	0.84	0.686
Residual	179(1)	7482.88	41.80		
Total	278(1)	42058.99			

Table 4.4.b.

The mean estimate values for ***osmotic potential*** of 5 field grown *Acacia* species.

Variate: osmotic potential (bars)

Grand mean: 28.49

time	1	2	3	4	5	6	7
	27.20	26.78	25.98	24.77	33.52	26.98	34.24

species	anceps	aneura	gilli	iteaphylla	myrtifolia
	31.28	30.24	27.15	26.43	27.37

stress	ww	str
	21.10	35.89

time	species	anceps	aneura	gillii	iteaphylla	myrtifolia
1		26.70	32.18	26.12	27.32	23.65
2		25.05	29.44	25.47	27.67	26.25
3		23.10	27.11	25.80	25.98	27.90
4		26.89	28.35	25.05	21.55	22.03
5		39.74	35.66	30.73	27.79	33.66
6		35.52	26.04	26.09	23.76	23.47
7		41.98	32.87	30.78	30.92	34.64

time	stress	ww	str
1		25.37	29.02
2		22.26	31.30
3		17.92	34.04
4		20.58	44.01
6		19.66	34.29
7		18.86	49.61

species	stress	ww	str
anceps		21.01	41.56
aneura		24.48	35.99
gillii		19.78	34.52
iteaphylla		21.12	31.74
myrtifolia		19.09	35.66

Table 4.5.a

Analysis of variance for ***turgor pressure*** data of 5 field grown *Acacia* species.

Variate: turgor pressure (bars)

Source of variation	df	ss	m.s	v.r	F.pr
plant stratum					
species	4	876.59	219.15	9.00	<.001
stress	1	3.06	3.06	0.13	0.725
species.stress	4	154.48	38.62	1.59	0.204
Residual	30	730.52	24.35		
plant.time stratum					
time	6	5327.14	887.86	41.21	<.001
time.species	24	1148.73	47.86	2.22	0.002
time stress	6	1030.93	171.82	7.98	<.001
time.species.stress	24	858.22	35.76	1.66	0.034
Residual	179(1)	3856.50	21.54		
Total	278(1)	13976.58			

Table 4.5.b.

The mean estimate values for ***turgor pressure*** of 5 field grown *Acacia* species.

Variate: turgor pressure (bars)

Grand mean: 14.82

time	1	2	3	4	5	6	7
	22.94	14.68	11.35	19.15	13.79	12.53	9.33

species	anceps	aneura	gillii	iteaphylla	myrtifolia
	14.85	18.23	13.54	13.53	13.98

stress	ww	str
	14.93	4.72

time	species	anceps	aneura	gillii	iteaphylla	myrtifolia
1		22.78	29.03	22.37	21.45	19.08
2		10.66	19.92	14.01	15.26	13.54
3		8.71	13.49	9.38	11.93	13.24
4		21.51	23.96	18.41	13.79	18.07
5		14.40	17.47	13.10	9.13	14.87
6		16.17	13.03	11.35	11.73	10.37
7		9.73	10.71	6.12	11.39	8.70

time	stress	ww	str
1		21.16	24.73
2		15.60	13.75
3		11.93	10.77
4		15.55	22.74
5		14.85	12.74
6		13.54	11.52
7		11.86	6.80

species	stress	ww	str
anceps		13.50	16.20
aneura		18.86	17.60
gillii		14.17	12.91
iteaphylla		13.77	13.29
myrtifolia		14.35	13.61

continued next page

Table 4.5.b continued:

	species	anceps		aneura		gillii		iteaphylla		myrtifolia	
time	stress	ww	str	ww	str	ww	str	ww	str	ww	str
1		19.30	26.25	28.72	29.34	20.23	24.52	18.88	24.03	18.68	19.49
2		12.13	9.18	19.71	20.13	15.18	12.83	14.67	15.85	16.32	10.76
3		7.49	9.92	17.66	9.33	10.77	8.00	12.44	11.41	11.32	15.17
4		13.62	29.39	18.55	29.36	14.97	21.85	15.50	12.08	15.12	21.02
5		13.34	15.45	18.43	16.52	15.66	10.54	10.23	8.04	16.58	13.16
6		14.45	17.89	15.96	10.09	12.83	9.88	10.80	12.67	13.68	7.05
7		14.17	5.30	13.03	8.40	9.53	2.72	13.85	8.93	8.75	8.65

Note: For turgor pressure, these means are displayed here (repeated as in Table 4.2.b), since the anova showed that differences between them are significant. See text.

b. At any time, one species differs from another species given the same treatment, but these differences are the same, whether the plants are watered or stressed.

As shown in Figure 4.3.a, at harvest 7 for example, the differences between well-watered *A.iteaphylla* and its stressed treatment is not significantly different from the difference between well-watered *A.anceps* and stressed *A.anceps*. As another example, from Table 4.3.b, the expected difference between water potential of a well-watered *A.anceps* and a well-watered *A.aneura* is -0.08 MPa at the first harvesting, and -0.49 MPa at the second harvesting. The expected difference between a stressed *A.anceps* and a stressed *A.aneura* is also -0.08 MPa and -0.49 MPa at the first and the second harvesting. The magnitude of the water potentials also differs significantly between stress levels for all species. But this magnitude is not significantly different between species. Take an example, the expected differences between a well-watered and a stressed *A.anceps* is -0.008 MPa and -1.09 MPa at harvesting one and two; the expected differences of *A.aneura* between the same treatments and harvesting also would be the same i.e. -0.008 MPa and -1.09 MPa. No interaction was found between the three variates time, species and stress.

Table 4.4 revealed a similar pattern of osmotic potential as water potential, i.e. a well-watered plant differs from a stressed plant of the same species, but the difference is the same for all species. Also, the magnitude of the difference between two species is the same whether the two are well-watered or stressed. The statistical analysis, as before, did not show interactions between the variates time, species and stress.

For turgor pressure, however (Table 4.5), interactions were found between the three variates i.e. time, species and stress, thus an interaction model could be retained. This means that:

a. Turgor pressure varied over time

b. At any time, a watered plant had a different turgor pressure from a stressed plant of the same species, and these differences differed among species.

c. At any time, one species differed from another species given the same treatment, and these differences differed between watered and stressed plants.

As an example of these significant differences, in Table 4.5 and Figure 4.3.c, the expected difference in turgor pressure, between a well-watered *A.anceps* and a well-watered *A.aneura* is 0.94 MPa at harvest I, and 0.76 MPa at harvest II, while the expected difference in turgor pressure between a stressed *A.anceps* and a stressed *A.aneura* is 0.31 MPa at harvest I and 1.1 MPa at harvest II. Therefore, fluctuations of turgor pressure during the summer season occurred, and the magnitude of the fluctuations varied between species and water treatment.

4.4.3.b. *Evidence of osmotic adjustment*

Osmotic adjustment may be seen if osmotic potential fluctuates against water potential changes in such a way as to generate turgor maintenance. For the detection of seasonal osmotic adjustment, these fluctuations should be seen over a season where the stress may occur intermittently.

However, the declining of osmotic potential itself is not always an indication of osmotic adjustment, since the loss of water from cells would itself be followed by the decline in osmotic potential due to the passive solutes concentration effect (Turner and Jones, 1980; Morgan, 1984).

Table 4.5.b and Figure 4.3.c show the different magnitude of turgor pressure within treatments and species. For example, at fourth harvest, water potentials were similar for stressed and unstressed plants, but in all but *A.iteaphylla*, osmotic potential was more

negative, hence, turgor pressure was greater in the stressed plants. This indicates that some osmotic adjustment had previously taken place, because the stressed plants had greater turgor when their water potential values were approximately the same as the watered controls. The exact degree of osmotic adjustment cannot be calculated but these results are suggestive.

Turgor pressure variations differed significantly among species and between treatments. For *A.anceps*, the stressed plants' turgor was greater than the control at 5 of the 7 harvests. For other species, stressed plant turgor was below the control in most cases, but rose above occasionally. For all, stressed turgor was less than control on the last harvest, when stress was greatest. This evidence suggests that the turgor pressure is not simply changing in response to changes in water potential and consequently in osmotic potential, but is being modified by osmotic adjustment.

The evidence for field osmotic adjustment is shown more clearly in next Section, by examining the relationship between water potential and osmotic potential, using the same exponential relationship model as used in Chapter 3.

4.4.3.c. *Exponential relationship between water potential and osmotic potential*

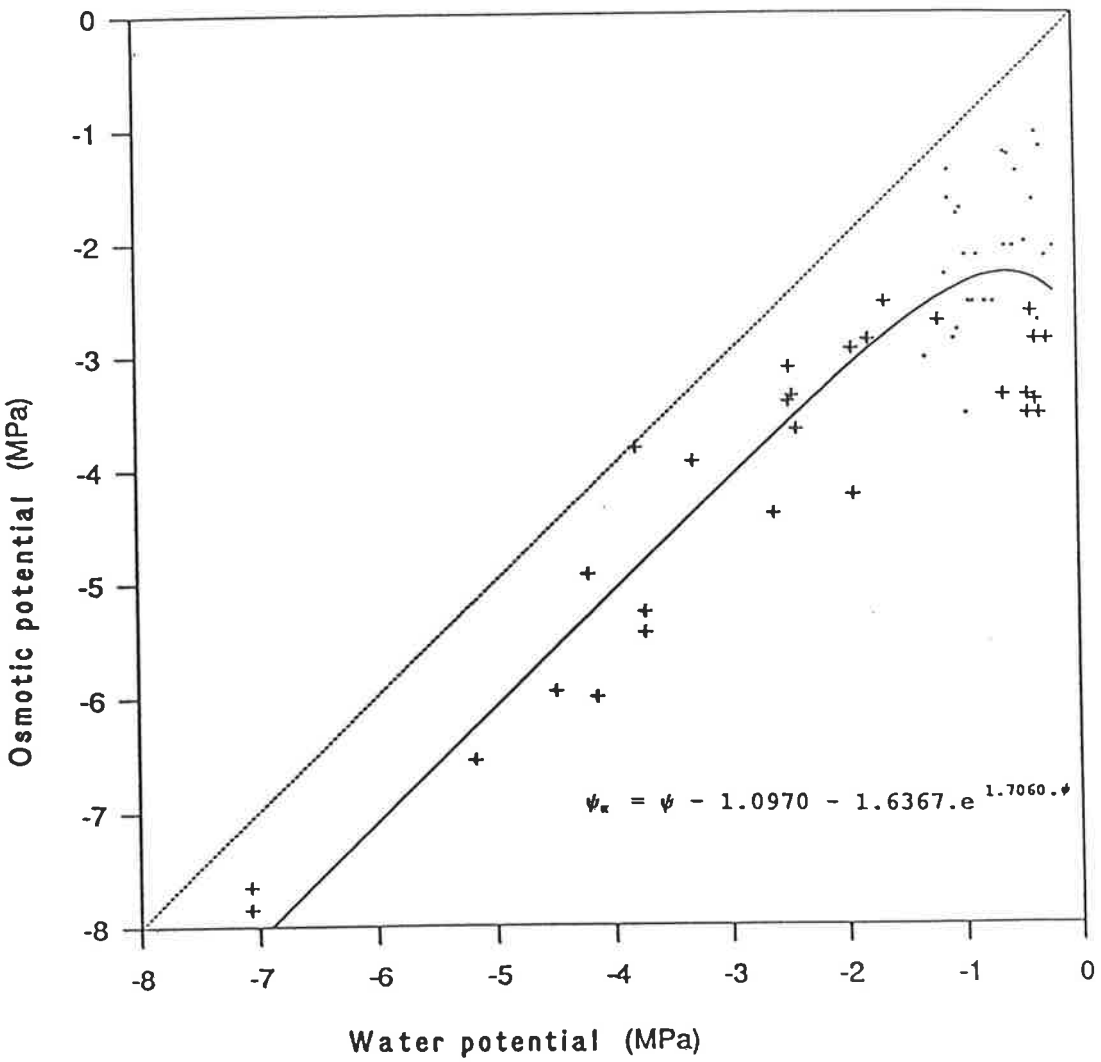
Equation (6) Section 3.3 represented the relationship between water potential and osmotic potential in an exponential form. Figure 4.4 displays this exponential relationship for each species of field grown plants (as was done for the glasshouse experiment, in Chapter 3). These curves were fitted using all the data points gathered from both well-watered and stressed treatments during the seven harvests (n = 56, for each species, except for *A.myrtifolia* where n = 55). Table 4.6 presents the equation and coefficients of the exponential regressions. It was found that the exponential lines fitted

Figure 4.4

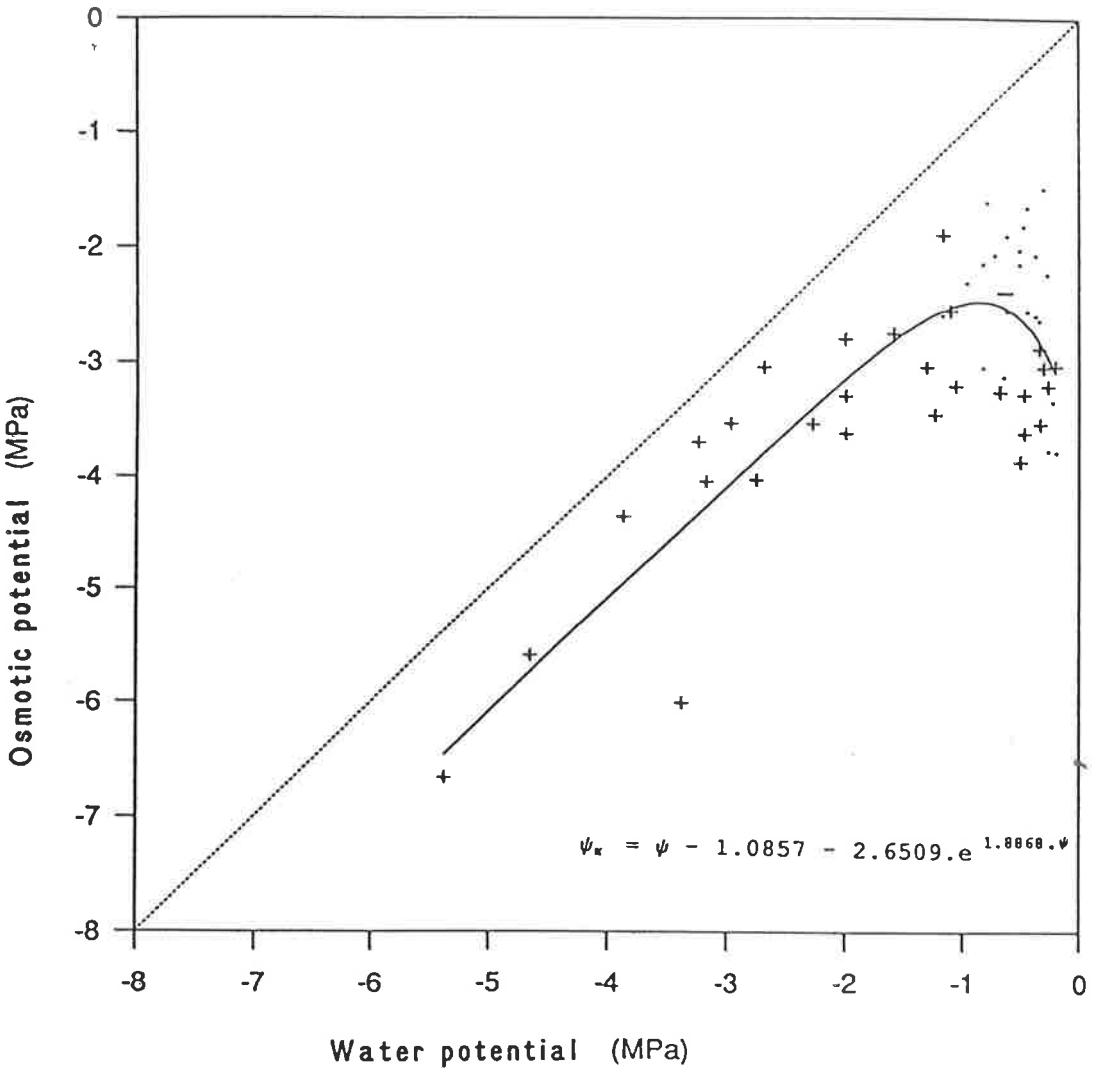
The exponential relationship between the two variables water potential and osmotic potential during seven harvests in the field grown plants of five *Acacia* species. Units are (MPa).

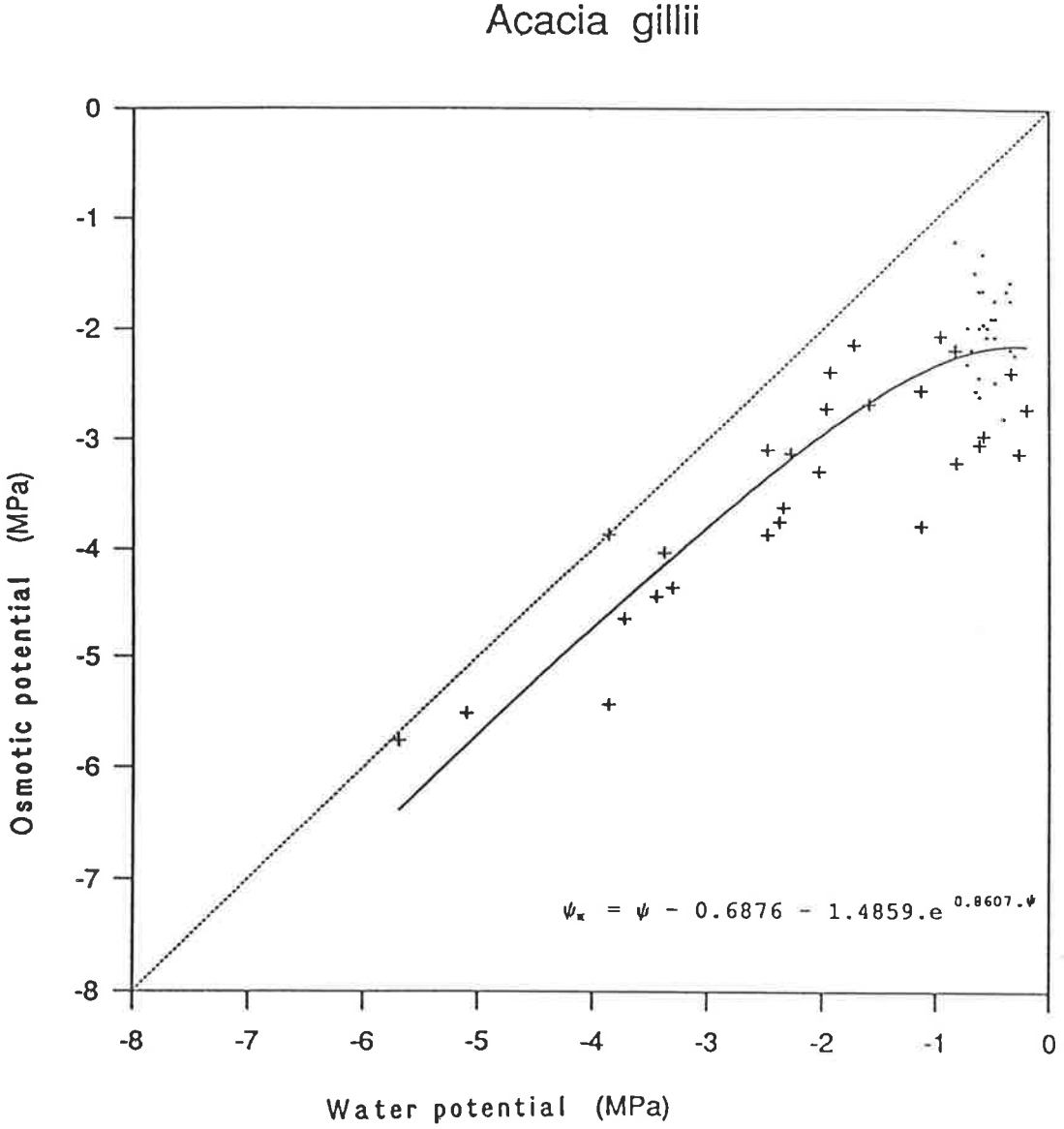
• well-watered

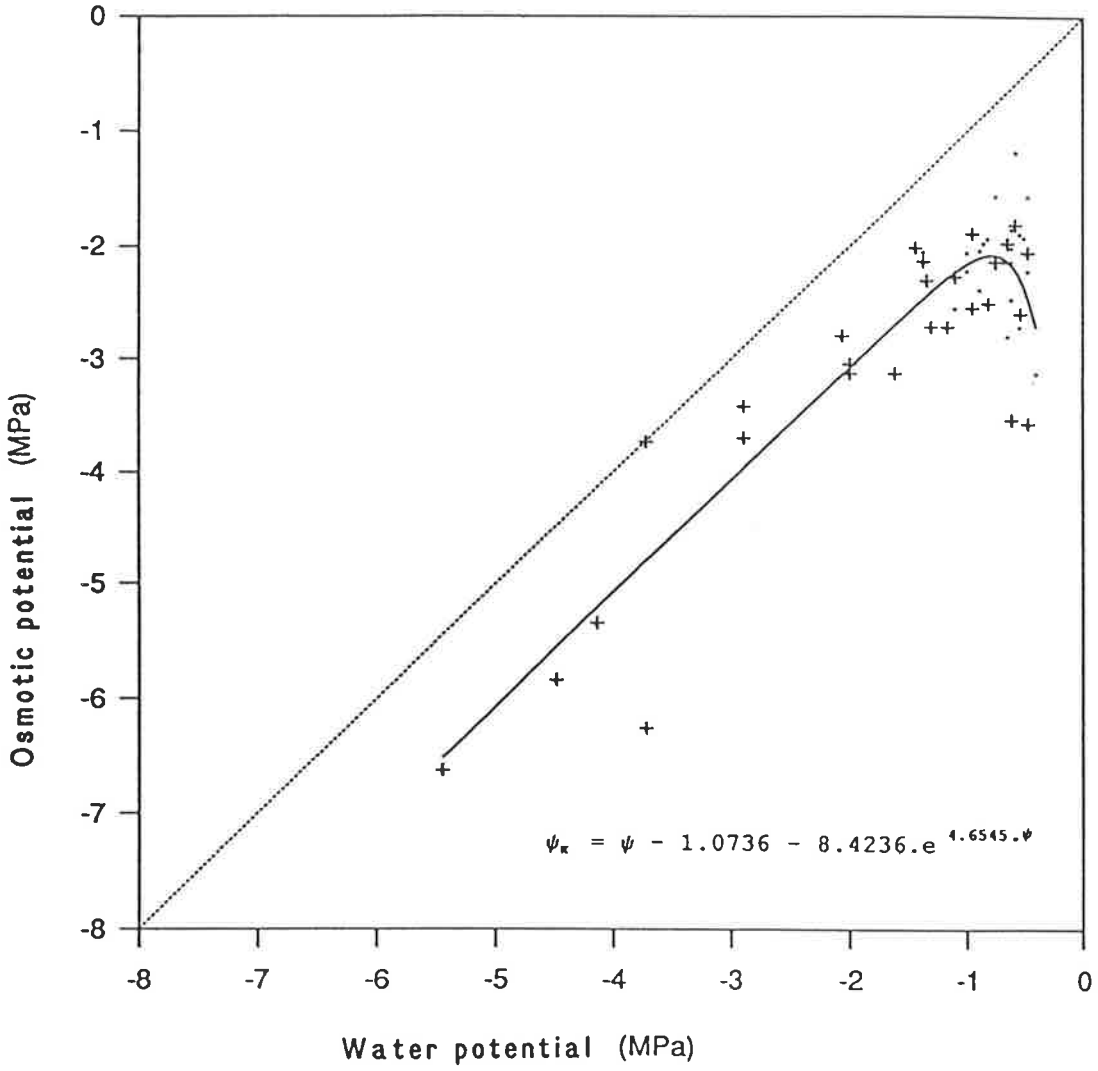
+ stressed


Acacia anceps
0
-1
-2
-3
-4
-5
-6
-7
-8
Osmotic potential (MPa)
-8
-7
-6
-5
-4
-3
-2
-1
0
Water potential (MPa)
$\psi_\pi = \psi - 1.0970 - 1.6367.e^{1.7060.\phi}$

Acacia aneura




Acacia gillii
0
-1
-2
-3
-4
-5
-6
-7
-8
Osmotic potential (MPa)
$\psi_\pi = \psi - 0.6876 - 1.4859.e^{0.8607.\psi}$
-8
-7
-6
-5
-4
-3
-2
-1
0
Water potential (MPa)


Acacia iteaphylla
0
-1
-2
-3
-4
-5
-6
-7
-8
Osmotic potential (MPa)
-8
-7
-6
-5
-4
-3
-2
-1
0
Water potential (MPa)
$\psi_\pi = \psi - 1.0736 - 8.4236.e^{4.6545.\psi}$

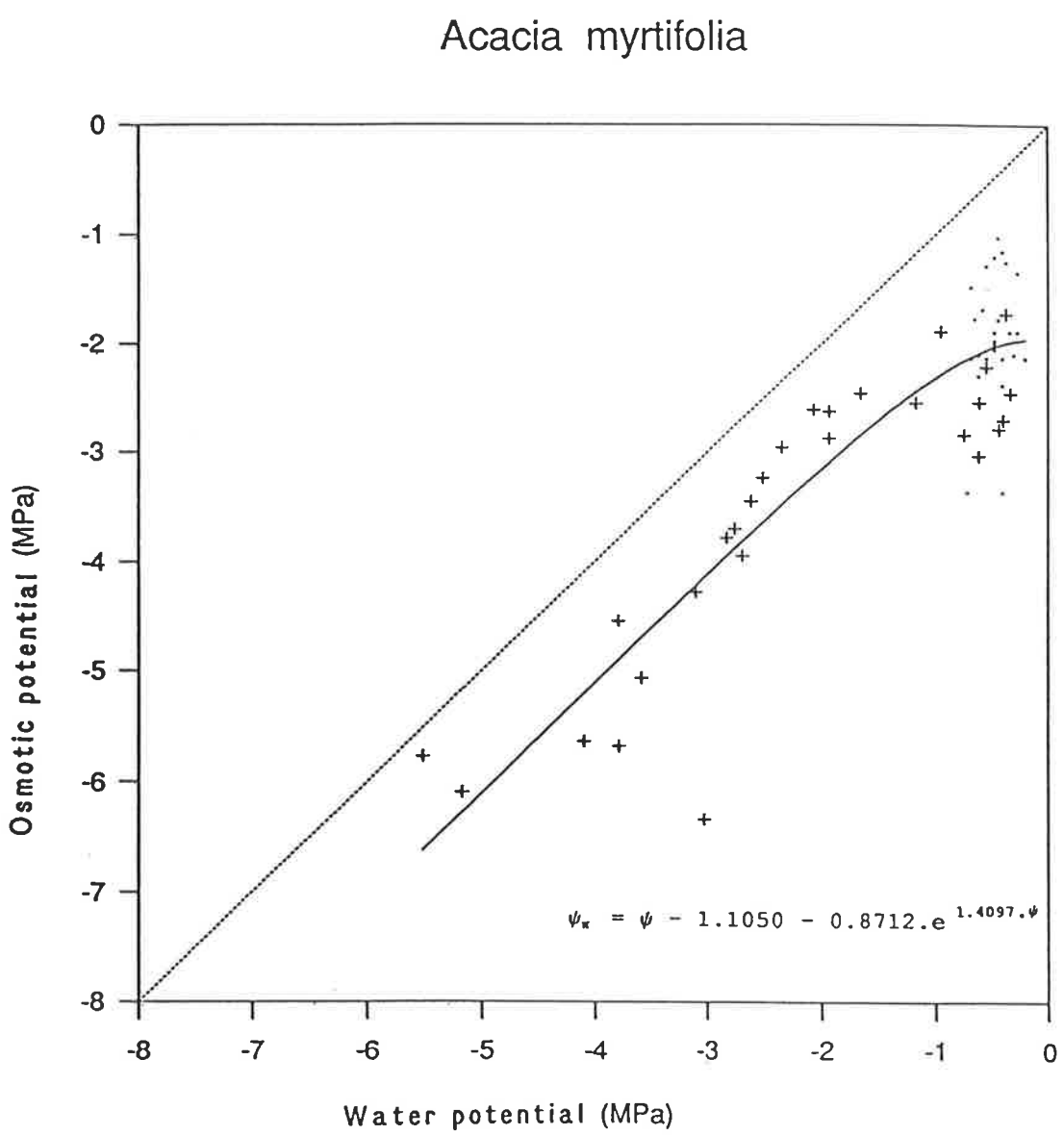

Acacia myrtifolia
0
-1
-2
-3
-4
-5
-6
-7
-8
Osmotic potential (MPa)
-8
-7
-6
-5
-4
-3
-2
-1
0
Water potential (MPa)
$\psi_\pi = \psi - 1.1050 - 0.8712.e^{1.4097.\psi}$

Table 4.6

The coefficients of the exponential regression describing the relationship between water potential and osmotic potential of *field grown* plants of 5 *Acacia* species. The two variables were measured during seven harvests throughout a summer season. The equation of the regression has the form:

$$\Psi = \Psi_\pi - \beta_0 - \beta_1 e^{\gamma\Psi}$$

where Ψ is water potential, Ψ_π is osmotic potential and β_0, β_1 and γ are constants. Units are MPa. (*cf Table 3.9*).

Species	β_0	β_1	γ	SE(β_0)	SE(β_1)	SE(γ)	r^2
A.anceps	1.0970	1.6367	1.7060	0.17975	0.65409	1.64395	0.8112
A.aneura	1.0857	2.6509	1.8868	0.18089	0.57814	1.01104	0.6443
A.gillii	0.6876	1.4859	0.8607	0.25061	0.29185	0.62501	0.7735
A.iteaphylla	1.0736	8.4236	4.6545	0.11359	8.65344	6.83476	0.8097
A.myrtifolia	1.1050	0.8712	1.4097	0.18615	0.58809	2.63546	0.7926

reasonably well to the scattered data points of all species. Their r^2 values provide evidence of a good relationship between the two parameters.

As mentioned in Chapter 3, to achieve the qualification of having an osmotic adjustment, it is required that β_0 (in Table 4.6) must be at least twice its standard error. Values in the Table show that all species have fulfilled this requirement. Hence, significant osmotic adjustment occurred in all species.

The term β_0 represents approximately the difference between osmotic potential and water potential at low water potential. Hence it is a measure of the degree of adjustment. Table 4.6 shows that the osmotic adjustment was about the same, namely 1.1 MPa in 4 of the species. *A.gillii* was somewhat lower, at 0.7 MPa. Thus the exponential relationship demonstrates turgor maintenance in all species during this field experiment.

4.5. *Discussion*

4.5.1. *Field osmotic adjustment*

The water potentials and osmotic potentials of stressed plants changed over time in response to the stress; but the measurement could not detect significant differences between species in the amount of stress shown by the lowered water potential and osmotic potential. However, for turgor pressure, significant differences do show up between species in the amount of change in turgor pressure between watered and stressed plants at each harvest.

Overall, Figures 4.3 a,b,c and Table 4.2.b revealed that osmotic potential fluctuations followed the pattern of water potential, suggesting turgor maintenance did occur. The existence of osmotic adjustment within these *Acacia* species throughout a season was confirmed by the non-linear analysis of the relationship between water potential and osmotic potential. The analysis revealed that all species developed osmotic adjustment during stress

periods. The magnitudes of osmotic adjustment among these 5 species were similar ranging from 0.7 to 1.1 MPa (represented as β_0 in Table 4.6). The four species which showed osmotic adjustment in the glasshouse experiment (Chapter 3) retained their behaviour in the field as well. Also the degree of osmotic adjustment of the plants of these 4 species in the field was rather greater than in the glasshouse where the range was between 0.3 to 1 MPa (compare β_0 in Table 3.9 and Table 4.6). *A.anceps* retained its magnitude in the field (1.10 MPa) compared to glasshouse (1.04 MPa), but the other 3 species developed a bigger osmotic adjustment when grown in the field: *A.Aneura* (0.86, 1.08 MPa: 0.23 MPa different), *A.gillii* (0.36, 0.69 MPa: 0.33 MPa different), and *A.myrtifolia* (0.26, 1.11 MPa: 0.85 MPa different). *A. iteaphylla*, which did not show significant osmotic adjustment in the glasshouse, did so in the field (1.07 MPa). These differences in magnitude may be due to differences in the tissue age, or the rate and pattern of stress development (Myers and Neales, 1986).

The result revealed that even though these 5 species are distributed in a wide range of areas with different environmental backgrounds (see Section 3.2.1), they all demonstrated the pattern of osmotic adjustment. In spite of these differences in distribution pattern and rainfall requirements, these species behaved similarly in the degree of osmotic adjustment. *A.anceps* is distributed along coastal areas, *A.aneura* is widespread throughout inland Australia; *A.myrtifolia* found in higher rainfall areas. *A.gillii* is endemic to a small area in Eyre Peninsula (South Australia), and *A.iteaphylla* is distributed across the Flinders Ranges (South Australia) and is endemic to this area (Whibley, 1980).

Water potentials in the well-watered treatments varied very little, while in stressed treatment the variations were governed by the rainfall. Thus these young plants mainly rely on precipitation, and these fluctuations are a response to the changing soil moisture content. This feature proved that the plants were still at a very vulnerable stage, where they would

hydrate during abundant rain but lose water drastically in a rainless period. This contrasts with the report of Monson and Smith (1982) in their study of Sonoran Desert native plants. They showed that the fluctuations of water potential of *Baccharis* did not parallel soil moisture changes, and suggested that the deep tap root may be in contact with perennial underground water supplies.

The fluctuations of the water potential components of these acacias appeared to be almost fully controlled by the "soil surface' moisture fluctuations following precipitation, indicating their inability to absorb permanent ground water. This is another example of why the seedlings of arid zone species are always a high risk stage in the regeneration processes of the species itself.

4.5.2. *Comparison with other arid zone species*

The features of the stressed plants data may represent (nearly) the actual behaviour of plants grown from seed in the field, since they were allowed to undergo the climatic fluctuations without any treatment.

I could not find much data for field grown Australian acacia on the fluctuation of water relations with rainfall. This lack of reports has limited a comparative discussion of the results of this experiment. The intensive study of *A.harpophylla* by Tunstall and Connor (1975) did not include rainfall data which made it difficult to compare with these results. Also, their records of water potential for *A.aneura* and *A.harpophylla* can not be used as a comparison, since they were obtained by artificial desiccation in the laboratory.

However, early findings of Slatyer (1967b) from a six months intensive study of an *A.aneura* stand in Central Australia may give some figures. Even though without rainfall records, he found that the dawn water potential of non-irrigated stands ranged between -1.5 and -11.5 MPa. As a comparison, the lowest individual value of *A.aneura* recorded in this

experiment was -5.38 MPa. Tunstall and Connor (1975) reported the lowest dawn water potential from *A.harpophylla* was -6.7 MPa, while the most negative values of an individual plant recorded from this Black Hill experiment reached -7.07 MPa, i.e. in *A.anceps* at the seventh harvest. Thus the values from these 5 *Acacia* species were within the range of others that have been reported.

The lowest values of turgor pressure under stress recorded here seem comparable to data for *A.harpophylla* reported by Tunstall and Connor (1975). They found that under the greatest stress, the plants they studied retained a turgor pressure of 0.5 MPa.

Result from Slatyer (1967b) were used here as a comparison in discussing osmotic aspects of mulga (*A.aneura*). However, Slatyer's data should be treated carefully since the osmotic potential values in that work were obtained from a hypothetical relationship with relative turgidity, and an extrapolation based on only three original osmotic potentials measured in the field, to represent the whole trend of osmotic potential over a wide range of relative turgidity. Water potential was plotted using all data of natural and artificial dehydration (in the laboratory).

Slatyer found that osmotic potential at full turgor was about -2.2 MPa, while zero turgor was achieved at about -2.8 MPa. As relative turgidity decreased further, the (hypothetical) osmotic potential also continued to decrease and reached about -11 MPa at 20% relative turgidity. Measured water potentials were more negative than calculated osmotic potentials at relative turgidity values below the zero turgor point thus showing apparent "negative turgor".

Sinclair (pers. comm.) recorded a turgor pressure of about 3 MPa which was maintained during a two year study of fluctuating water potentials in a mature field grown *Eucalyptus cladocalyx* tree. This is a higher level of turgor maintenance than was recorded from individual *Acacia* plants in this study. As shown in Table 4.2.b, all values of turgor

pressure have fallen below 2 MPa by the third harvest, and dropped below 1 MPa at the seventh harvest.

Myers and Neales (1986) summarized some reports on other field grown arid zone species: 0.5 MPa osmotic adjustment in evergreen desert species (Monson and Smith, 1982), 1.2 MPa in *Tsuga canadensis* (Tyree et al, 1978), and 1.5 MPa in *Eucalyptus* (Myers and Neales, 1984). Nielsen et al (1983) measured a diurnal osmotic adjustment as big as 0.5 to 0.7 MPa in a desert species *Prosopis glandulosa*, and a seasonal fluctuation between 1.51 to 3.30 MPa.

Among data for non-arid zone species, Ford and Wilson (1981) measured 3 tropical grasses and 1 legume. They found the range of osmotic adjustment in green panic as big as 0.55 MPa., buffel grass 0.71 MPa, speargrass 0.39 MPa and the legume siratro 0.34 MPa. Among cultivated species, Wenkert et al (1978) detected a 0.4 MPa seasonal adjustment in field grown soybean. Jones et al (1980) detected 0.49 MPa and 0.25 MPa adjustment of osmotic potential at moderate and severe stress in sorghum, 0.22 MPa and 0.17 MPa respectively in sunflower. A significant field osmotic adjustment was also recorded in 4 sorghum genotypes by Premachandra et al (1992). At moderate water stress, the adjustment lay between 0.25 and 0.48 MPa, while at severe stress, between 0.47 and 0.79 MPa.

These comparisons revealed that the magnitude of osmotic adjustment found in *Acacia* in this study, are within the range of published values for other field grown species.

4.5.3. *Physiological-Ecology significance of osmotic adjustment*

Plants exposed to water stress may develop either physiological or morphological adaptations (see Turner and Kramer, 1980). Among the physiological adaptations, two processes of adjustment may be found in plant cell water relations. The first is the decrease in leaf osmotic potential (osmotic adjustment) to balance the decrease in

water potential, and thus maintain favourable turgor pressure; the second is a biophysical change in cell wall elasticity which affects the magnitude of changes in cell turgor pressure as water content changes (Monson and Smith, 1982).

In answer to the first two questions posed for this field *Acacia* experiment (Section 4.1) it was found that osmotic adjustment really does play a role in the five species response to water stress. All species developed a similar level of osmotic adjustment which ranged from 0.7 to 1.1 MPa.

For various reasons, this *Acacia* research only concentrated on osmotic adjustment. However, it would be interesting to know how far the Australian *Acacia* seedlings balance various possibilities for survival during severe drought such as developing smaller cells or more elastic cell walls. This is another opportunity for research in the future.

Jones et al (1980) calculated the contribution of overall inorganic and organic solutes to osmotic adjustment which was only 84% and 53% in severely stressed sorghum and sunflower, while 100% in moderately stressed sorghum. The remaining "undetectable" values may be due to biophysical contributions such as developing smaller cells and more elastic cell walls. Pavlik (1984) estimated that in dunegrass *Elymus mollis* only 72% of the contribution to osmotic changes came from solute accumulation, while the remaining 28% was due to a decrease in symplastic water content. However, in *Ammophila arenaria*, all osmotic changes were due to decreasing symplastic water content (no solute contribution). This feature is similar to siratro as reported by Ford and Wilson (1981), where the osmotic adjustment was not due to solute accumulation but to the decrease of tissue hydration. A recent study (Fan et al, 1994), provided further evidence on how interactions between osmotic adjustment and bulk modulus of elasticity (elastic adjustment) contributed to turgor maintenance in 3 woody species. Jack pine (*Pinus banksiana*), a dehydration tolerant species, did not adjust its bulk modulus of elasticity when exposed to drought, while the less

drought tolerant black spruce (*Picea mariana*) and flooded gum (*Eucalyptus grandis*) could develop elastic adjustment to maintain turgor.

Osmotic adjustment by solute synthesis involves higher energy consumption than when solutes are re-translocated (Wyn-Jones and Gorham, 1983). Seedlings may face more risk by this method since, in order to survive they have to utilize more energy in the process of solute synthesis for adjustment when rain is scarce.

One or a combination of two or more physiological or biophysical features may have contributed to the survival under stress of the 5 *Acacia* species in this study.

However, besides water potential components, only one other aspect was investigated, namely solute accumulation, as described in the coming Chapters.

As in Chapter 3, no account has been taken in this study of the possibility that the elasticity of cell walls varied over the course of the treatments. If there had been changes in the modulus of elasticity ε of cells, this may have modified the conclusions about osmotic adjustment, but it was not possible to measure ε in this study. See p13a.

CHAPTER 5

SOLUTES: SEASONAL FLUCTUATIONS IN FIELD-GROWN PLANTS

As explained in section 2.3.2 both inorganic and organic solutes could be involved as osmotic agents in the process of osmotic adjustment. Table 2.1 summarized a number of reports on kinds of solutes which may be accumulated due to water stress. These solutes were generally considered as having effects on the osmotic values of the cells.

The field experiment reported in Chapter 4 showed evidence of osmotic adjustment in the five *Acacia* species. As a continuation, solutes possibly involved in the process of this adjustment were determined in the laboratory. For inorganic solutes, potassium and sodium were chosen to be analyzed, while proline, betaines and other solutes detectable by the same method were the organic compounds of interest.

5.1 *Inorganic solutes:*

5.1.1 *Potassium and sodium*

Reports provide information that in field grown higher plants these two ions may or may not be accumulated under stress (for example Cutler and Rains, 1978). Therefore, the purpose of this Section was to find out whether or not potassium and sodium concentrations would increase as a response to water stress in *Acacia,* during a summer season, and if so, whether the increase depended upon the plant species.

5.1.1.1 *Materials and Methods*

As mentioned previously, solute determinations were made only for the last four harvests mainly due to the availability of samples. The same samples for K^+ and

Na^+ study were used as for water potential and osmotic potential determinations. Solutes were determined for each of the plants harvested, then mean values calculated.

Approximately 10 to 20 mg oven dry leaf samples were boiled in 10 ml 1 N nitric acid for about 15 to 20 minutes. Loss by evaporation during boiling was replaced with deionized water. Potassium and sodium then were determined with a flame photometer. Calibration curves were produced using a series (0, 0.25, 0.5, 0.75 and 1 mM) of KCl and NaCl solutions. Results were expressed as umol g^{-1} dry weight of tissue.

5.1.1.2 *Statistical Analysis*

Six models

The data were subjected to a statistical analysis to determine the way the variates (potassium, sodium and proline content) depended upon the plant species, water treatment applied and the water potential of plants. Full details are given in McNamara (1990) See also Appendix 4, B2. The analysis starts by comparing six regression models which describe the relationship between water potential and the variates. Each model has its own assumption. The six models vary from the most complex to the simplest. The final analysis indicates which model it is valid to apply.

a. The most complex model (species * stress in Table 5.2) tests the hypothesis that at each time the average concentration of each ion consisted of a base level plus a part due to the level of water potential. Therefore, at any time (t), the concentration C of K^+ or Na^+ is

$$\mathbf{C = Constant + \mu\Psi} \quad \ldots\ldots \quad (8)$$

where μ is the gradient and Ψ is water potential. *Constant* is different for each species, each treatment and each time, and μ for each species and treatment. The part due to the water

potential was taken to be a linear function of the water potential where the gradient and *constant* depended on the species and water treatment in a non-parallel way.

Thus, for the five species and two water treatments there would be ten groups of four parallel lines, since there were ten treatment combinations at each time and four times of harvests. The slope of each line is different for each treatment combination, but constant over time.

For the k_{th} plant of species i, subjected to treatment j at time t,

the concentration = C_{ijkt}, the corresponding water potential is Ψ_{ijkt}.

Then, the expected concentration, E(C_{ijkt}) is:

$$C_{ijkt} = m_t + a_{it} + b_{jt} + g_{ijt} + (d + e_i + k_j + l_{ij})\,\Psi_{ijtk} \quad \ldots\ldots \quad (9)$$

where

m_t is the grand mean at time t,

a_{it} refers to the effect due to species i at time t,

b_{jt} is the effect due to water treatment j at time t,

g_{ijt} is the interaction effect between species i and water treatment j at time t,

d corresponds to the "base value" of the gradient - an analogue of the

grand mean;

e_i refers to the part of the gradient due to the species.

k_j is the part of the gradient due to the water treatment, and

l_{ij} is the part of the gradient due to the interaction between

species and treatment.

These parameters are constrained by

$$a_{1t} = b_{1t} = g_{1jt} = g_{i1t} = 0$$

$$e_1 = k_1 = l_{1j} = l_{i1} = 0$$

Therefore, the a_{it}, b_{jt} and so on represent additional contributions to the mean due to the experimental factors and the e_i, k_j etc. represent additional contribution to the gradient due to a factor.

b. The second model is simpler (shown as species + stress, see Table 5.2). It assumes that the gradient depended additively on treatments. This would generate ten groups of parallel lines; but here, $l_{ij} = 0$ for all combinations of species and water treatments.

c. The next model (referred to as stress in Table 5.2) has an assumption that species does not affect the change in concentration as water potential changes. This would correspond to 2 groups of 20 parallel lines, where $e_i = 0$ for both water treatments.

d. This model (species in Table 5.2) is similar to *c*, but in this case water treatment does not affect the changes in concentration as water potential fluctuates. Therefore, it would produce five groups of eight parallel lines. Here, k_j are 0 for all species.

e. This model (mean, Table 5.2) assumes the gradient is independent of the treatments, which corresponds to 20 parallel lines. Both e_i and k_j values are 0.

f. The last model (no water potential effect, Table 5.2) is the simplest with no water potential effect at all, thus producing 20 lines parallel to the *x-axis*. The value d = 0.

5.1.1.3 *Results and Discussion*

Table 5.1 presents the original data for potassium (a) and sodium (b) concentrations on a dry weight basis. Table 5.1.c and Table 5.1.d show the average values of potassium and sodium at each harvest. These fluctuations can be seen in Figure 5.1, which shows the concentrations of potassium and sodium at the four harvests namely the fourth, fifth, sixth and seventh plotted against time with plant water potentials shown for comparison.

Table 5.1.a

The original* values of potassium concentrations (μmol g^{-1} DW) extracted from 5 species of *Acacia* (*A.anceps, A.aneura, A.gillii, A.iteaphylla and A.myrtifolia*) field grown seedlings. The data were from two treatments, gathered during 4 harvests.

Table 5.1.b

The equivalent data for sodium.

Note:

** The underlined values on the Table were actually blank originally, due to accident during the processing of samples, or other reasons. But when the statistician was doing the analysis, these missing values were inserted using the default method of the Genstat 5 anova.*

Table 5.1.a. Potassium

Species	Water treatment	Harvesting			
		4	5	6	.7
A.anceps	Ww	400.0	275.0	400.0	345.2
		357.1	313.3	317.6	251.4
		281.9	263.5	261.5	318.2
		324.3	215.6	203.8	275.9
	STR	226.7	346.1	428.4	390.8
		207.3	180.7	328.7	276.0
		105.5	183.0	281.7	114.9
		262.5	260.4	349.4	320.0
A.aneura	WW	262.5	109.3	244.4	298.3
		228.2	350.9	254.6	229.9
		272.1	258.1	226.0	238.1
		305.3	291.4	223.5	317.6
	STR	242.4	207.3	228.9	299.0
		322.1	363.6	191.0	195.4
		346.7	201.3	235.3	231.2
		177.5	115.6	211.2	211.1
A.gillii	WW	95.2	97.6	360.0	362.6
		299.3	207.3	289.2	446.9
		97.0	278.5	300.0	423.5
		98.8	79.1	258.8	414.6
	STR	191.6	195.5	278.7	241.0
		223.5	204.8	363.6	271.2
		152.9	112.4	180.0	275.9
		387.1	294.5	205.9	298.1
A.iteaphylla	WW	235.3	223.5	201.3	232.6
		173.9	258.8	186.7	265.1
		210.5	207.8	201.2	246.9
		205.1	269.2	162.8	255.8
	STR	189.4	141.2	255.8	261.4
		155.8	151.9	136.4	135.6
		233.8	223.5	111.7	158.2
		191.1	259.7	169.7	189.9
A.myrtifolia	WW	202.8	250.0	239.5	300.0
		170.7	113.2	188.2	227.3
		130.7	171.8	171.4	266.7
		70.6	48.8	160.0	312.1
	STR	220.8	206.1	97.6	122.7
		169.7	186.7	112.4	129.9
		142.9	162.8	176.1	226.0
		253.2	236.7	158.2	179.5

Table 5.1.b. Sodium

Species	Water treatment	Harvesting			
		4	5	6	7
A.anceps	Ww	146.7	162.5	200.0	214.3
		130.9	144.8	117.7	125.7
		147.6	131.7	261.5	227.3
		108.1	107.8	114.6	114.9
	STR	93.3	89.7	168.8	91.9
		85.6	84.3	165.7	95.8
		120.7	117.6	223.4	80.5
		87.5	35.5	120.5	125.7
A.aneura	WW	37.5	65.6	55.5	77.3
		67.1	70.1	72.7	57.5
		68.0	64.5	56.5	83.3
		45.8	53.0	129.8	58.8
	STR	48.5	36.6	72.8	74.5
		40.3	36.4	56.2	57.5
		66.7	113.2	70.6	69.4
		35.5	34.7	62.1	77.8
A.gillii	WW	136.0	109.7	140.0	152.1
		95.2	61.0	108.4	145.3
		84.8	164.6	150.0	129.4
		74.1	33.9	82.3	121.9
	STR	71.9	104.0	150.0	84.3
		82.3	84.3	127.2	79.1
		82.3	101.1	140.0	69.0
		116.1	85.9	132.0	87.0
A.iteaphylla	WW	78.4	55.9	88.1	151.2
		161.5	117.6	93.3	84.3
		78.9	51.9	82.8	74.1
		64.1	76.9	81.4	69.8
	STR	82.8	70.6	69.8	71.4
		142.9	126.6	79.8	124.3
		77.9	105.9	78.2	69.0
		114.6	90.9	72.7	87.0
A.myrtifolia	WW	77.6	62.5	131.7	100.0
		61.0	88.0	117.7	102.3
		78.4	85.9	80.0	84.8
		58.8	24.4	80.0	115.6
	STR	39.0	84.8	73.2	98.2
		36.4	80.0	112.4	116.9
		23.8	58.1	100.6	79.1
		75.9	59.2	79.1	115.4

Table 5.1.c

The average (n=4)* values of potassium concentrations (μmol g^{-1} DW) during 4 harvests in 5 species of field grown plants *Acacia*.
WW = well watered, STR = stressed.

Species and Treatment		Time of harvesting IV	V	VI	VII
A. anceps	WW	340.83	266.82	295.75	297.68
	STR	232.16	242.56	315.54	275.30
A.aneura	WW	267.03	252.38	237.11	270.99
	STR	272.19	221.96	216.60	221.54
A.gillii	WW	147.57	165.61	301.99	411.91
	STR	238.80	201.80	257.03	271.54
A.iteaphylla	WW	206.21	239.81	187.97	250.08
	STR	192.51	194.09	168.40	186.28
A.myrtifolia	WW	124.01	145.94	189.79	276.52
	STR	196.25	198.05	136.05	164.51

* For few points, n less than 4 as presented in Table 5.1.a.

Table 5.1.d

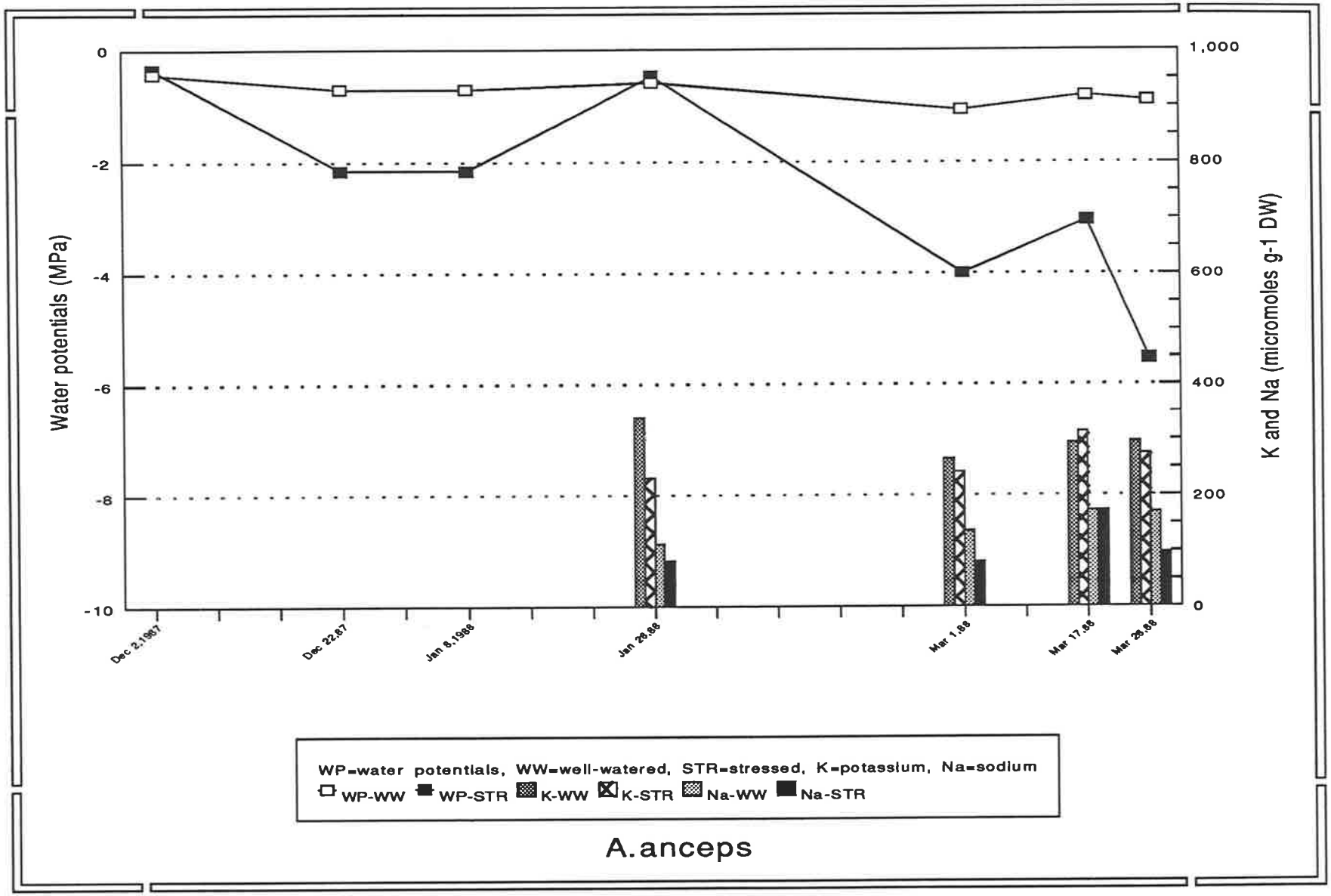
The average (n=4)* values of ***sodium*** concentrations (μmol g^{-1} DW) during 4 harvests in 5 species of field grown plants *Acacia*.
WW = well-watered, STR = stressed.

Species and Treatment		Time of harvesting IV	V	VI	VII
A.anceps	WW	113.34	136.65	173.46	170.55
	STR	88.73	81.81	172.91	98.48
A.aneura	WW	54.61	63.30	78.63	69.24
	STR	47.73	55.21	62.29	69.78
A.gillii	WW	97.55	92.30	120.19	137.16
	STR	88.17	93.85	137.30	79.84
A.iteaphylla	WW	95.62	75.60	86.40	94.83
	STR	104.57	98.49	75.06	88.26
A.myrtifolia	WW	66.08	65.21	102.35	100.68
	STR	43.64	70.54	91.31	102.38

* For few points, n less than 4, as presented in Table 5.1.b

Figure 5.1

The seasonal fluctuation of potassium and sodium (μmol g^{-1} DW) in the field grown plants of 5 *Acacia* species gathered during four harvests, plotted against time. Mean water potentials (-MPa) of the well-watered and stressed treatments are displayed for comparison.
For legends, see Figure.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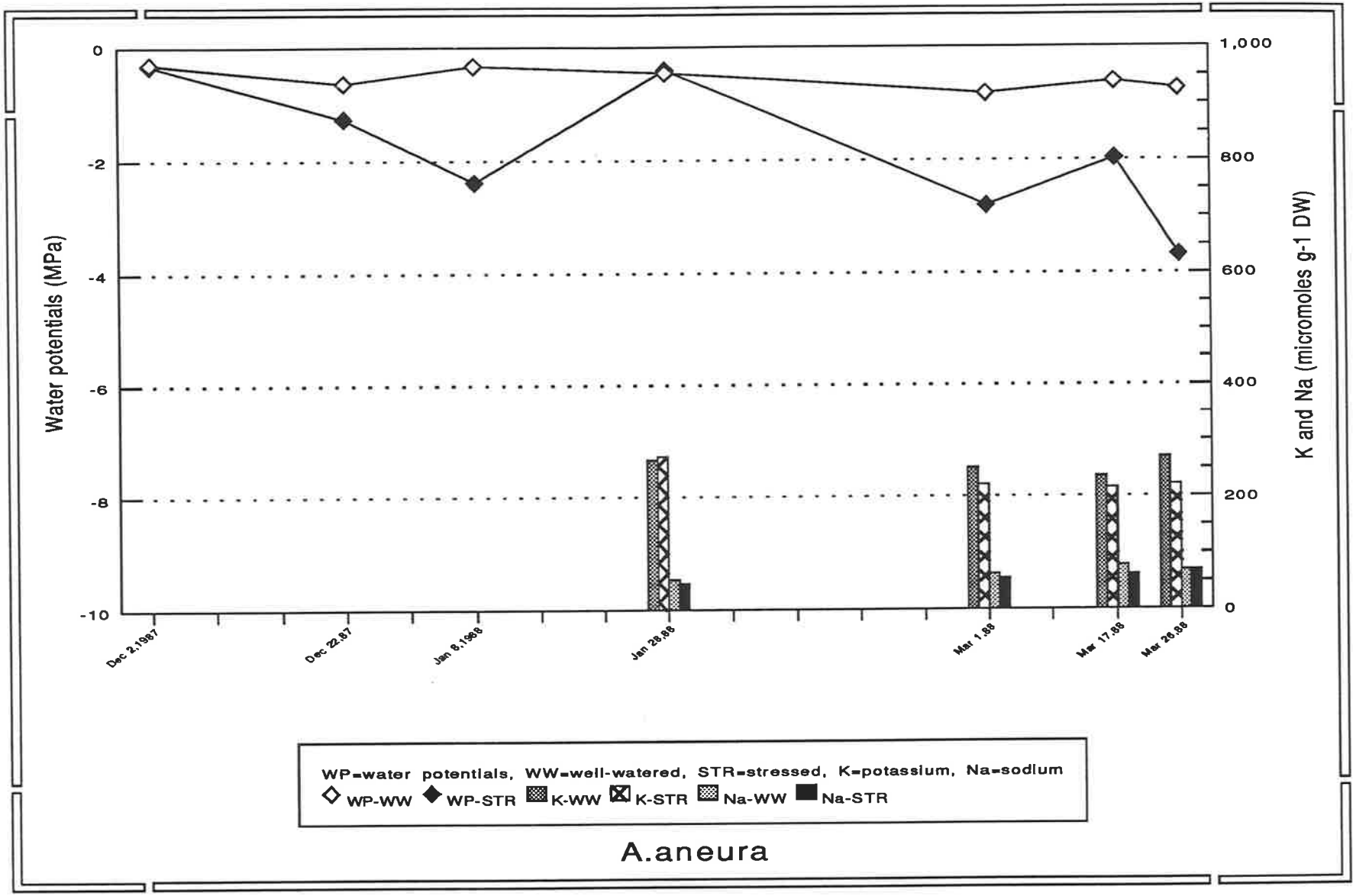
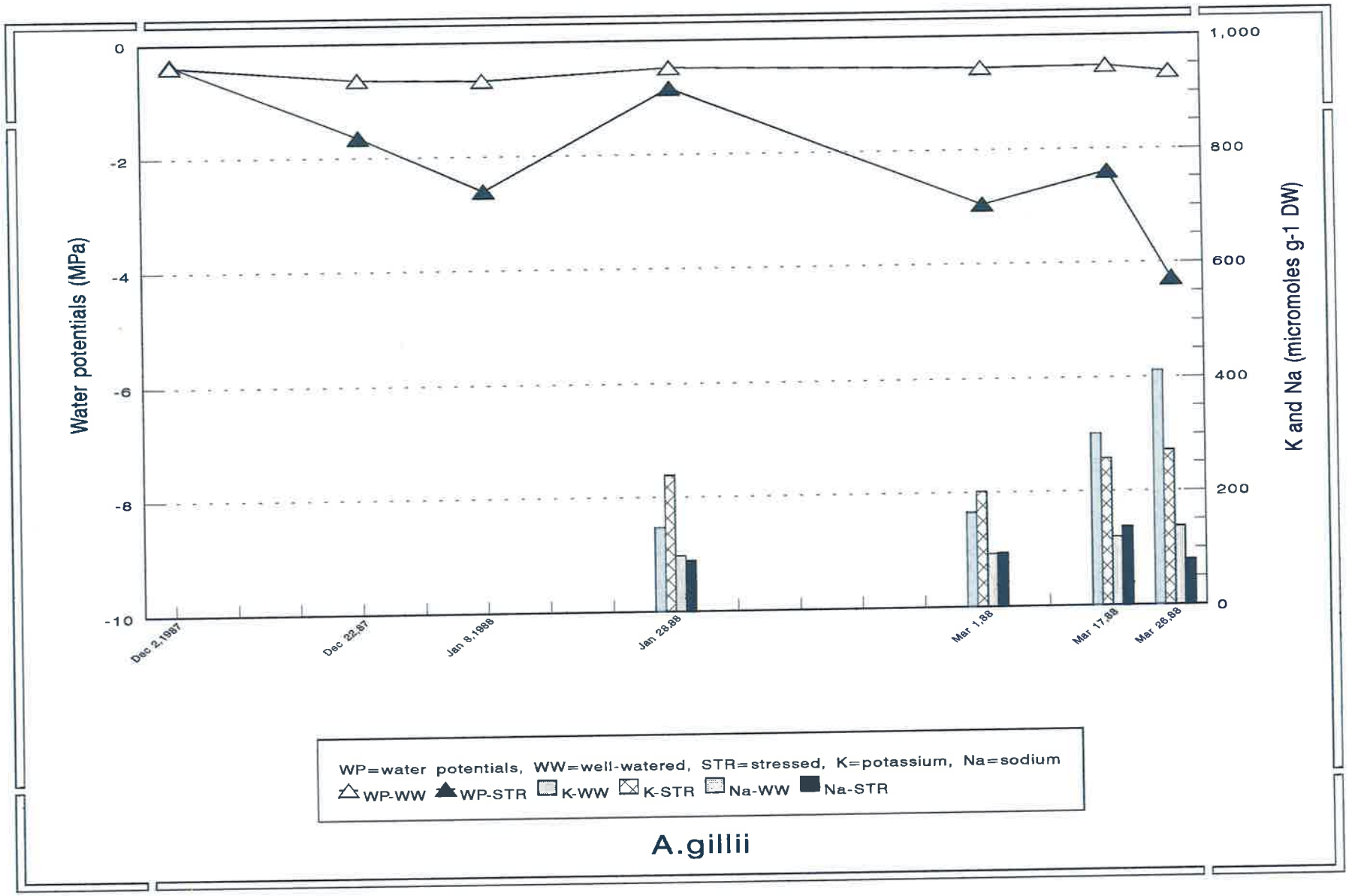
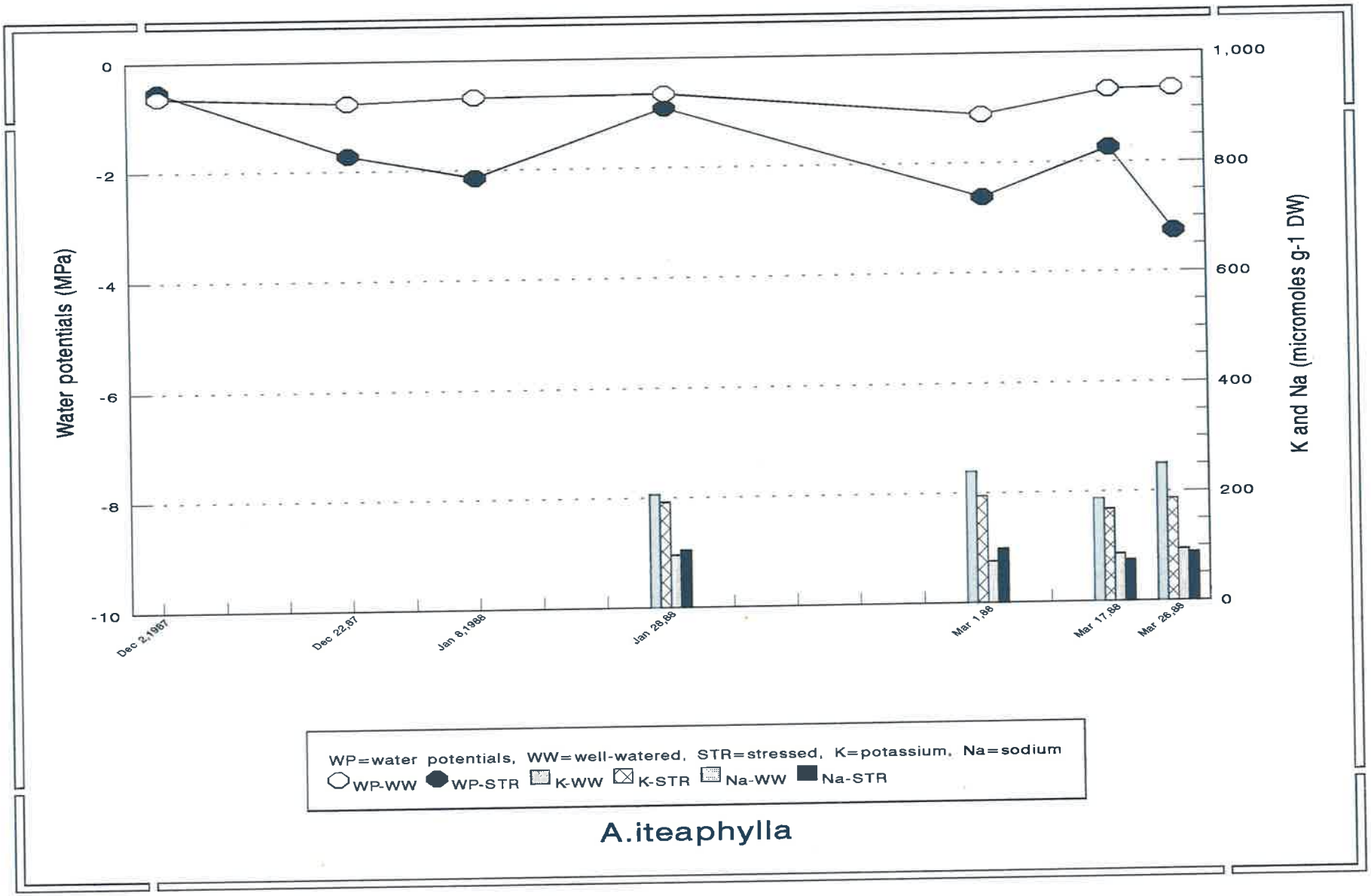
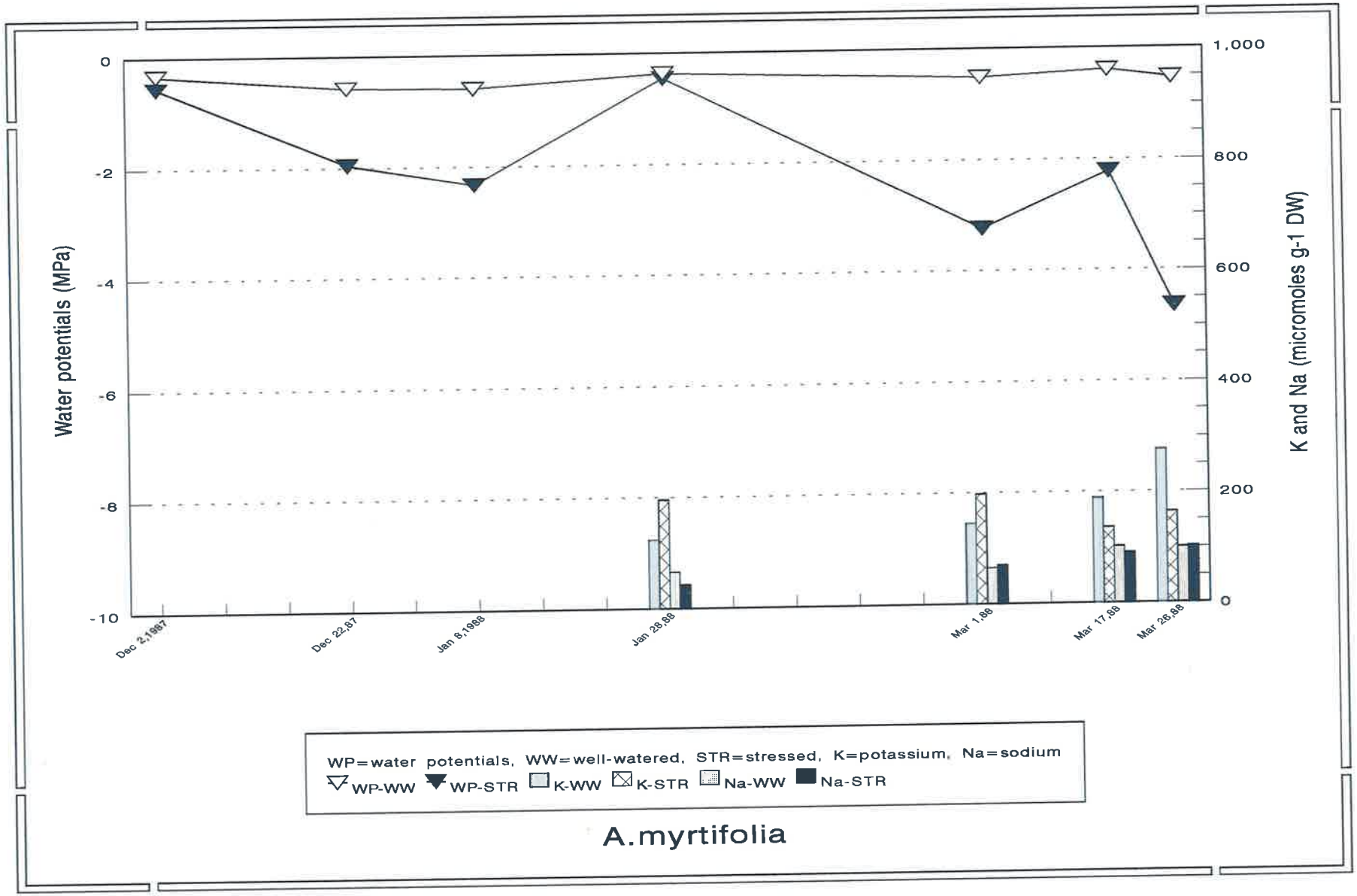
0
-2
-4
-6
-8
-10
K and Na (micromoles g-1 DW)
1,000
800
600
400
200
0
Dec 2,1987
Dec 22,87
Jan 6,1988
Jan 20,88
Mar 1,88
Mar 17,88
Mar 29,88
WP=water potentials, WW=well-watered, STR=stressed, K=potassium, Na=sodium
WP-WW
WP-STR
K-WW
K-STR
Na-WW
Na-STR
A.anceps


Water potentials (MPa)
0
-2
-4
-6
-8
-10
K and Na (micromoles g-1 DW)
1,000
800
600
400
200
0
Dec. 2,1987
Dec. 22,87
Jan 8,1988
Jan 28,88
Mar 1,88
Mar 17,88
Mar 28,88
WP=water potentials, WW=well-watered, STR=stressed, K=potassium, Na=sodium
WP-WW
WP-STR
K-WW
K-STR
Na-WW
Na-STR
A.aneura


Water potentials (MPa)
0
-2
-4
-6
-8
-10
K and Na (micromoles g-1 DW)
1,000
800
600
400
200
0
Dec 2,1987
Dec 22,87
Jan 8,1988
Jan 28,88
Mar 1,88
Mar 17,88
Mar 26,88
WP=water potentials, WW=well-watered, STR=stressed, K=potassium, Na=sodium
WP-WW
WP-STR
K-WW
K-STR
Na-WW
Na-STR
A.gillii


Water potentials (MPa)
0
-2
-4
-6
-8
-10
K and Na (micromoles g-1 DW)
1,000
800
600
400
200
0
Dec 2,1987
Dec 22,87
Jan 8,1988
Jan 28,88
Mar 1,88
Mar 17,88
Mar 26,88
WP=water potentials, WW=well-watered, STR=stressed, K=potassium, Na=sodium
WP-WW
WP-STR
K-WW
K-STR
Na-WW
Na-STR
A.iteaphylla


Water potentials (MPa)
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WP=water potentials, WW=well-watered, STR=stressed, K=potassium, Na=sodium
WP-WW
WP-STR
K-WW
K-STR
Na-WW
Na-STR
A.myrtifolia

It is clear that the seasonal change of potassium concentration did not appear to correlate with the increase of stress in plants as indicated by the fall of water potential. The same effect is also displayed by sodium, where the fluctuations of this ion are not controlled by the decrease of plant water potential especially in stressed treatment plants.

For example, at fourth harvest (the first harvest for potassium and sodium) where the available soil water was abundant due to the recent rainfall, hence the water potentials were least negative, the concentrations of potassium in well-watered plants of *A. anceps* and *A. iteaphylla* were higher than stressed plants, while in *A. aneura, A. gillii* and *A. myrtifolia* the concentrations in stressed plants were higher. This trend was not consistent, since at the fifth harvest it is clear in *A. aneura* that when water potential had declined (as a result of less rainfall), potassium concentrations in stressed plants were lower; but not in *A. gillii* and *A. myrtifolia* where the concentrations remained higher.

Even though water potential had moved back to less negative in the sixth harvest the trend of potassium concentration in *A. anceps* was upwards, with potassium of the stressed plants more concentrated than well-watered plants, while other species show a contrasting trend.

At the seventh harvest where all plants had undergone the most stress, the concentration of potassium in well-watered plants of all species was higher than stressed ones.

The fluctuation of sodium also was not consistent with the water treatment. At the fourth harvest where water potential was least negative, the concentration in well-watered plants of *A. anceps* was much higher than stressed plants. At further stresses, the concentration did not follow the fluctuation of water potential.

A relatively steady state of sodium concentration was shown by the other four species, with no large changes during four harvests, except for *A. gillii*, where both

treatments were slightly higher in the sixth harvest and well-watered plants in the seventh harvest.

Statistical test of the results

The appearance of no correlation between ion levels and water potential, was tested by the statistical analysis. The results of testing the various hypotheses are presented in Table 5.2. Initially, the hypotheses were tested with a split-plot method, however it was found to be inappropriate. Hence, a multivariate analysis with time changing covariate was employed. See Appendix 4, B.2 or McNamara (1990).

a. The slopes

The test for the slopes of potassium and sodium shows there was insufficient evidence to reject the hypotheses that d, e_i, k_j and l_{ij} all equal zero. Hence, the concentrations of these ions were independent of water potential. Therefore, lines are parallel to the *x-axis*, (model f).

b. The constants

To determine the constant term for K^+ and Na^+ a simple multivariate analysis of variance was sufficient. The test statistics used were approximate chi-squared and F-distributed. For potassium at 5% significance level, there was insufficient evidence to reject parallelism in the concentration.

Thus, $g_{ij} = 0$.

Therefore,

$$[K^+] = m_t + a_{it} + b_{jt} \quad \ldots\ldots\ldots\ldots \quad (10)$$

Table 5.2

Generalized ANOVA table for sodium, potassium and proline concentration data.

Model: Response = species*stress + water potential model

Water potential model	-2 ln (Generalized Likelihood Ratio)			
	df	Sodium	Potassium	Proline
Species*stress vs species + stress	4	5.56	4.33	3.92
Species + stress vs stress	4	2.22	5.65	42.8
Species + stress vs species	1	0.252	0.0485	3.90
Stress vs mean	1	0.201	0.0666	4.79
Species vs mean	4	2.17	5.66	43.7
Mean vs no water potential effect	1	1.44	3.20	50.9

For sodium, at 5% significance level, there was sufficient evidence to retain the interaction model, since at least one of the g_{ij} is non-zero.Therefore,

$$[Na^{+}] = m_t + a_{it} + b_{jt} + g_{ijt} \quad \ldots\ldots\ldots\ldots \quad (11)$$

Overall, statistical analysis confirms the evidence of Figure 5.1 that the seasonal fluctuations of potassium and sodium concentrations, on a dry weight basis, were not controlled by the tissue water potential fluctuations in the field grown plants of *Acacia.* In contrast to what was expected, potassium and sodium did not have higher concentrations in plants under water stress.

As the variations in osmotic potential were similar to water potential for the last 4 harvests (see Figures 4.3.a and 4.3.b; also statistical results for these two aspects in section 4.4.3, Tables 4.3.a and 4.4.a), therefore it appears there was no consistent pattern of correlation between osmotic potential and potassium or sodium concentrations within any species.

This phenomenon may be due to several reasons:

a. The stress was not heavy enough to promote the accumulation of these two ions. In other words, the threshold water potential for the accumulation of these two ions during stress was still not achieved. Due to their metabolic toxicity (Flower and Yeo, 1986) the plants may only accumulate these two ions as a last resort.

b. The concentration of these two ions in the soil (growth medium) was not high enough for them to be transported to the regions that need accumulation.

c. The water supply under dry conditions may have affected the transport of ions. The volume of flow is likely to be reduced and so supply could be maintained only by an increase in the concentration of ions (Flower and Yeo, 1986).

These results, where potassium and sodium did not respond to the fluctuations of water potential, were similar to reports of some species but not others. During a simultaneous study of field grown plants, Ford and Wilson (1981) showed that under stress potassium increased greatly in buffel grass (*Cenchrus ciliaris*), only slightly increased in spear grass (*Heteropogon contortus*), and did not change in green panic (*Panicum maximum*) or the shrub legume siratro (*Macroptilium atropurpureum*); sodium concentration did not change in any species.

5.1.1.4 *Role and contribution of potassium and sodium to the osmotic potential of cells*

Potassium is a very mobile ion, the most abundant cation in cytoplasm, while sodium is relatively immobile. Potassium salts make a big contribution to the osmotic potential of glycophytic plants (Marschner, 1986). Potassium is retained in a relatively high concentration in the cytoplasm and chloroplast to neutralize the soluble and insoluble macromolecular anions. Also, its presence would retain the pH between 7 to 8 which is the optimum level for enzymatic reactions (Marschner, 1986). Potassium may be important in generating osmotic potential in both xerophytes and mesophytes but its involvement in osmotic adjustment varies among species (Ford and Wilson, 1981). Its role as an osmoregulator in lower plants is well known and documented (see review by Hellebust, 1976). Sodium may also be absorbed for the adjustment of osmotic potential (Kylin and Quatrano, 1975; Gale, 1975).

The data presented here suggest that for these five *Acacia* species potassium and sodium were not accumulated at all as inorganic solutes in osmotic adjustment in the field grown plants. This may be related to the mobility of these two ions as mentioned above.

However, since there were no noticeable changes in potassium and sodium concentration in stressed plants on a dry weight basis, therefore it seems that the two

inorganic ions were retained within the cells during desiccation. As a consequence, their osmotic effects would be unavoidably increased as the symplastic water volume decreased, due to the concentration effect (cf. Flower and Yeo, 1986). Thus their contribution to the osmotic values have to be seen in relation to the changing symplastic water content. These ions may not be retained in very high concentration since they are known to be quite toxic. In the case of potassium for example, a concentration of about 125 mmol would inhibit protein synthesis (Marschner, 1986), while about 250 mmol of monovalent ions as K^+ and Na^+ within the cytoplasm is the toxic level for metabolism (Flower and Yeo, 1986).

Although the symplastic water volumes were not measured during the droughting experiment, it is still possible to estimate the osmotic contribution of these ions to the osmotic potentials of the field grown plants using fresh weight-dry weight data (fw/dw ratio) gathered later, and making the assumption that all the water content of the tissues is cell water.

The FW data were gathered from these 5 *Acacia* species (each species 3 samples) at the Black Hill Experimental Site in February 1990, while DW were obtained by drying and weighing. These FW-DW data do not correspond to the previous treatments (i.e. well-watered and stressed), since the automatic watering system was stopped more than six months before; they are taken to be representative values, and the calculations based on them are approximations only.

Table 5.3 presents the estimates of K^+ and Na^+ contributions to osmotic potentials of the 5 species. K^+ and Na^+ dry-weight concentrations (column 2, Table 5.3) are means for both well-watered and stressed plants from all four harvests shown in Tables 5.1.c and 5.1.d. The fresh weight : dry weight ratios derived from the February 1990 measurements (column 3) were used to calculate approximate fresh weight concentrations (column 4).

Table 5.3

Contributions of potassium and sodium to the osmotic potentials of the field grown plants of 5 *Acacia* species. C_{K+Na+} : potassium and sodium concentrations; FW, DW : fresh weight, dry weight; WC: water content; KNa_{cntb}: potassium and sodium contributions to ψ_π.

Species	C_{K^+,Na^+} (µmol g^{-1} DW)	FW/DW ratio	$C_{K^+Na^+}$ at FW (umol g^{-1} FW)	Total WC (%)	$C_{K^+Na^+}$ (mM)	ψ_π KNa_{cntb} (-MPa)	Actual measured ψ_π (-MPa)	Total K, Na cntb (-MPa), %
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
A.anceps	K 283.33 Na 129.66	2.05	138.21 63.25	51.11	270.42 123.75	1.2 (33) 0.5 (14)	3.60	1.7 (47)
A.aneura	K 245.97 Na 62.60	1.85	132.95 33.83	46.12	288.27 73.35	1.3 (42) 0.3 (10)	3.07	1.6 (52)
A.gillii	K 249.53 Na 105.79	2.75	90.74 38.47	63.64	142.58 60.45	0.6 (22) 0.2 (7)	2.75	0.8 (29)
A.iteaphylla	K 203.22 Na 89.83	2.03	100.12 44.25	50.78	197.16 87.14	0.9 (35) 0.4 (15)	2.60	1.3 (50)
A.myrtifolia	K 178.88 Na 80.27	2.0	89.44 40.13	49.96	179.02 80.32	0.75 (26) 0.4 (14)	2.85	1.15 (40)

Note: **Values in:**

Column

(2) Mean concentrations of the four harvests, both well-watered and stressed, from Tables 5.1.c and 5.1.d (µmol g^{-1} DW).

(3) Mean Fresh Weight : Dry Weight ratio from measurements made in February 1990.

(4) Mean concentrations on a FW basis (column 2 / column 3) (µmol g^{-1} FW).

(5) Mean total water content, % FW.

(6) Ion concentrations (column 4 / column 5) assuming all water, and all ions, are symplastic (mM).

(7) Osmotic potentials of these solutions, assuming the ions are present as KCl and NaCl, and using Figure 5.2. (-MPa). *

(8) Mean measured osmotic potentials, including both watered and stressed treatments (Table 4.2.b). (-MPa).

(9) Estimated contributions of potassium plus sodium to total osmotic potential (-MPa)*.

*Values in parentheses are % of the total osmotic potentials shown in column 8.

Column 5 shows total water content as a percentage of fresh weight. Assuming that all this water and all the ions are within the cells (i.e. symplastic), then the fresh weight ion concentrations were converted to mM concentrations (column 6). Assuming that the ions are present as KCl and NaCl, the corresponding osmotic potentials (-MPa) are given in column 7. These are derived from Figure 5.2, which shows the relationship between concentration and osmotic potential for KCl, NaCl, sucrose and proline.

Column 8 gives the average values of total osmotic potential for the plants over the four summer harvests, combining data from both watered and stressed treatments (Table 4.2.b). Finally, column 9 shows the estimated combined contribution of potassium and sodium to these osmotic potentials based on the FW:DW ratios measured in February 1990. The values in parentheses in column 7 and 9 are the % contributions of the two ions.

It can be seen that the contribution of potassium ranged from 22% (*A.gillii*) to 42% (*A.aneura*), while that of sodium ranged from 7% (*A.gillii*) to 15% (*A.iteaphylla*). The total contribution varied from 29% (*A.gillii*) to 52% (*A.aneura*).

From these order-of-magnitude estimates, it can be seen that these two ions are among the important solutes of these *Acacia* species in spite of the lack of evidence that changes in their concentrations contribute to osmotic adjustment.

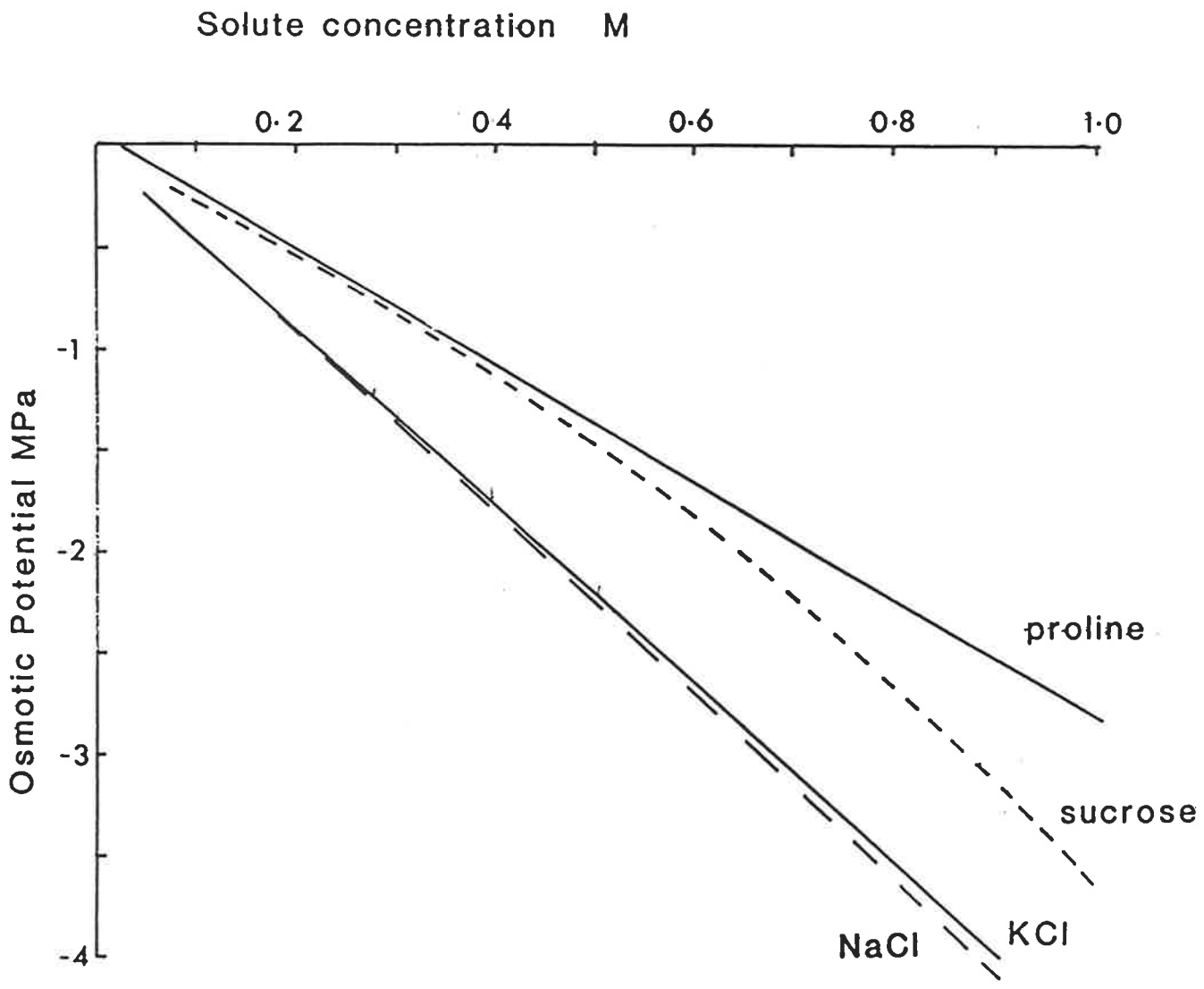
No attempt has been made to sub-divide water content between symplast and apoplast. No doubt some of the ions would also be in the apoplastic water, so the true concentrations inside cells may differ from these estimates.

The Table shows that the highest concentration of potassium was 288 mM (*A.aneura*) and of sodium 123 mM (*A.anceps*). These values seems quite high compared to the common concentrations within plants (cf Marschner, 1986; Flower and Yeo, 1986) where 125 mM K^+ inhibited protein synthesis while 250 mM K^+ or Na^+ was toxic for

Figure 5.2

The osmotic potential (MPa) curves for four solutions namely potassium chloride, sodium chloride, sucrose and proline.

Note: Data for KCl and NaCl from Wiebe et al (1971), for sucrose from Handbook of Physics and Chemistry (1972), for proline measured in this study (see Section 5.2.1.4c)


Solute concentration M
0·2
0·4
0·6
0·8
1·0
-1
-2
-3
-4
Osmotic Potential MPa
proline
sucrose
KCl
NaCl

cytoplasmic metabolism. Hence, a high proline content (see next Section) may be needed to balance the high K^+ and Na^+ in the vacuoles.

Further experiments (Chapter 6) will provide evidence in some pot grown seedlings of the relationship between symplastic water volume and the contribution of potassium to the osmotic potential.

5.2 *Organic solutes*

Various organic solutes have been reported to be accumulated in higher plants during water stress conditions (See Table 2.1).

5.2.1 *Proline*

One main reason that proline was measured in this experiment was that it is one of the commonest organic solutes reported to accumulate in wilted higher plants. Moreover, proline has invited some controversy regarding its role as an osmotic constituent. For example Singh et al (1973), confirmed by Aspinall and Paleg (1981), claimed that proline has a role as an osmotic agent and could be used as a drought resistance indicator at least in barley; this suggestion was contradicted by Hanson and Tully (1979), who could not find evidence in barley to support this concept. Ibarra-Caballero et al (1988) came to the same conclusion when working with maize.

Therefore, this section was proposed to study possible changes in proline concentration in the field grown *Acacia* when subjected to water stress, and whether species possess different abilities to accumulate proline.

5.2.1.1 *Materials and Methods*

Proline content was estimated by the colorimetric method, following procedures established by Troll and Lindsay (1955); Singh et al (1973); confirmed by Parameshwara (1984); and Naidu et al (1987).

Tissue was taken from the same samples as used for water potential, osmotic potential, K^+ and Na^+ determinations.

Phyllode tissue was freeze dried for two to three days. Approximately 100 to 400 mg samples were homogenized in ten ml of extraction solution, i.e. methanol/chloroform/ water (12:5:3) in a large glass centrifuge tube, using an ultraturrax. The tube was placed in an ice bath during extraction in order to counteract heat possibly generated by the ultraturrax. Excessive heat generation can cause the breakdown of chloroform with production of HCl which would change the supernatant pH which should be about 6 to 7.

Another tube was prepared, filled with 10 ml distilled water to wash out the grinding head of the ultraturrax. The emulsion which was produced was then added to the first homogenate.

The homogenate then was centrifuged at 3 to 3.5 x 10^3 r.p.m for 10 minutes at 20°C. The supernatant ($MeOH/H_2O$ phase) was removed and stored for further steps.

Two ml, 1 ml, 0.5 ml or 0.25 ml of solution (depending on the level of water stress), were transferred into a boiling tube containing 3 to 4 beads to prevent bumping. It was found in some *Acacia* species that if the water potential of plants ranges between 0 to -2 MPa, one or two ml of solution would give a good reading by the colorimeter, while 0.5 ml was used for water potential ranges between -2.1 to -4 MPa, and 0.25 ml for more than -4.1 MPa.

To that sample, 10 ml distilled water was added followed by 5 ml of ninhydrin solution (see Appendix 1), and 5 ml of glacial acetic acid.

A series of standard proline solutions for calibration was prepared as well (See Appendix 2).

The tubes (including standard calibration solutions) were thoroughly mixed, covered with marbles, boiled in a water bath for approximately 45 minutes, then cooled to room temperature.

Five ml of toluene was added to the solution, and shaken well.

The optical density (OD) of the toluene-extracted ninhydrin product was measured at 520 nm, using a Brinkmann P/C 600 colorimeter.

The proline content was then estimated using a calibration line produced by the standard proline solutions.

5.2.1.2. *Statistical analysis*

For proline analysis, the same methods as for potassium and sodium were used (Section 5.1.1.2) - six models varying from the most complex to the simplest (Table 5.2). The hypotheses were tested with all models. A multivariate analysis with time changing covariate was employed.

5.2.1.3. *Results*

Seasonal fluctuations

The proline concentration in the field grown plants of these five species was measured at the same four harvests as the other variables. Table 5.4.a presents the original values of four harvests for all individuals of all species, Table 5.4.b the average values. As shown in Figure 5.3, the proline concentration did respond to the fluctuations of water potential except for *A.iteaphylla,* which only shows small differences.

Table 5.4.a

The original* values of ***proline*** (μmoles g^{-1} DW) from 5 species of *Acacia* field grown seedlings, in 2 treatments, over 4 harvests.

Note:
* The underlined values on the Table were actually blank originally, due to accident during the processing of samples, or other reasons. But when the statistician was doing the analysis, these missing values were replaced using the default method of the Genstat 5 anova.

Table 5.4.a. Proline

Species	Water treatment	Harvesting			
		4	5	6	7
A.anceps	Ww	1.05	1.64	1.17	1.67
		0.79	2.39	1.10	0.77
		0.09	1.82	0.88	1.15
		0.44	1.56	1.14	0.67
	STR	2.33	1.54	1.41	1.95
		1.32	2.24	1.28	0.94
		1.01	4.92	6.31	7.00
		3.43	4.97	5.07	5.36
A.aneura	WW	13.24	10.14	10.68	3.36
		0.17	3.78	9.77	3.60
		6.23	4.53	11.78	7.59
		4.98	9.98	20.51	5.90
	STR	4.66	30.98	17.15	16.95
		9.56	33.98	20.61	17.45
		8.26	75.11	30.71	41.54
		7.49	10.14	60.43	40.53
A.gillii	WW	1.40	0.86	1.15	1.10
		0.86	2.95	0.89	1.26
		1.13	1.36	0.98	0.44
		1.02	0.44	1.34	0.99
	STR	7.28	14.01	16.03	15.43
		2.38	6.87	5.96	12.30
		2.25	14.26	3.68	18.99
		1.34	2.01	2.27	2.45
A.iteaphylla	WW	14.29	11.34	15.34	11.13
		3.81	8.71	19.85	19.44
		8.70	38.44	15.89	16.45
		7.61	7.71	1.62	17.53
	STR	13.82	12.26	30.14	8.60
		5.45	21.71	10.25	19.96
		8.32	9.04	11.48	23.32
		5.08	21.14	11.16	21.30
A.myrtifolia	WW	1.18	1.10	0.63	0.72
		0.67	2.19	1.19	5.55
		0.57	1.00	0.61	2.77
		2.30	4.79	3.68	0.91
	STR	0.74	13.26	7.49	29.21
		3.51	29.65	13.41	37.67
		4.31	31.07	20.49	44.61
		0.94	19.24	7.79	30.31

Table 5.4.b

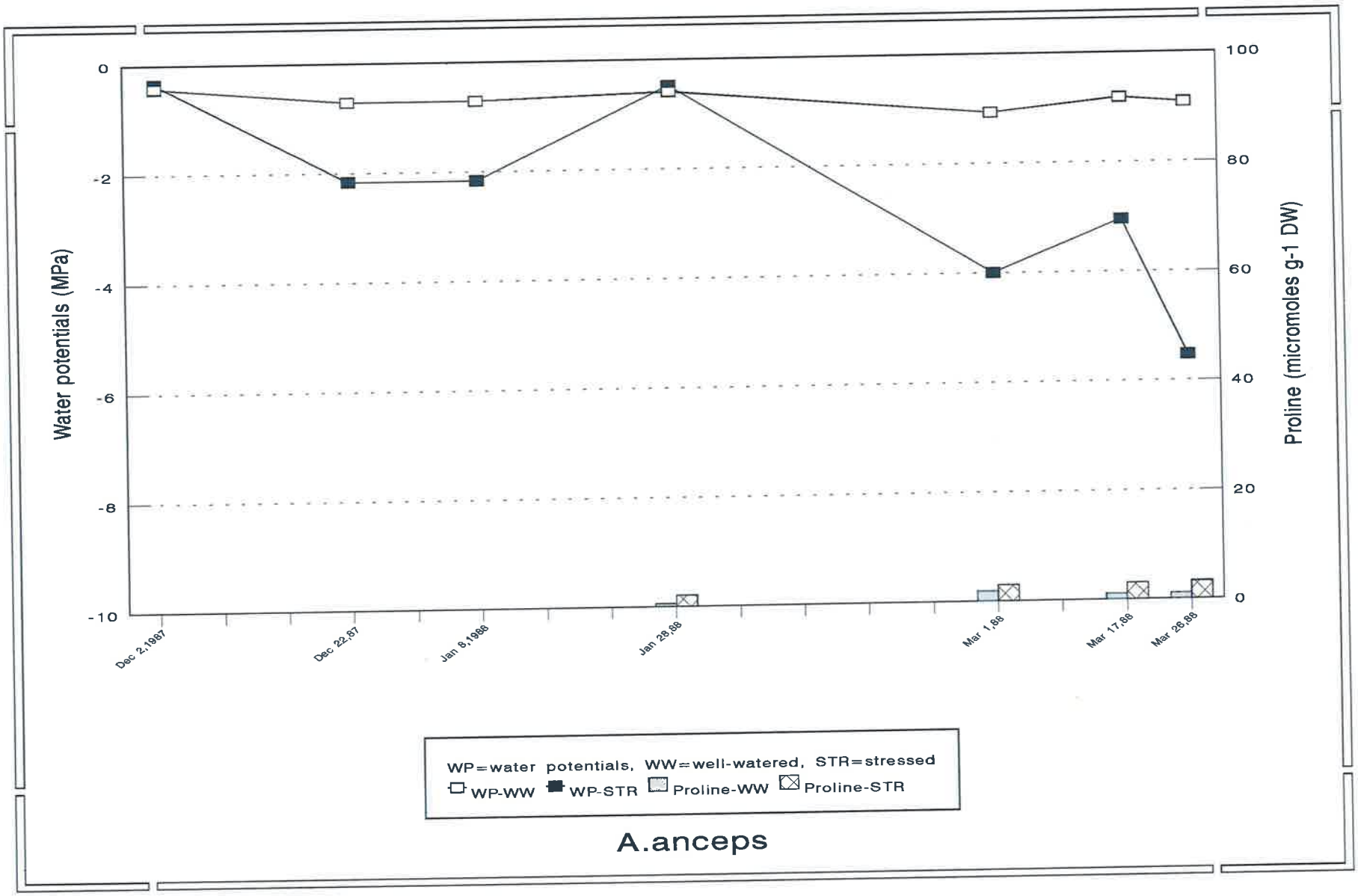
The average (n=4) * values of ***proline*** concentrations (µmol g^{-1} DW) at 4 harvests in 5 species of field grown plants of *Acacia*. WW = well watered, STR = stressed.

Species and Treatment		Time of harvesting IV	V	VI	VII
A. anceps	WW	0.54	1.85	1.06	1.06
	STR	2.02	2.90	3.00	3.20
A.aneura	WW	6.15	7.11	13.18	5.11
	STR	7.49	37.55	32.22	29.12
A.gillii	WW	0.55	1.40	1.09	0.95
	STR	3.31	9.29	6.98	12.29
A.iteaphylla	WW	8.60	16.55	15.42	16.14
	STR	8.17	16.04	15.76	18.29
A.myrtifolia	WW	1.18	2.27	1.53	2.49
	STR	2.37	23.30	12.29	35.45

* For few points, n less than 4 as presented in Table 5.3.a.

Figure 5.3

The seasonal fluctuations of proline (μmoles g^{-1} DW) in the field grown plants of 5 *Acacia* species measured in two treatments during four harvests, plotted against time. Mean water potentials (-Mpa) of the well-watered and stressed treatments are displayed for comparison. For legends, see Figure.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-8
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Proline (micromoles g-1 DW)
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60
40
20
0
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WP=water potentials, WW=well-watered, STR=stressed
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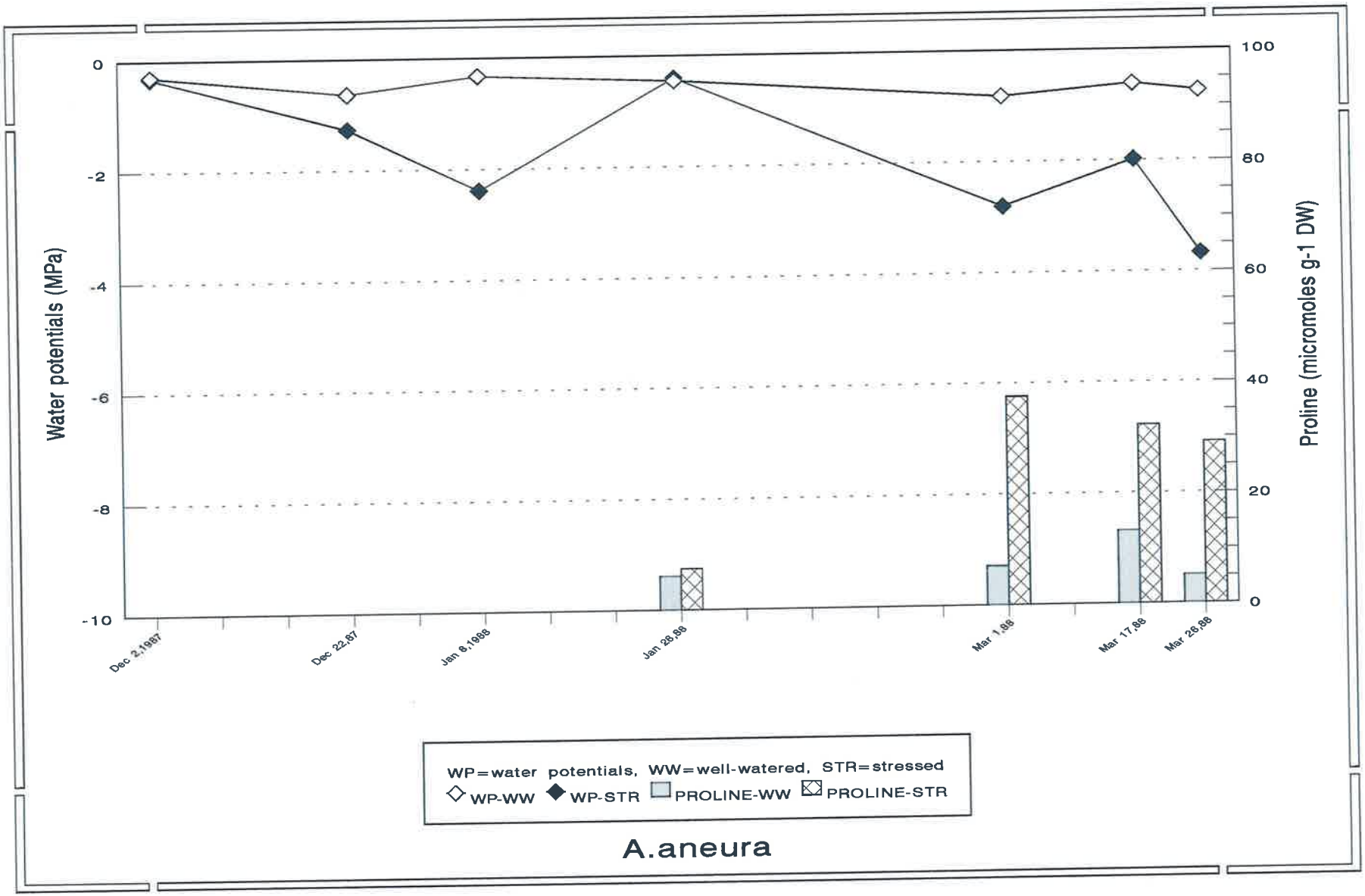
WP-STR
Proline-WW
Proline-STR
A.anceps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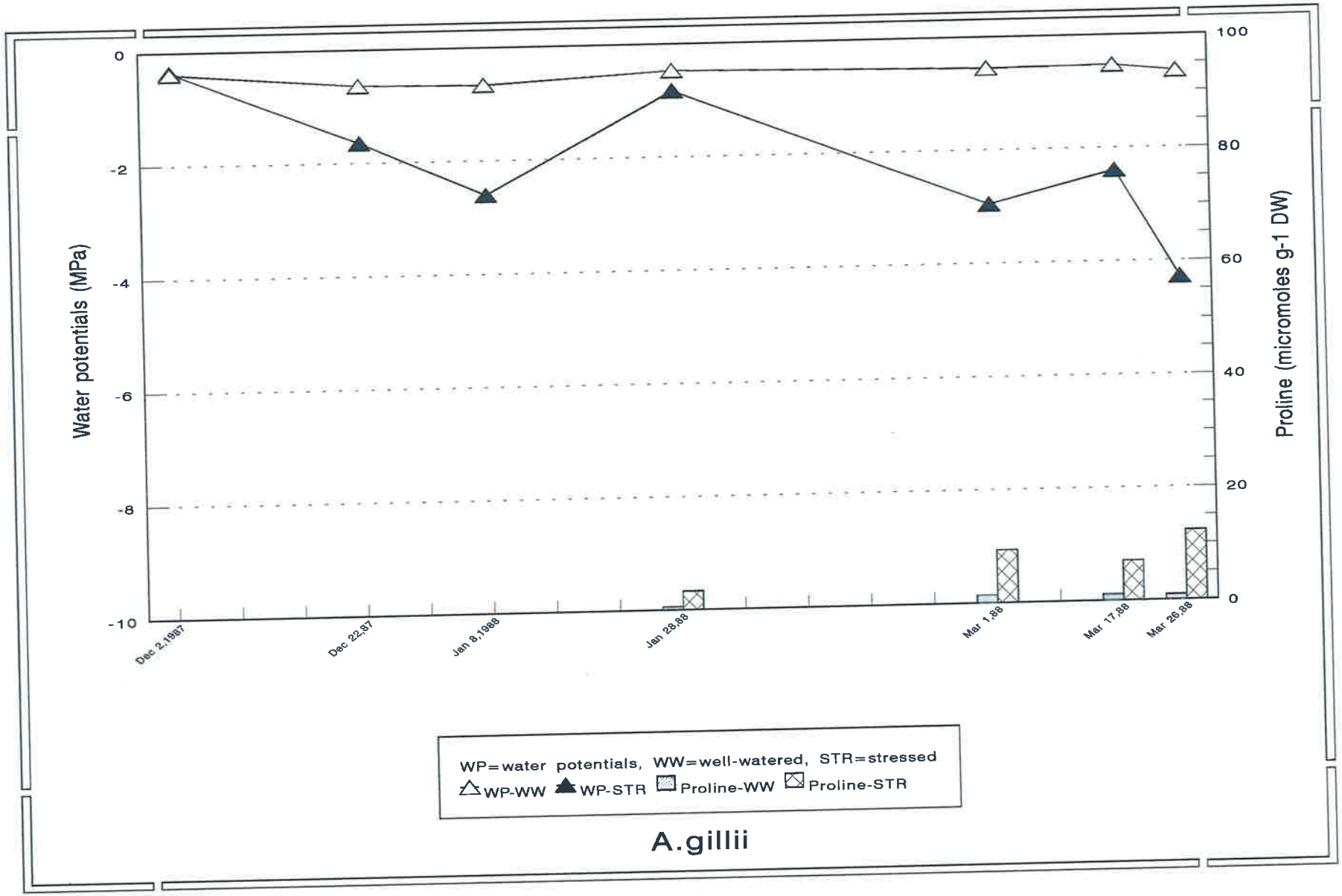
WP-STR
PROLINE-WW
PROLINE-STR
A.aneura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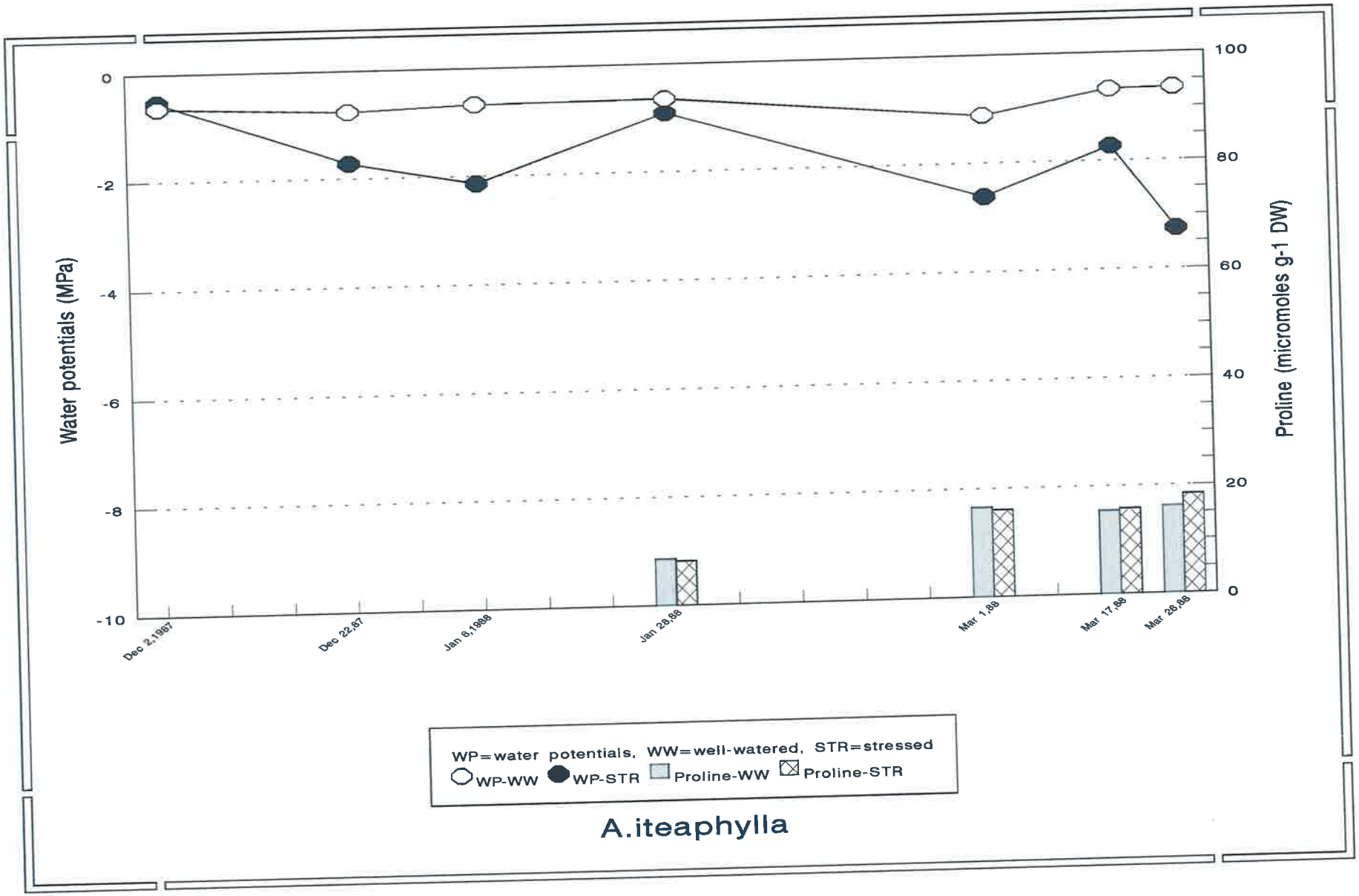
WP-STR
Proline-WW
Proline-STR
A.gillii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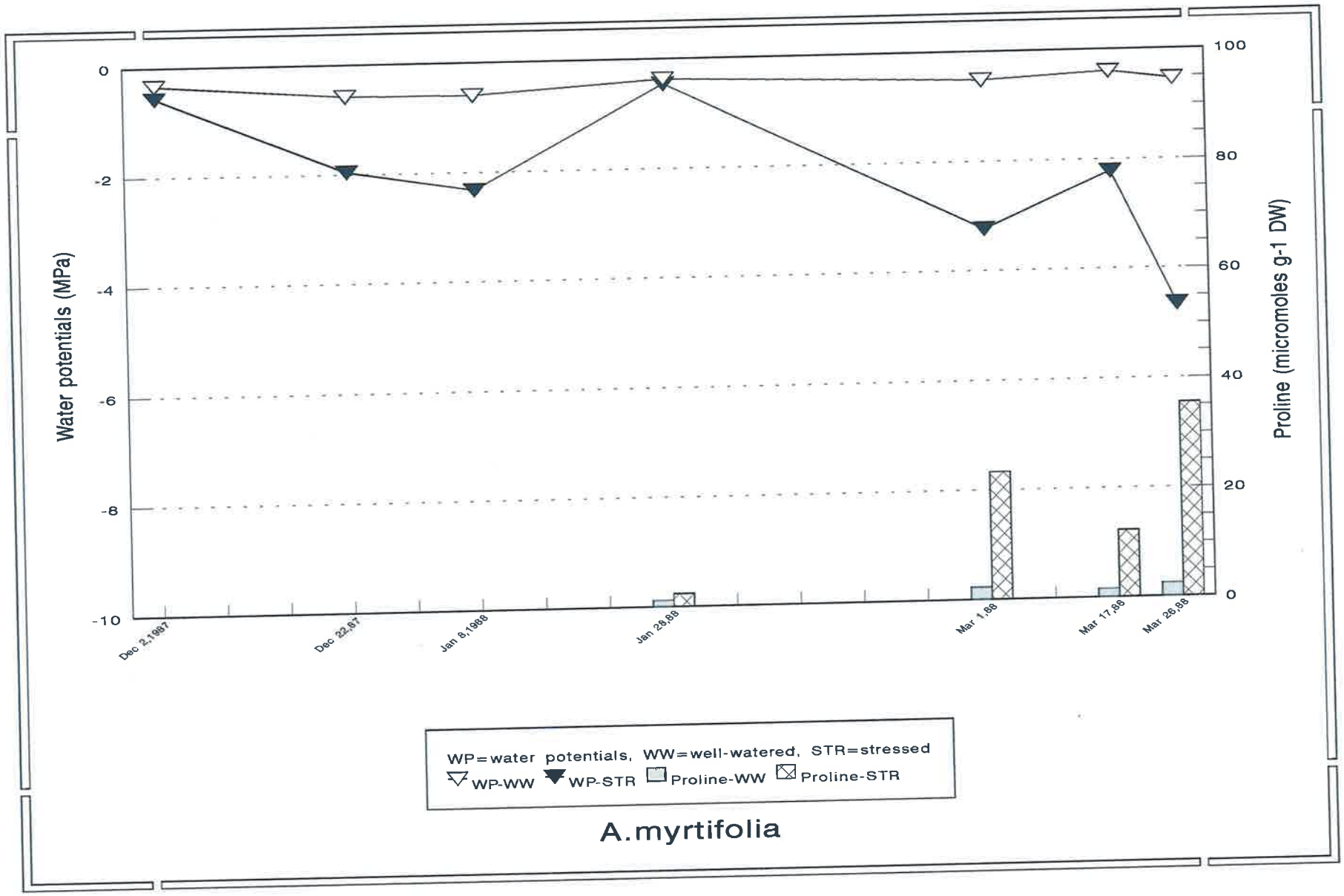
A.iteaphylla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-4
-6
-8
-10
Proline (micromoles g-1 DW)
100
80
60
40
20
0
Dec 2,1987
Dec 22,87
Jan 8,1988
Jan 28,88
Mar 1,88
Mar 17,88
Mar 26,88
WP=water potentials, WW=well-watered, STR=stressed
WP-WW
WP-STR
Proline-WW
Proline-STR
A.myrtifolia

Under well-watered conditions proline was at a lower level. The concentration generally increased when water potential declined due to the loss of water from the tissues.

The analysis to test the *slope* in the case of proline (Equation 12) presented sufficient evidence to retain the hypothesis of a gradient of concentration which depends upon the plant species. Thus, there was a slope in the relationship between proline concentration and water potential, where in this case d and at least one e_i are non-zero.

A further test to determine the *constant* term for proline used the same multivariate analysis as for potassium and sodium. Here, water potential has an effect due to the water treatment. Table 5.5 shows that there was sufficient evidence to retain an additive model for the concentration, i.e. $g_{ijt} = 0$. Therefore,

$$[P] = m_t + a_{it} + b_{jt} + (d + e_{it})\,W \quad \text{.....} \quad (12)$$

Figure 5.4 displays the linear relationship between water potential and proline concentration. Data points were separated to show the tendency on each species, harvest and water treatment. Table 5.6 presents the slopes and the intercept values of all lines.

5.2.1.4 *Discussion*

In contrast to potassium and sodium, proline evidently responded to the changes in plant water potential (Figures 5.3 and 5.4). At the first harvest for proline both well-watered and stressed plants in all species were at similar levels. This may have been due to an abundant rainfall (30 mm) about one week before. At the later harvests the difference between species and treatments started to appear.

The slope values from the statistical analysis (Table 5.6) proved that the magnitude of response in proline accumulation is different in different species. Thus, it suggests that there

Table 5.5

Generalized ANOVA table for proline

Model: Response = Anova + species.stress

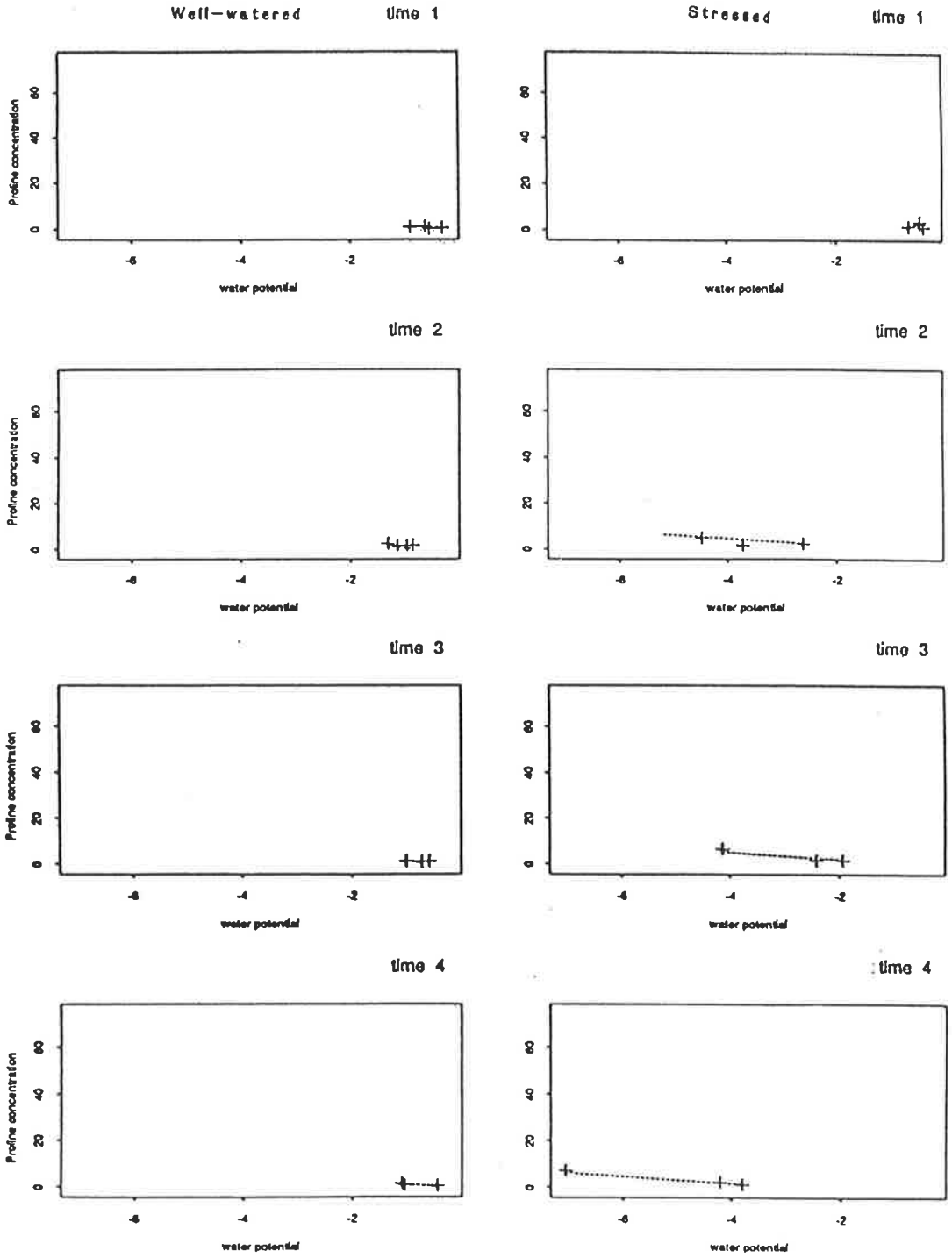
Anova model	df	-2 ln (Generalized Likelihood ratio)
species*stress vs species + stress	16	16.8
species + stress vs stress	16	108.0
species + stress vs species	4	11.0
stress vs mean	4	3.15
species vs mean	16	99.7

Figure 5.4.

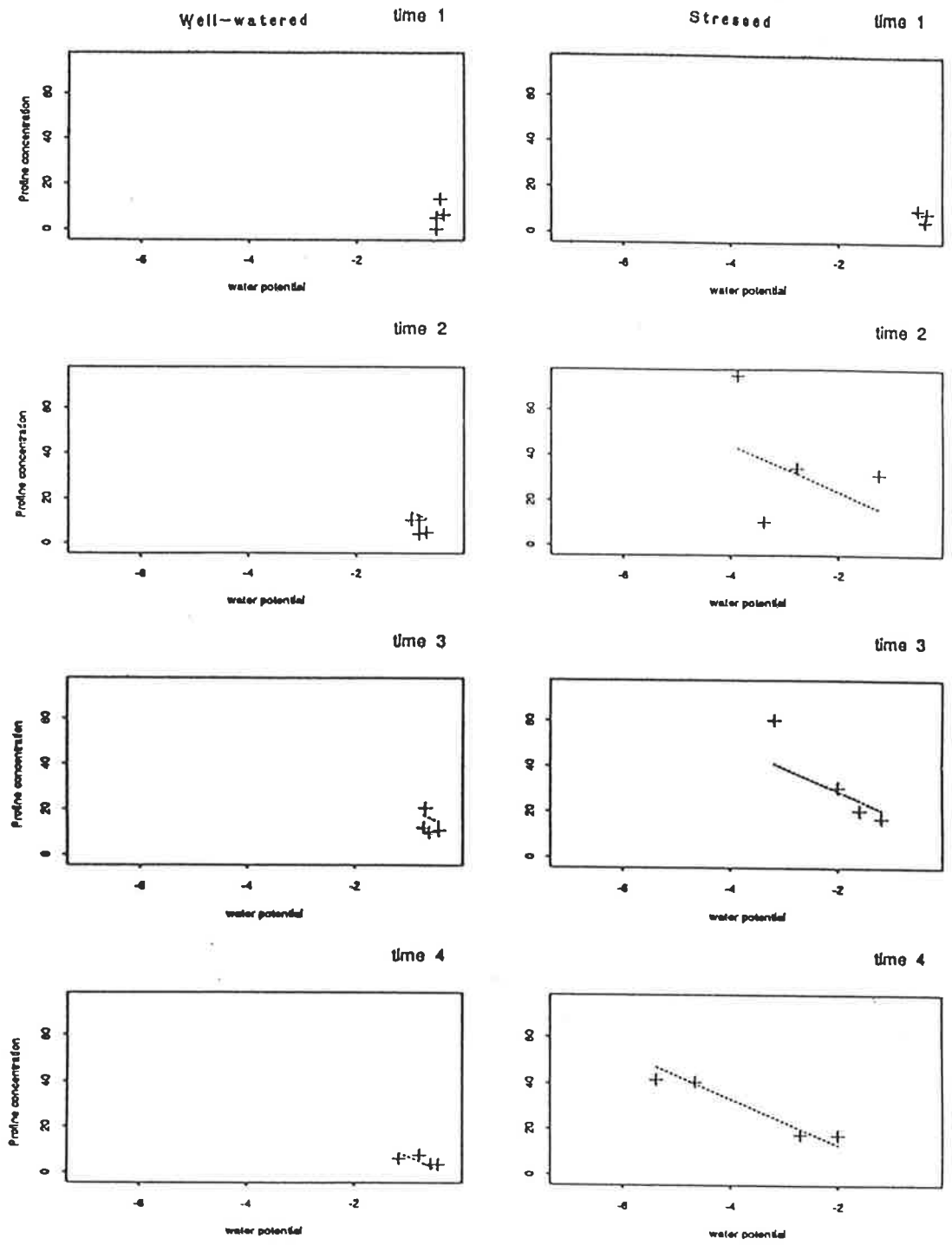
The relationship between proline concentration (μmoles g^{-1} DW) and plant water potential (MPa) at each (four) harvest and each (two) treatment over time for 5 field grown *Acacia* species.

Note: the lines represent a linear relationship between the two variates.

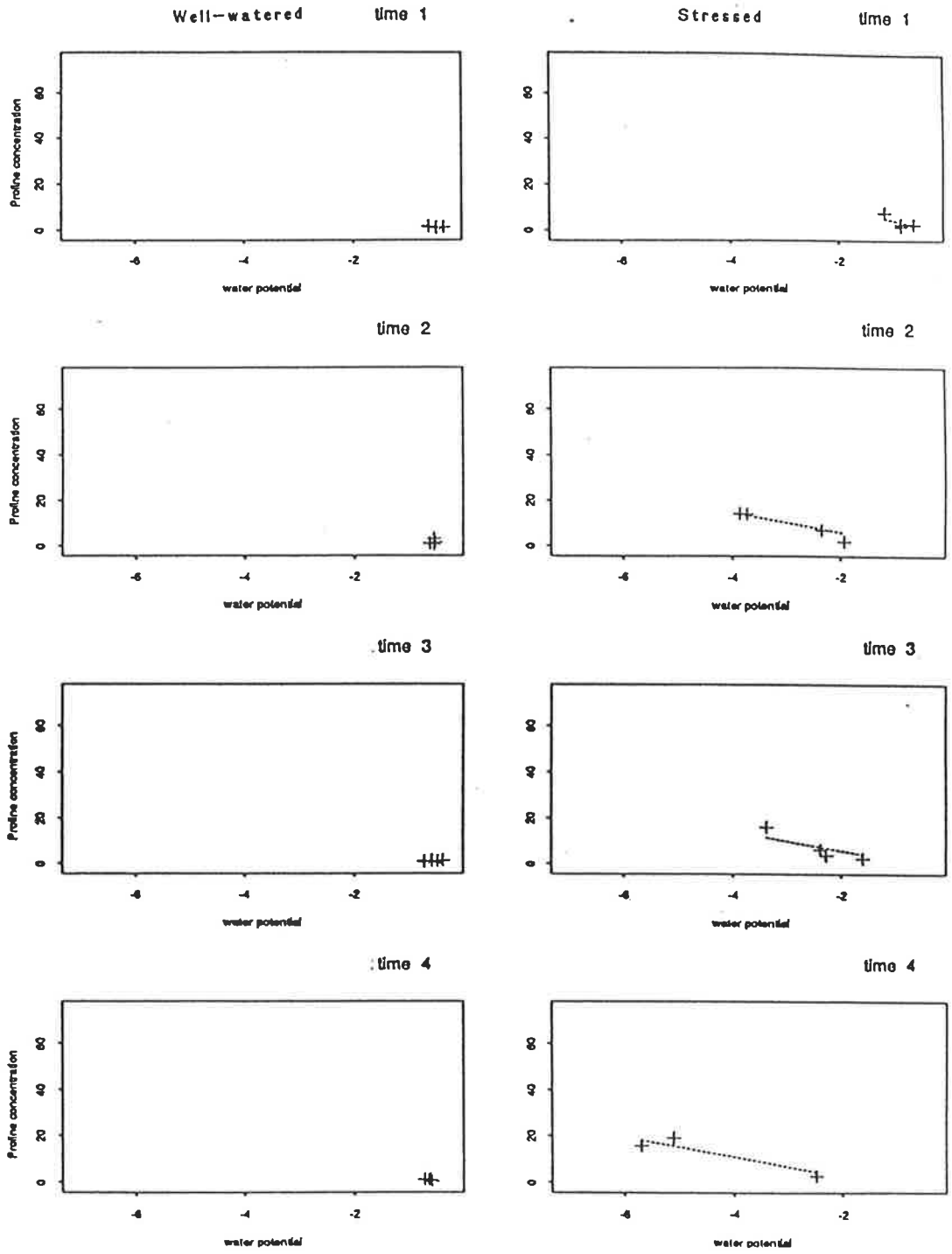
A. anceps


Well-watered
time 1
Stressed
time 1
time 2
time 2
time 3
time 3
time 4
time 4
Proline concentration
water potential
0
20
40
60
-6
-4
-2

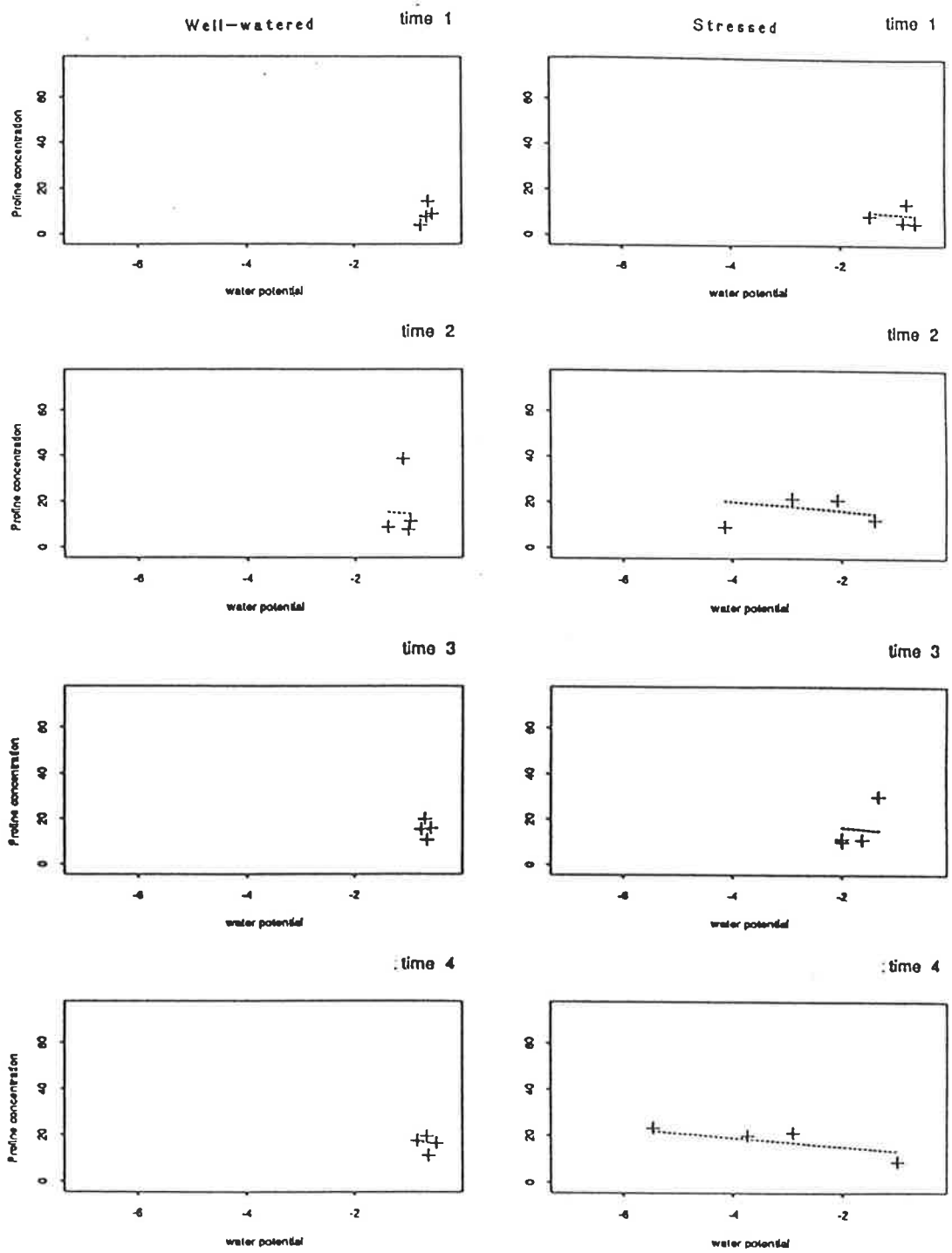
A. aneura


Well-watered
time 1
Stressed
time 1
time 2
time 2
time 3
time 3
time 4
time 4
Proline concentration
water potential
0
20
40
60
80
-6
-4
-2

A. gillii


Well-watered
time 1
Stressed
time 1
time 2
time 2
time 3
time 3
time 4
time 4
Proline concentration
water potential
0
20
40
60
-6
-4
-2

A. iteaphylla



A. myrtifolia

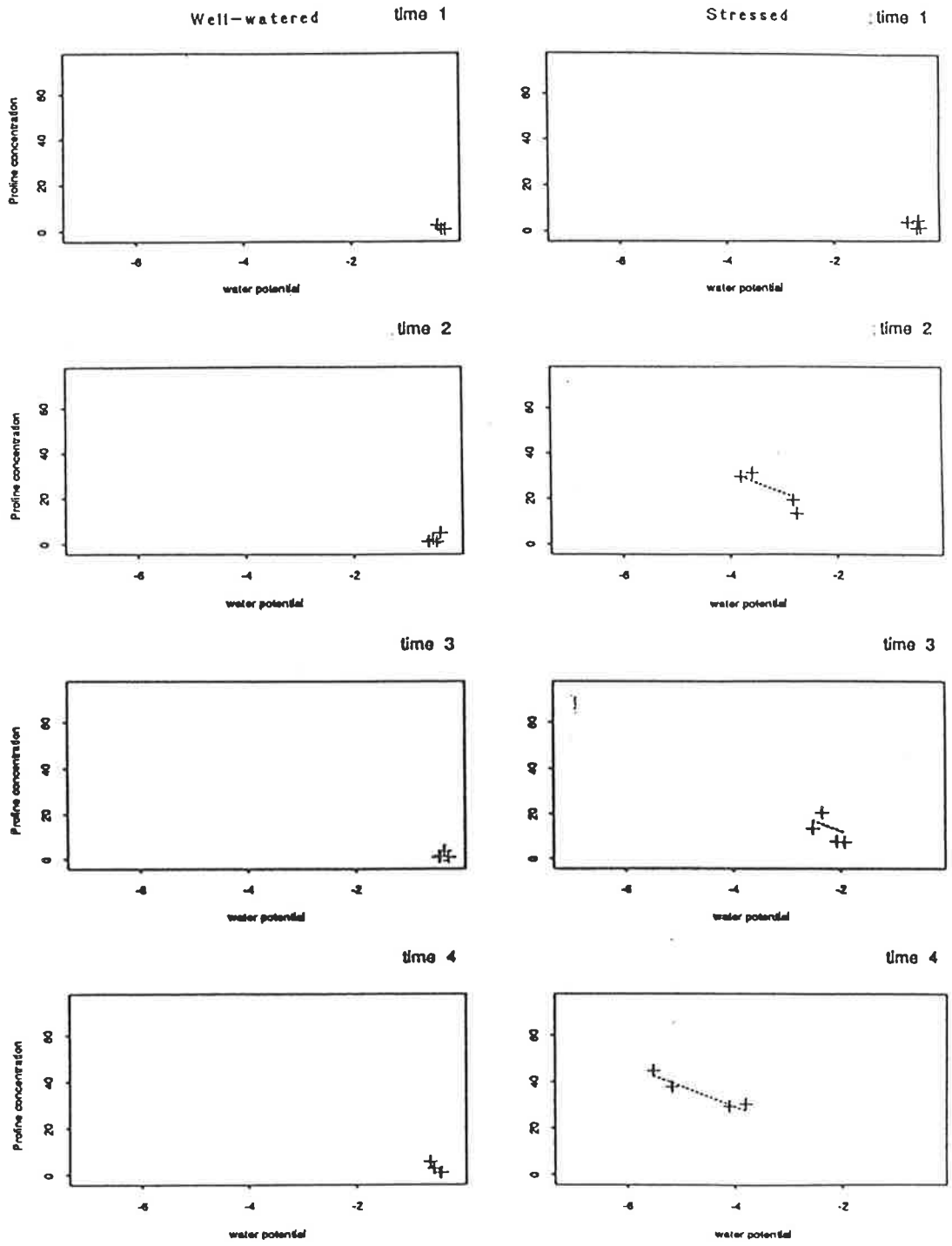


Table 5.6

Parameter estimates for proline concentration data

Fitted model

Slope estimates

anceps	0.148
aneura	1.02
gillii	0.421
iteaphylla	0.196
myrtifolia	0.878

Constant term estimates (values of estimation at zero water potential, μmol g^{-1} DW)

Water treatment: well-watered

	Harvesting			
Species	1	2	3	4
anceps	-0.0246	-1.0082	-0.0975	-0.2949
aneura	2.4971	3.9379	9.9173	-3.3819
gillii	-1.1210	-1.9640	1.7116	-1.9589
Iteaphylla	6.3251	12.7372	13.6820	15.4202
myrtifolia	-2.6405	-3.6033	-4.1390	-1.7718

Water treatment: Stressed

	Harvesting			
Species	1	2	3	4
anceps	1.0444	-1.2268	-1.0039	-4.3733
aneura	3.5662	3.7193	9.0109	-7.4603
gillii	-0.0519	-2.1826	-2.6180	-6.0373
iteaphylla	7.3942	12.5186	12.7756	11.3418
myrtifolia	-1.5714	-3.8219	-5.0454	-5.8502

Variance - Covariance matrix

6.94	-0.0524	6.29	-2.42
-0.0524	78.3	-9.37	-3.57
6.29	-9.37	25.28	-3.85
-2.42	-3.57	-3.85	5.18

is a common pattern in the field grown *Acacia* that when plants are in water stressed conditions, they accumulate more proline than those grown with sufficient water, however this accumulation would differ between species. This pattern seems to support the report of Treichel et al (1984) over a five year field study in the Namib Desert, Africa.

5.2.1.4.*a Differences between species*

During the four harvests the lowest concentration of proline in stressed plants was found in *A. anceps* (Table 5.4.b). The highest was *A. aneura* followed by *A. myrtifolia, A. iteaphylla* and *A. gillii.* These values seem comparable to those of Poljakoff-Mayber et al (1987) who found in a survey of native South Australian plant species that proline concentrations in some naturally field grown *Acacia* were varied, and ranged around the values from this experiment. In *A. pycnantha* the concentrations were (4, 12 and 15 μmoles g^{-1} DW), *A. colletioides* (8, 25 and 34), *A. oswaldii* (16, 15 and 14), *A. estrophiolata (*0.6 to 1.4). *A. aneura* samples which were from near Alice Springs, (Central Australia) ranged between 4.8 to 5.3 μmol g^{-1} DW, which is much lower than the same species reported here. During five years of records, Treichel *et al* (1984) found that the concentration of proline in field grown plants of *Acacia erioloba* only ranged from 1 to 4.4 μmol g^{-1} DW.

It was expected that species with a distribution covering a lower rainfall area would be more responsive to producing high proline concentrations since proline is considered an adaptation to drought (Aspinall and Paleg, 1981). In turn, species which occupy higher rainfall areas might be less responsive to accumulating proline. Thus more responsive species would accumulate more proline when the water potential moved to more negative levels.

A.aneura, a species distributed over low rainfall areas of 150 to 250 mm $year^{-1}$ (Whibley, 1980) behaved consistently with this expectation in that its proline content under

stress was higher than *A.myrtifolia* (distribution within rainfall limits of 500 to 1200 mm) and the other three species.

Interestingly however, the other three species distributed in lower rainfall areas had lower proline concentrations than the first two. In fact these three are the South Australian endemics. Whether or not there is any physiological implication between their endemic status and their lower capability to produce proline as field grown plants, still remains as a question.

A. iteaphylla showed a particular pattern. The average values of proline concentration during the four harvests (Figure 5.3) showed only small differences between control and stressed plants. This is not a result of 'drought killing' or firing leaves as suggested by Hanson and Tully (1979) who reported that the concentration of proline which remains high after stress could be due to the proline being 'locked up' in dead cells of shoot or leaves. During harvesting the shoots or leaves were carefully selected to avoid this possibility; also the water potential of this plant showed reasonable levels - although big differences existed between control and stressed plants. This unusual response led to an extended experiment with pot grown seedlings to show whether or not this pattern would be repeated under more controlled conditions (see Chapter 7).

5.2.1.4.*b* *Concentration in other field grown species*

Few reports have been found of seasonal fluctuation of proline in water stressed field grown plants. Besides the reports on *Acacia* in Poljakoff-Mayber et al (1987), Naidu et al (1987) recorded concentrations of only 1.3 and 5.8 μmol g^{-1} DW in *Callistemon pauciflora* and *C. brachiandus* at two independent winter harvests. *Melaleuca* spp. (15 species) for two winter and one summer measurements ranged between 0.2 to 5.6 with only one species reaching 2.4 μmol g^{-1} DW in summer. The lowest water potentials of

those samples in summer were between -2 and -2.6 MPa. Proline concentrations in all these species were lower than *Acacia* in this experiment.

5.2.1.4.c *The contribution of proline to osmotic potential*

Proline has become one of the most interesting organic solutes in plant water stress studies. It has drawn attention as a subject of research and discussion since it was first reported by Kemble and MacPherson (1954) to accumulate in water stressed ryegrass. Aspinall and Paleg (1981) reviewed a wide range of reports dealing with proline accumulation in various plant species.

It seems that in its early days, proline was considered to play an important role as an osmotic constituent, responsible for decreasing osmotic potential values. Singh et al (1973) after a study of pot grown seedlings of barley plants, suggested that genetically, proline could be used as a standard to select varieties better adapted to drought conditions. However, this finding was not repeatable by Hanson et al (1977) even using the same varieties used by Singh et al. Reports on other species also did not support the correlation between proline accumulation and drought resistance (Tully et al, 1979; Ibarra-Caballero et al, 1988). Ibarra-Caballero et al (1988) questioned the rôle of proline as an osmotic constituent at least in maize, and suggested that proline accumulation is only a sympton of drought stress. Stewart and Hanson (1980) noted that proline accumulation may result from the breakdown of other products such as protein or membranes; but this is still difficult to accept since the amount accumulated is greater than the possible proteolitic by-products.

Hence, to estimate the "actual" contribution of proline to the osmotic potential in plant cells, the osmotic potential of proline was measured (Figure 5.2). A series of proline concentrations was prepared of 0.1, 0.3, 0.5, 0.7 and 1 M respectively (chemical supplied by

Sigma, USA). The osmotic potential of each concentration was measured by thermocouple psychrometer.

It is clear that proline osmotic potential is rather less negative than sucrose (the common solute in plants) and much less negative than potassium and sodium. The curve for sucrose was generated from 'Handbook of Physics and Chemistry' (1972), those for pôtassium and sodium chloride from Wiebe et al (1971).

As has been mentioned (cf. Section 5.1.1.4) to estimate the contribution of a compound to the osmotic potential of the plant cells or tissues, the level of symplastic water must be known. Unfortunately, as mentioned above, no direct values of symplastic water were obtained from the samples of these *Acacia* species.

However, it can be seen by comparing Table 5.4.b (proline) with Tables 5.1.c (potassium) and Table 5.1.d (sodium) that the proline concentrations on a dry weight basis are an order of magnitude or more lower than potassium concentrations, and in most cases several times lower than sodium.

Table 5.7 presents similar calculations as Table 5.3 for K^+ and Na^+, to estimate the osmotic contribution of proline within these 5 field grown *Acacia* species. As shown (columns 7 and 9), the proline osmotic potential was very small (<0.01 MPa) as was its contribution to the total osmotic potential (<0.3%). Even taking the highest individual concentration, say the stressed *A. myrtifolia* (Table 5.4.b, 7th harvest) i.e. 35 μmol g DW^{-1}, the contribution was still less than 0.01 MPa. Assuming an error of say up to 30% in the calculation due to over estimation of symplastic : apoplastic water ratio, yet the concentration is still not sufficient for proline to have significant osmotic role.

Since proline is much less osmotically active than the two ions (K^+ and Na^+), it can be concluded that the contribution of proline to the cell osmotic potential would be very low, if it were free in the vacuole. However, since proline is considered to be a cytoplasmic solute,

Table 5.7

Contributions of proline to the osmotic potentials of the field grown plants of 5 *Acacia* species. C_p : proline concentrations; FW, DW : fresh weight, dry weight; WC: water content; P_{cntb}: proline contributions to ψ_π.

Species	C_p (μmol g^{-1} DW)	FW/DW ratio	C_p at FW (umol g^{-1} FW)	Total WC (%)	C_p (mM)	ψ_π P_{cntb} (-MPa)	Actual measured ψ_π (-MPa)	Total P cntb (-MPa), %
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
A.anceps	1.95	2.05	0.95	51.11	1.86	<0.01	3.60	<0.3
A.aneura	17.24	1.85	9.34	46.12	20.25	<0.01	3.07	<0.3
A.gillii	4.48	2.75	1.63	63.64	2.56	<0.01	2.75	<0.3
A.iteaphylla	14.37	2.03	7.08	50.78	13.94	<0.01	2.60	<0.3
A.myrtifolia	10.11	2.0	5.05	49.96	10.11	<0.01	2.85	<0.3

Note: **Values in:**

Column

(2) Mean concentrations of the four harvests, both well-watered and stressed, from Tables 5.4.b (μmol g^{-1} DW).

(3) Mean Fresh Weight : Dry Weight ratio from measurements made in February 1990.

(4) Mean concentrations on a FW basis (column 2 / column 3) (μmol g^{-1} FW).

(5) Mean total water content, % FW.

(6) Solute (proline) concentrations (column 4 / column 5) assuming all water, and all molecules, are symplastic (mM).

(7) Osmotic potentials of this (proline) solution, using Figure 5.2. (-MPa).

(8) Mean measured osmotic potentials, including both watered and stressed treatments (Table 4.2.b). (-MPa).

(9) Estimated contributions of proline to total osmotic potential (%).

and since the cytoplasm occupies only 5 to 10% of a cell (Flowers and Yeo, 1986), therefore for cytoplasmic osmotic adjustment, proline may have a substantial role. Here, proline is best seen as a 'compatible solute' (cf. Weimberg et al 1982; Tyree and Jarvis, 1982).

As mentioned in Section 5.1.1.4, where potassium and sodium were nearly up to metabolically damaging concentrations, there is a need for proline in the cytoplasm as a compatible solute.

5.2.2 *Betaines and other ammonium compounds*

Unlike proline, betaine is not commonly accumulated in water stressed plants. This compound tends to accumulate in salt stressed plants (Hanson and Hitz, 1982; Robinson and Jones, 1986). However, a few reports provided evidence that it could increase to a significant concentration when plants were in drought conditions (Hanson and Nelsen, 1978) in barley. Hence, screening tests were carried out to find out whether or not *Acacia* belongs to the plant group which accumulates betaine (and other compounds such as choline, etc.) during water stress. Moreover, other new compounds may be accumulated by the *Acacia* plants during drought.

5.2.2.1 *Materials and Methods*

This work was carried out in Professor L.G.Paleg's laboratory in the Department of Plant Physiology, Waite Agricultural Research Institute, The University of Adelaide.

Betaines and other ammonium compounds were determined using H^+ NMR (Nuclear Magnetic Resonance) spectroscopy. The steps for final preparation of samples were carried out following procedures by Jones *et al* (1986) and Naidu *et al* (1987).

About 10 ml of the supernatant which was obtained for proline analysis was run through a column (approx. 30 cm long and 1.5 cm diameter) containing 5 g Dowex 50 W for ionic exchange.

However, before running the sample, the resin contained in the column was converted to the H^+ form by flushing with approximately 25 ml 8 M HCl, then washing with distilled water until the eluent pH reached 5. An appreciable residual of H^+ in the column can cause the loss of retention ability for betaine and other compounds.

The sample was then loaded onto the column and washed with approximately 100 ml distilled water to free the sample from sugars.

Quarternary ammonium compounds and amino acids were eluted by using 100 to 150 ml 4 M HCl.

The eluent eventually was vacuum-dried, using a rotary evaporator and a water bath at 50 to 60°C.

The residue which attached to the inner wall of the flask was dissolved with approximately 5 ml ethanol (as a step to remove residual hydrochloric acid), and re-dried. The residue of ethanol and probably acid, was removed by adding approximately 5 ml distilled water, to avoid interference during further NMR reading.

After redrying, the flask was placed in a 70°C oven for about 30 minutes, then sealed with a lid before cooling down at room temperature.

The sample was dissolved with 0.8 ml D_2O, removed into an eppendorf tube, and stored.

For NMR spectra reading, the sample was centrifuged for ten minutes, then 0.5 ml of the supernatant was transferred to the NMR tube, to which was added 2 µm *t*-butanol as an internal reference. The final pH ranged between 1 and 3.

The sample was loaded into the NMR machine and analyzed, as described by Jones et al (1986).

5.2.2.2 *Results and Discussion*

The results from the NMR spectra generated from all the field grown plant samples revealed *no betaine accumulation* in these five *Acacia* species. In the NMR spectra, the betaine peak usually appeared at 3.2 ppm (Jones et al 1986; Robinson and Jones, 1986). Other compounds such as choline also were not accumulated by these species as well.

5.2.3 *Phenylethylamine (PEA)*

5.2.3.1 *Results and Discussion*

The NMR spectra, however, displayed one unusual peak for a compound which was later identified as *phenylethylamine (PEA)*. Phenylethylamine was unexpectedly detected by the NMR spectroscopy from one of the five species used in the field experiment, namely *A. iteaphylla* (Figure 5.5). Since then a detailed study has been conducted of specific characteristics of its crystallography (Horn et al 1990). See Appendix 3.

Table 5.8 presents the original and the mean values of PEA during 4 harvests in *A.iteaphylla*. Figure 5.6 displays the average seasonal fluctuation of PEA concentration in field grown plants over four harvests in conjunction with the seasonal fluctuation of water potential. It reveals that the decline of water potential governed by the rainfall did not appear to promote the increase of PEA concentration.

The initial statistical model for PEA was similar to that for potassium, sodium and proline, except with no species effect. The model was a multivariate repeated measures with time changing covariates.

Figure 5.5

The Nuclear Magnetic Resonance (NMR) spectrum of an *A.iteaphylla* extraction, showing the peak of phenylethylamine (PEA) appearing at 7.38 ppm.

The peak at 1.245 ppm was from 2 μmol *t*-butanol which was used as an internal standard to evaluate the concentration of PEA and other ammonium compounds.

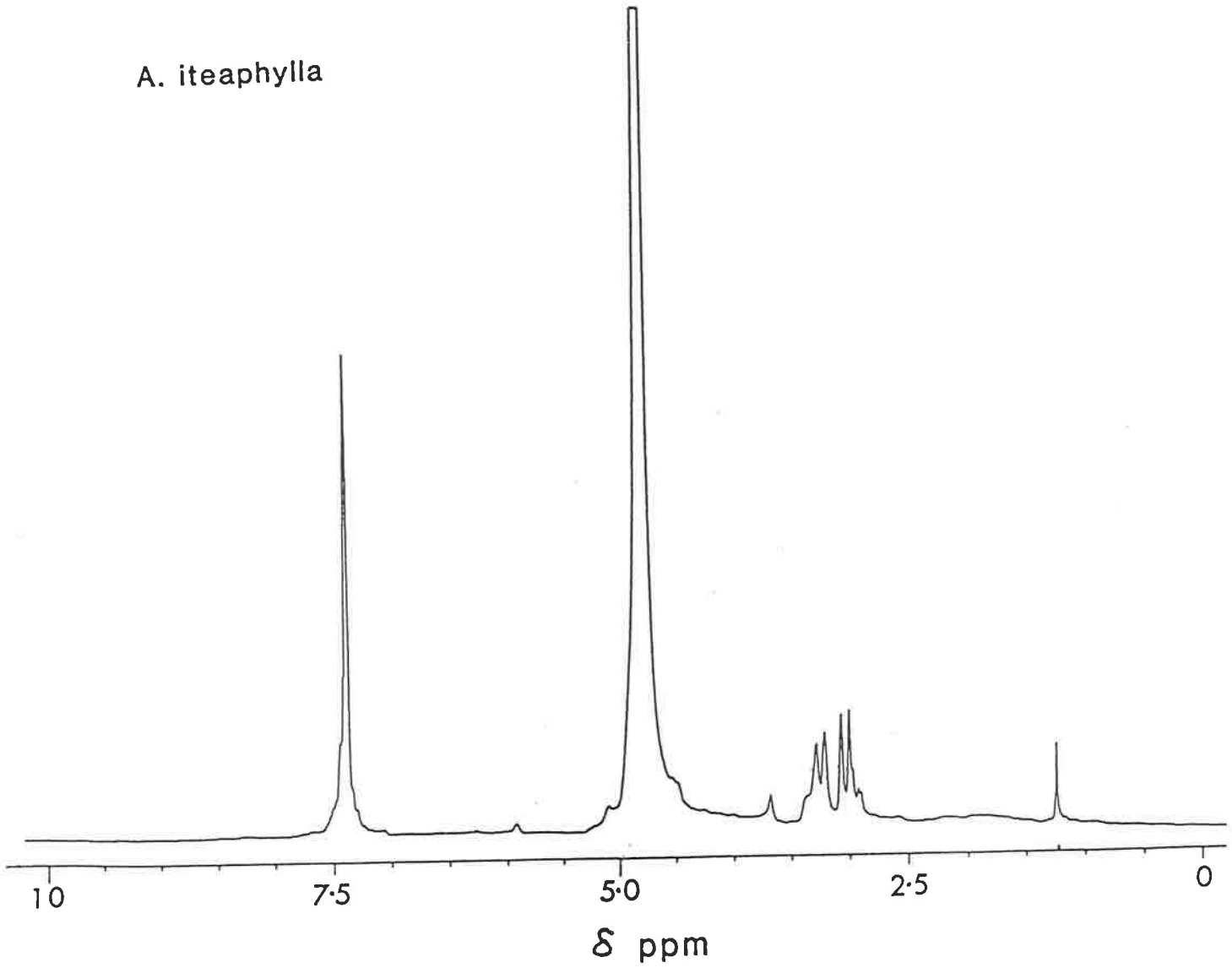

A. iteaphylla
10
7·5
5·0
2·5
0
δ ppm

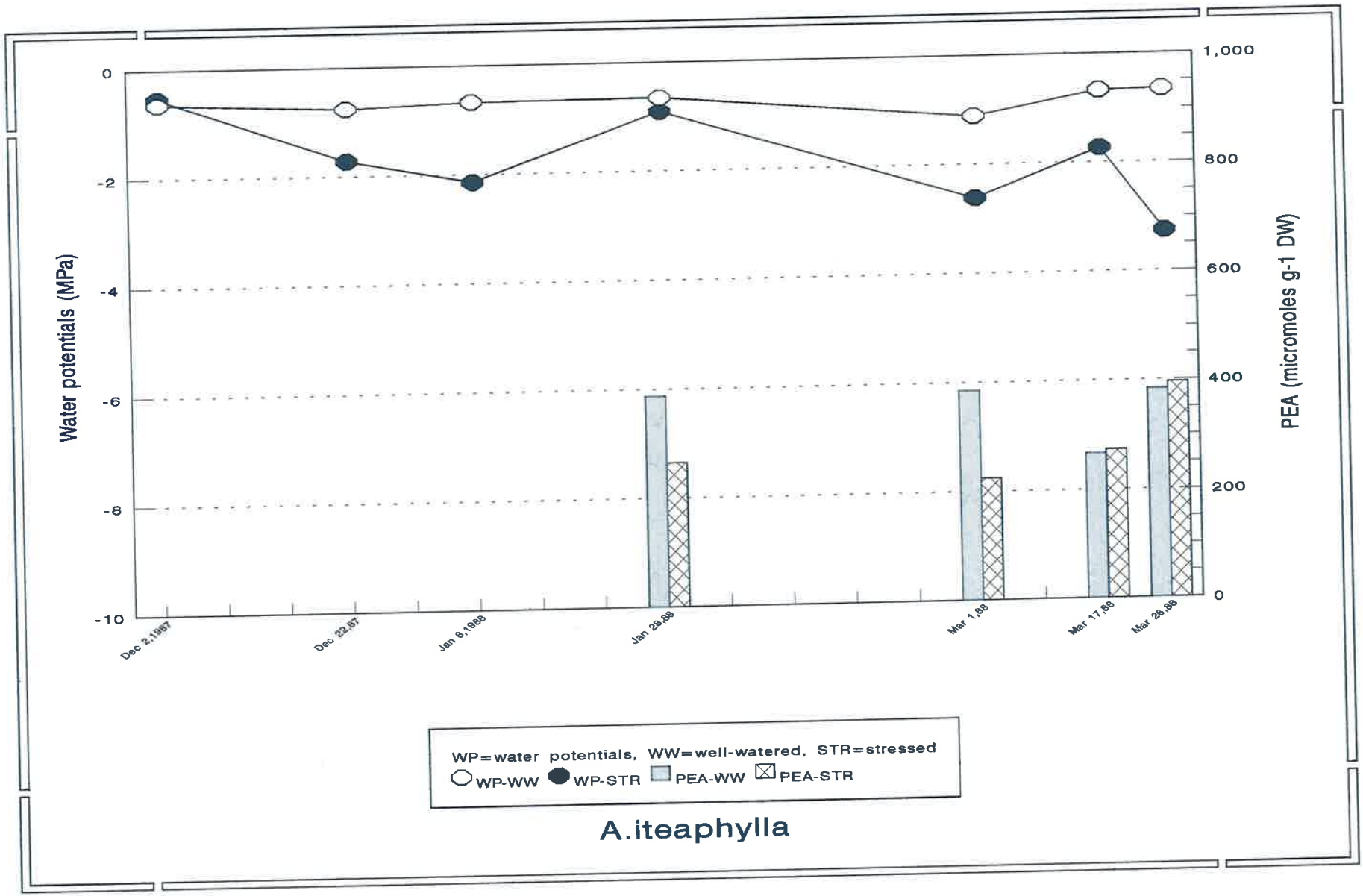
Table 5.8

The original and the mean values (n=4, except one harvest, n=3) of PEA concentrations (μmol g^{-1} DW) in the field grown *A.iteaphylla* during 4 harvests. *WW*, well-watered, *STR*, stressed treatments, *R*, replications.

Water treatment		Harvesting			
	R	4	5	6	7
WW	1	454.5	291.9	204.7	582.5
	2	376.7	815.7	301.2	215.7
	3	363.5	241.7	392.1	501.0
	4	354.9	192.8	168.1	237.7
Means		**387.4**	**385.5**	**266.5**	**384.2**
STR	1	86.3	103.9	316.6	369.0
	2	447.5	333.5	298.6	433.1
	3	188.4	234.8	128.5	360.1
	4	336.2	-	347.9	422.6
Means		**264.6**	**224.1**	**272.9**	**396.2**

Figure 5.6

The seasonal fluctuations of PEA (μmol g^{-1} DW) in the field grown plants of *Acacia iteaphylla* measured in two treatments during 4 harvests.
Mean water potentials (-MPa) of the well-watered and stressed treatments are displayed for comparison.
For legends, see Figure.


Water potentials (MPa)
0
-2
-4
-6
-8
-10
PEA (micromoles g-1 DW)
1,000
800
600
400
200
0
Dec 2,1987
Dec 22,87
Jan 8,1988
Jan 28,88
Mar 1,88
Mar 17,88
Mar 26,88
WP=water potentials, WW=well-watered, STR=stressed
WP-WW
WP-STR
PEA-WW
PEA-STR
A.iteaphylla

First of all, it has to be emphasized that there were not enough data to realistically show the relationship between PEA concentration and water potential. The analysis revealed a statistical condition to accept the occurrence of linear relationship between water potential and PEA concentration. However, the graphs and tables of estimation revealed the inadequacy of this model, which actually attempted to estimate 20 parameters from 28 data points.

Hence, a further analysis was conducted by ignoring the water potential information. A multivariate analysis of variance without time changing covariates was performed. At 5% significance level, Table 5.9 and Figure 5.7 provided evidence that the hypothesis of no stress effect could be retained. Therefore, it seems that PEA did not respond to the decline of water potential in the field grown plants of *A. iteaphylla.*

PEA occurrence within plants was reported quite a long time ago by White (1944) including some *Acacia* species but not *A. iteaphylla.* PEA and related compounds have been found distributed from algae, fungi to higher plants, and it has been reported in more than 300 species in this range covering 44 families (see review by Smith, 1977).

PEA is an aromatic compound; its architecture includes eleven protons, two methylated groups and one nitrate molecule. In the NMR spectroscopy spectrum the PEA peak which occurs at 7.38 ppm is due to its two methylated protons.

The concentration of this compound in *A. iteaphylla* was very high, up to 3% of dry weight, sometimes up to 7% (Horn et al, 1990). The highest concentration in individual field grown plants in this experiment was about 800 µmol g^{-1} DW.

5.2.3.2 *Extended experiments in PEA*

Since this study is concerned with water stress and solute accumulation as a response to water stress, further experiments with pot grown seedlings in a glasshouse

Table 5.9

Edited output from Genstat 5 Manova procedure

*** Multivariate analysis of variance***

Stress effect

SSP-matrix, with 1 degree of freedom

1	27375			
2	-2571	241		
3	-3885	365	551	
4	9557	-899	-1359	3349
	1	2	3	4

Test

Wilk's Lambda	0.2403					
Approximate Chi Sq	4.28	d.f	4			
Approximate F test	1.58	on	4	and	2	d.f
Pillai-Bartlett trace	0.7597					
Roy's maximum root test	0.7597					
Lawley-Hotelling trace	3.161					

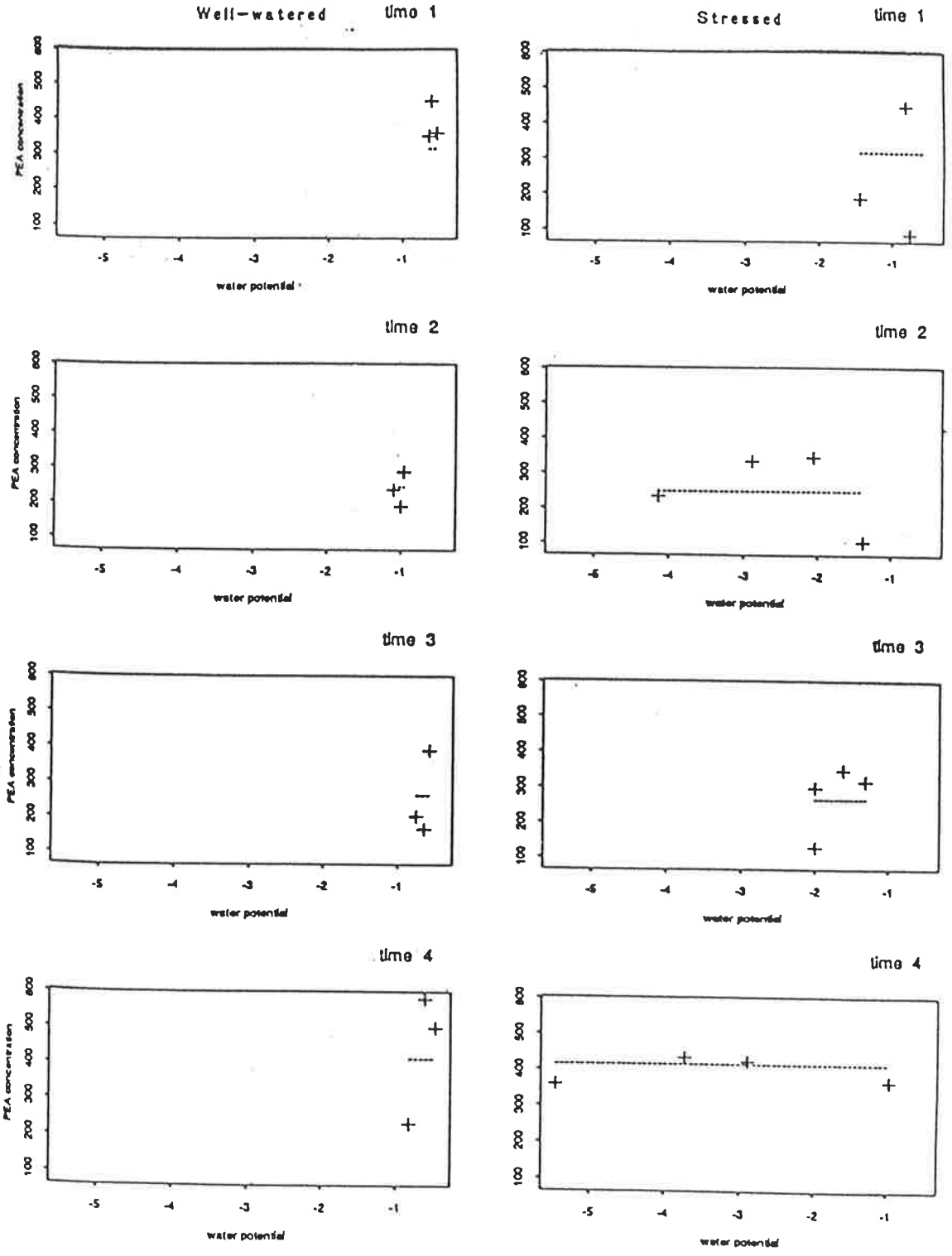
Residual SSP matrix, with 5 degree of freedom

1	82268			
2	54149	42194		
3	9455	6716	57925	
4	30914	27126	25728	69054
	1	2	3	4

Figure 5.7

The relationship between PEA concentration (μmol g^{-1} DW) and plant water potential (-MPa) at each harvest and each treatment for field grown *A.iteaphylla*.

A. iteaphylla



environment were conducted to investigate the PEA response to water stress. By using a pot experiment in the glasshouse, it was intended to isolate the plants from large fluctuations of environmental factors; therefore a single factor stress effect (in this case, water) could be more easily applied and might be better regulated and observed in relatively well-controlled conditions.

Two experiments were conducted. These are described in a later chapter. The first experiment was a preliminary investigation to reveal the response of PEA to water stress. Salt and cold (chilling) stresses were tested as well. Since the result from the water stress treatment was inconclusive, further observations were conducted in more detail.

From this study it is not clear what solutes caused osmotic adjustment, since K^+, Na^+, and PEA did not increase while osmotic adjustment was taking place, and the increase in proline was not enough to affect total Ψ_π. There must have been some other solutes involved such as sugars or other organic compounds which were not measured.

CHAPTER 6

POTASSIUM AND SODIUM

The effect of potassium concentration in nutrient solution on the osmotic adjustment of A.iteaphylla pot grown seedlings

As shown in section 5.1.1, neither potassium nor sodium concentrations changed as a response to water deficit in the field experiment. This further experiment was set up to investigate these ions in more detail with seedlings grown under controlled glass-house conditions.

Some reports show that potassium did not change noticeably with the change of plant water status (Cutler and Rains, 1978), while others provide evidence of potassium involvement in the regulation of osmotic pressure (for example Jones *et al*, 1980; Mengel and Arneke, 1982). Premachandra et al (1993) showed that high K^+ application reduced the leaf rolling during drought in maize. A low degree of leaf rolling was associated with high Ψ_p due to osmotic adjustment.

The aim of this experiment was to find out whether seedlings fed nutrients with different levels of potassium, would display different degrees of osmotic adjustment.

6.1 *Materials and Methods*

Seedlings of *A.iteaphylla* were established from seeds, and grown in pots (13.5 cm in height, 12 cm diameter at upper end, and 10 cm lower end, with no holes at the bottom). The growth medium was sterile sand, obtained from the Waite Agricultural Research Institute, University of Adelaide. For the first four months, they were fed by full strength Hoagland nutrient solution to promote early growth. During this period, the seedlings received 800 ml

full strength Hoagland solution given in four applications. The plants were watered with deionized water to field capacity by weighing the pots.

The treatments were then applied. Four regimes of Hoagland nutrient solutions were manipulated to contain different levels of potassium, i.e.

- *Hoagland without potassium*,
- *Hoagland with half strength potassium*
- *Hoagland with four times strength potassium* (*extra strength*), and
- *Hoagland with normal strength as control*.

Each litre of Hoagland solution with extra (4x) potassium, was enriched with 9 ml of (K_2SO_4) stock solution. Table 6.1 displays the composition of the Hoagland's nutrient solutions for the 4 treatments.

Forty-eight pots were randomized into 4 groups, and each group (12 plants) received one treatment. There were four applications, with 200 ml each over a three month period. At the end, only 11 pots of the 4x K^+ strength treatment were available due to an unexpected damaging.

After three months, all the seedlings were subjected to a drying cycle to investigate the osmotic adjustment process. Watering was stopped for all pots at the same time and the drying period lasted for about six weeks. On measurement days during this period 2 to 3 pots were selected and for each water potential (Ψ), osmotic potential (Ψ_π), K^+ and Na^+ contents were measured. Thus each pot generated one value of each variable. On each measurement day pots from different treatments were chosen, so that each treatment was sampled at intervals throughout the drying cycle.

Ψ was measured with the pressure chamber and Ψ_π by the pressure-volume curve method, as described in Chapter 2. Potassium and sodium content were estimated by flame

Table 6.1

Composition of four Hoagland's nutrient solutions with respect to the manipulation of potassium (K^+) concentrations for osmotic adjustment studies in *A.iteaphylla* seedlings Numbers in colums are the amount of stock solutions (ml) per 1 litre Hoagland

Stock solutions (M)	Treatments			
	Complete	Minus K^+	½ K^+	4 x K^+
KNO_3	5	-	2.5	5
Ca $(NO_3)_2$	5	5	5	5
$MgSO_4$	2	2	2	2
KH_2PO_4	1	-	0.5	1
NaH_2PO_4	-	1	0.5	-
$NaNO_3$	-	5	2.5	-
K_2SO_4	-	-	-	9
Micronutrients	1	1	1	1
Fe EDTA	1	1	1	1

photometer using solute extracted from 30 to 50 mg oven dry leaf samples, as described in Chapter 5.

In order to obtain the contribution of potassium to cell sap concentration, the symplastic water volumes were calculated from the pressure-volume (P-V) curves obtained from all the individual plants in all treatments during the drying cycle by the method described in Section 2.4.1.2.

6.2 *Results*

All the original data obtained from the pressure chamber are displayed in Table 6.2; Columns 1a to 4a for water potentials, 1b to 4b for osmotic potentials, columns 1c to 4c for potassium (K^+), and 1d to 4d for sodium (Na^+) concentrations. Figure 6.1 shows: (a) the relationship between the changes of potassium and sodium concentration, and (b) the decrease of water potential and osmotic potential in the four treatments over the drying cycle. Note that values in Table 6.2 and these histograms have been arranged in order of decreasing Ψ_π.

6.2.1 *Effects of drying on water potential and osmotic potential*

Table 6.2 and Figure 6.1 show the changes of water potential towards more negative values as a response to the water stress treatment. When water potential had declined to below -3 MPa, it seemed that all plants in all treatments could no longer lower their osmotic potential to values more negative than the water potential, thus turgor pressure approached zero.

Figure 6.2 shows the exponential relationship between water potential and osmotic potential using the same procedure as in Chapter 3 (Section 3.3 and 3.4.3.). Table 6.3 shows that the r^2 values indicated a good relationship between these two characters in all treatments.

Table 6.2

Measurements made during a drying cycle on *Acacia iteaphylla*, previously treated with 4 different potassium (K^+) concentrations in Hoagland's nutrient solution.ψ, water potential (-MPa), ψ_π. osmotic potential (-MPa), K^+, potassium and Na^+, sodium concentrations (μmol g^{-1} DW). CH, Complete Hoagland, -K^+, Hoagland minus potassium, ½ K^+, Hoagland with half strength potassium and 4xK^+, Hoagland with four times strength potassium. Values are arranged in order of decreasing ψ_π.

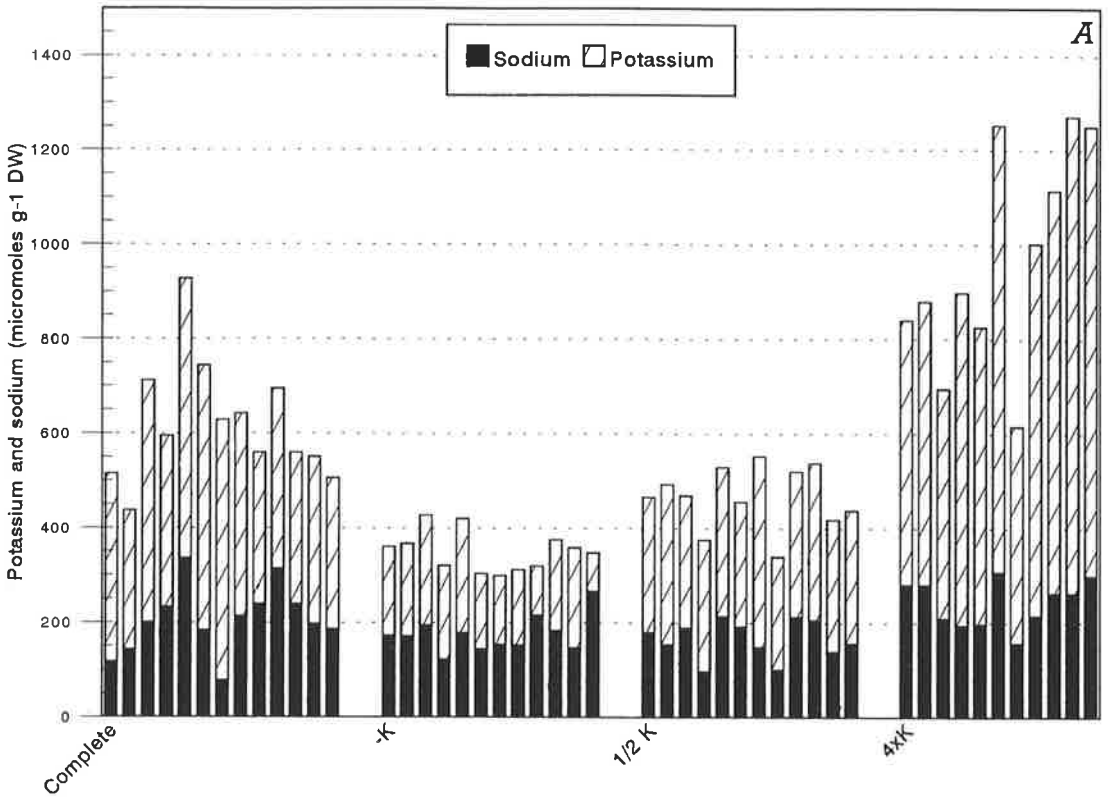
T R E A T M E N T															
1 CH				2 - K+				3 ½ x K				4 4 x K			
(a) ψ	(b) ψ_π	(c) K+	(d) Na+	(a) ψ	(b) ψ_π	(c) K+	(d) Na+	(a) ψ	(b) ψ_π	(c) K+	(d) Na+	(a) ψ	(b) ψ_π	(c) K+	(d) Na+
0.4	1.75	515.62	117.19	0.5	1.85	360.90	172.93	0.5	1.85	465.67	179.10	0.75	2.22	840	280
2.1	2.17	437.38	143.14	0.65	2.17	367.19	171.87	1.2	2.11	492.93	153.54	0.1	2.32	880	280
0.65	2.32	712	200	1.6	2.27	428.28	193.94	0.5	2.17	468.61	188.91	0.9	2.37	695.08	209.84
1.4	2.43	594	232.93	2.5	2.94	320.99	123.46	2.0	2.44	376	96	1.15	2.44	899.02	195.44
0.7	2.44	928	336	3.4	3.45	420.20	177.78	0.95	2.63	530.39	213.63	1.3	2.44	826.23	196.72
1.25	2.78	744	184	3.6	3.77	304	144	2.6	2.7	456	192	1.5	2.53	1253.33	306.67
3.2	3.33	628.99	76.90	3.8	4.08	300	154.16	2.65	2.92	553.09	148.15	3.2	3.23	616.39	157.38
3.25	3.51	643.90	214.63	3.95	4.08	312	152	2.9	3.03	340	100	3.3	3.54	1003.17	215.87
3.5	3.64	695.08	314.75	4.1	4.17	320	216	3.3	3.51	520	212.05	3.85	4.0	1114.75	262.29
3.4	3.77	560	240	4.5	4.76	376	184	3.6	3.61	538.46	205.13	4.1	4.17	1272.13	262.29
4.0	4.17	550.82	196.72	5.3	5.3	359.18	146.94	4.35	4.44	417.72	139.24	4.0	4.49	1250.81	299.67
5.2	5.41	506.67	186.67	5.65	5.65	347.47	266.67	4.58	4.76	437.79	157.38	---	---	---	---

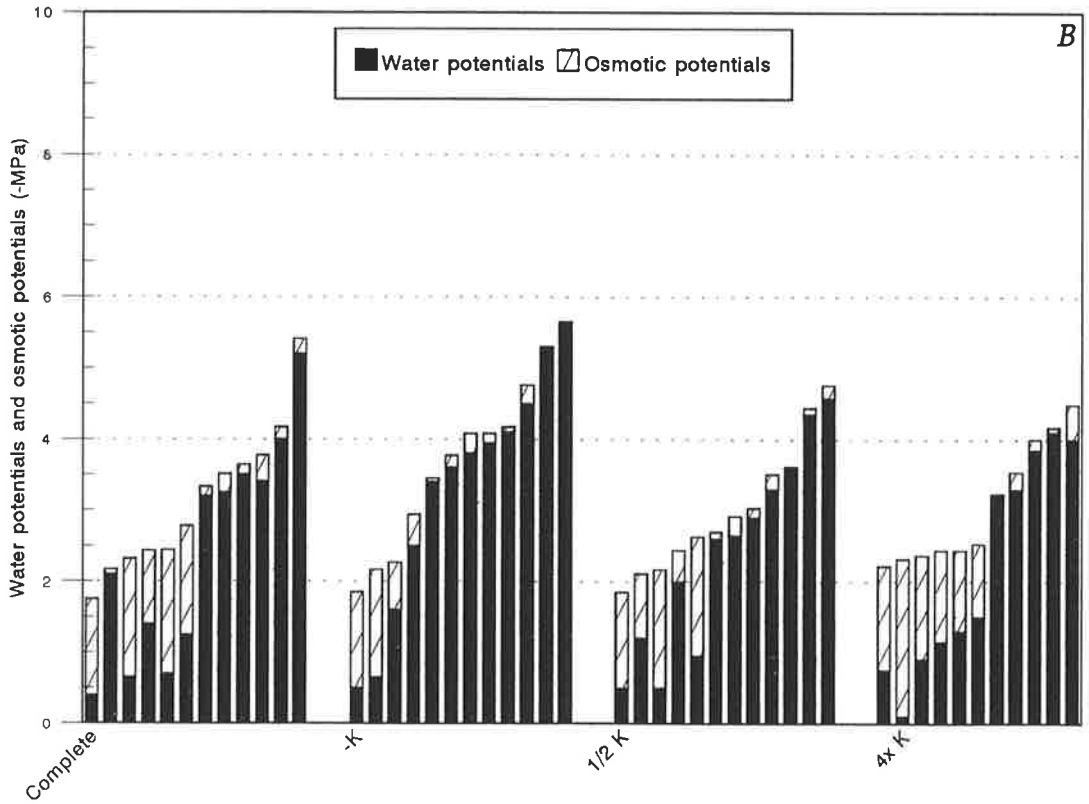
Figure 6.1

The trend of (A) potassium and sodium changes and (B) the corresponding water potential and osmotic potential during drying treatment in *A.iteaphylla* grown under different nutrient treatments.

Complete:		complete Hoagland
-K	:	without potassium
0.5 K	:	half strength potassium
4 x K	:	extra (four times) strength potassium

Note that values have been arranged in order of decreasing osmotic potential, not as a time sequence.





Potassium treatments

Figure 6.2

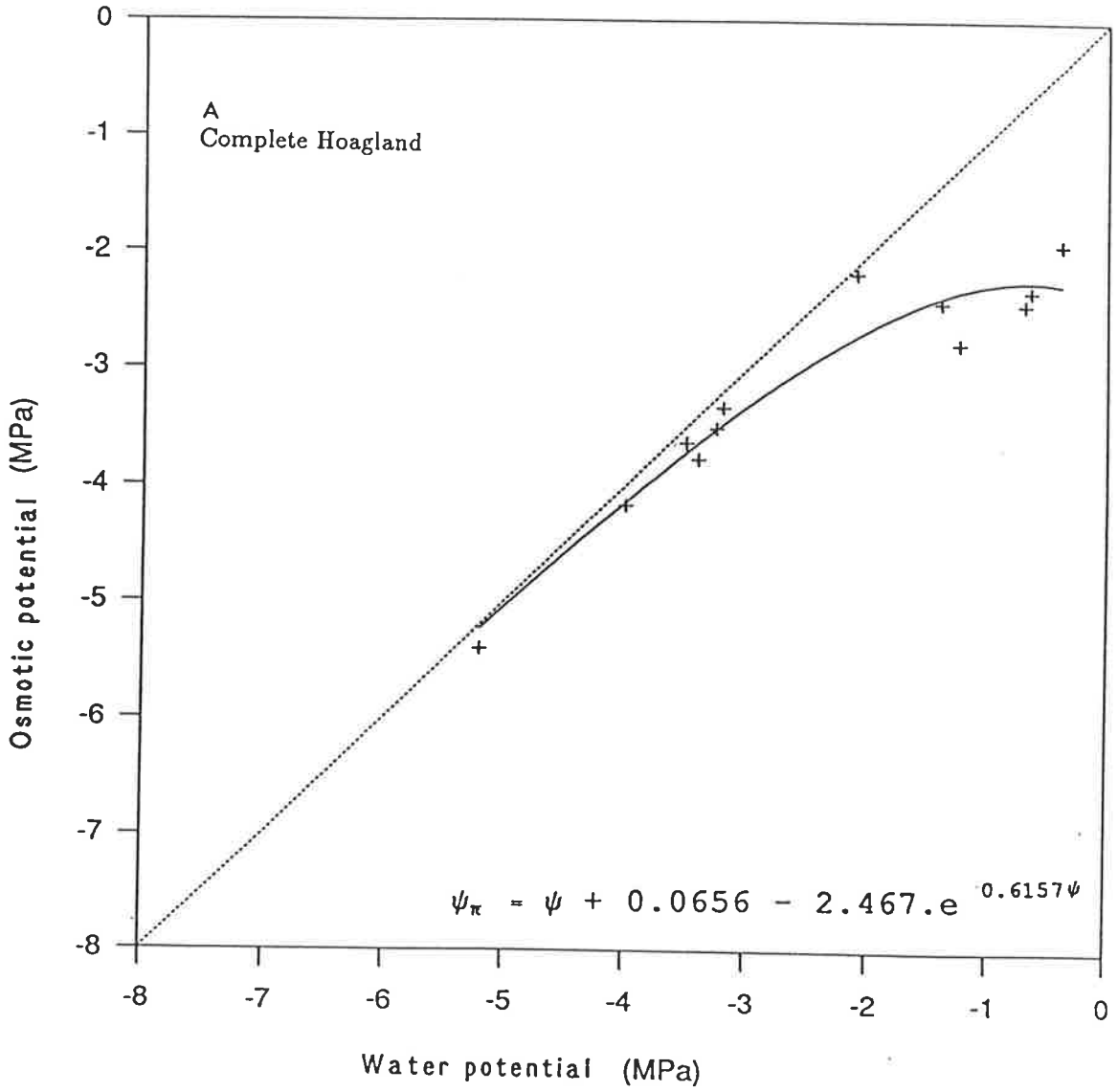
The exponential relationship between water potential (MPa) and osmotic potential (MPa) during a drying cycle, in *A.iteaphylla* seedlings grown under different concentrations of potassium in nutrient solution.

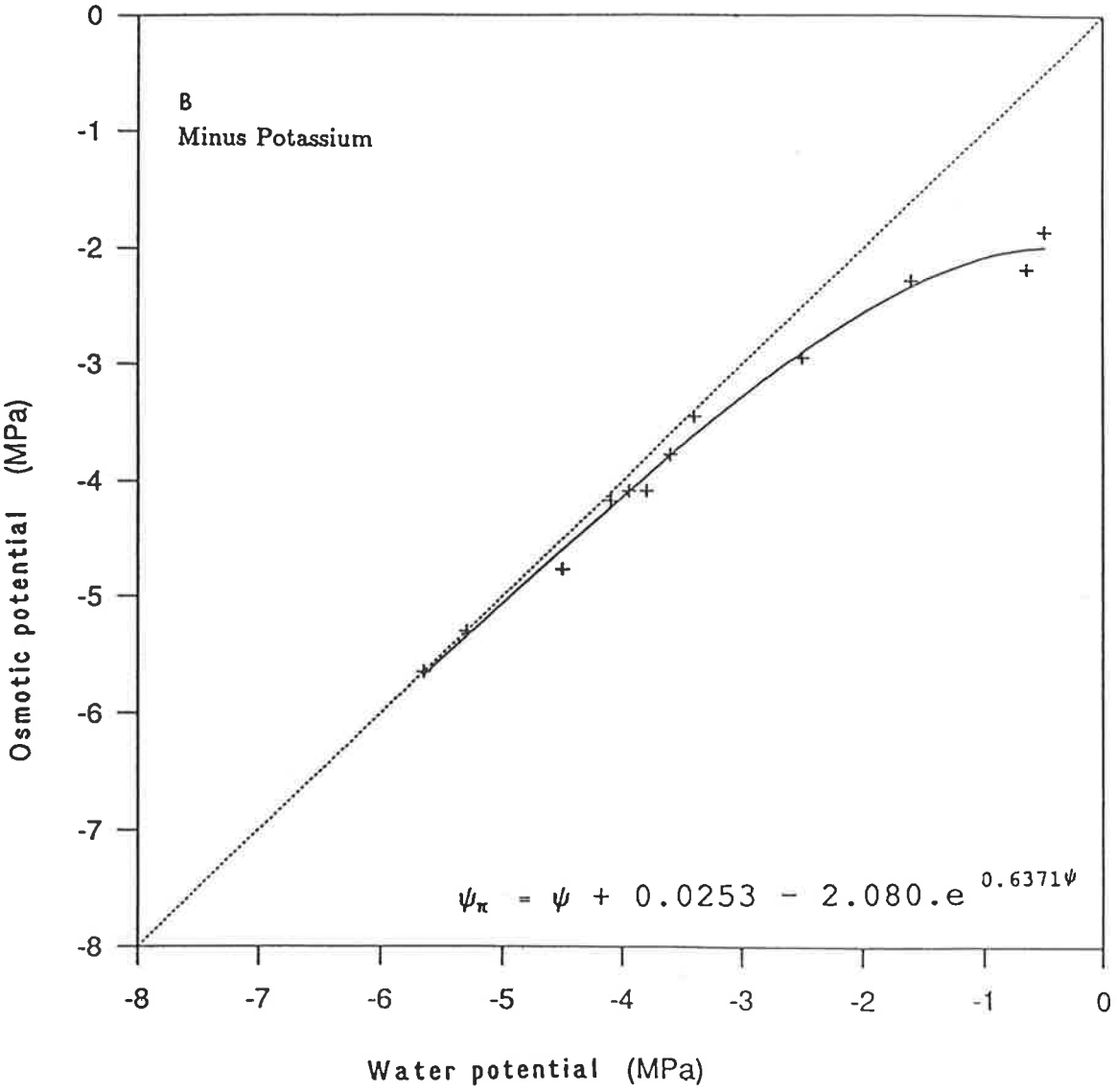
A: Complete Hoagland's

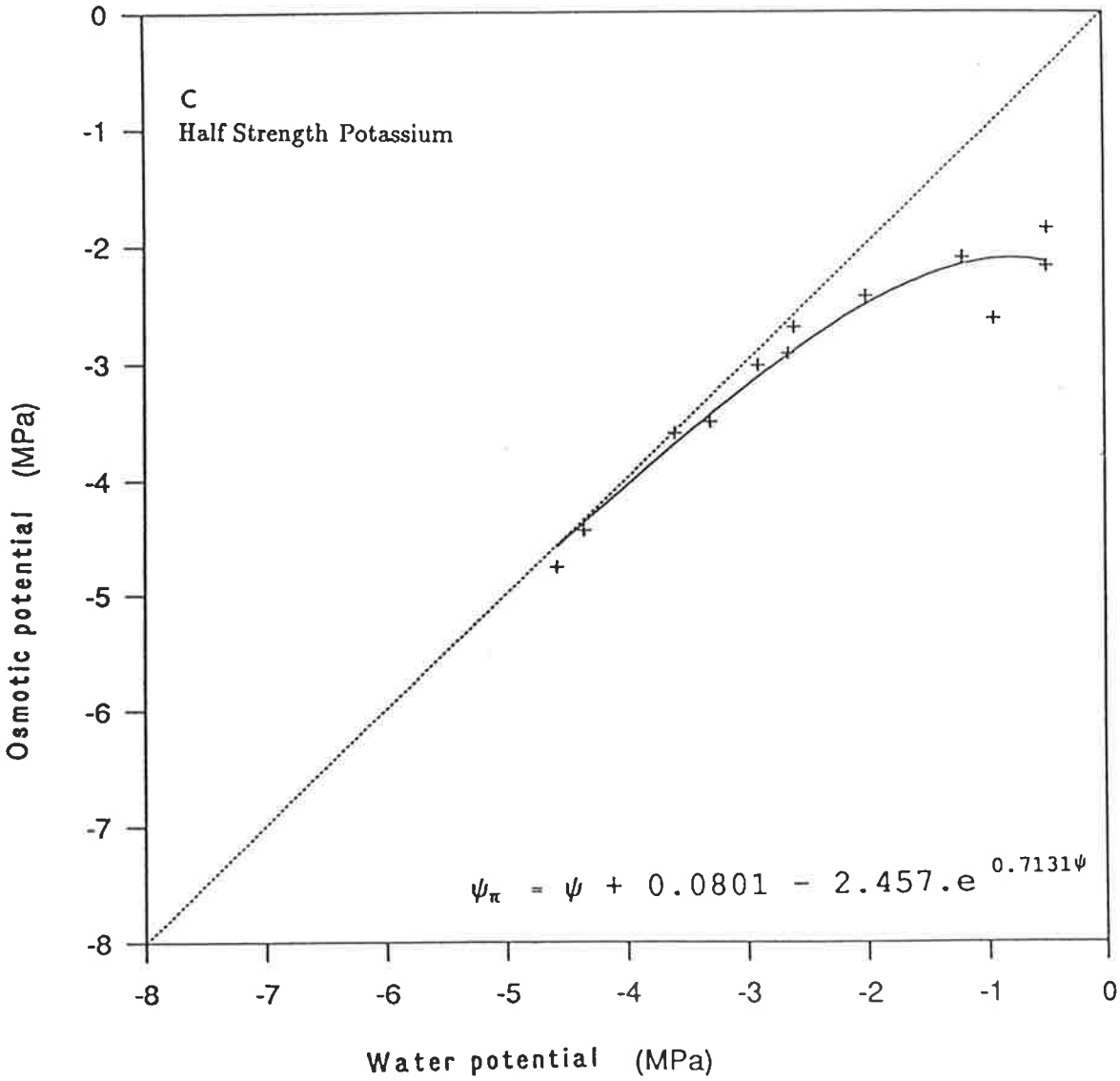
B: Hoagland's without potassium

C: Hoagland's with half strength potassium

D: Hoagland's with extra (four times) potassium


A
Complete Hoagland
$\psi_\pi = \psi + 0.0656 - 2.467.e^{0.6157\psi}$
Osmotic potential (MPa)
Water potential (MPa)
0
-1
-2
-3
-4
-5
-6
-7
-8


B
Minus Potassium
$\psi_\pi = \psi + 0.0253 - 2.080.e^{0.6371\psi}$
Osmotic potential (MPa)
Water potential (MPa)
0
-1
-2
-3
-4
-5
-6
-7
-8
-8
-7
-6
-5
-4
-3
-2
-1
0


C
Half Strength Potassium
$\psi_\pi = \psi + 0.0801 - 2.457.e^{0.7131\psi}$
Osmotic potential (MPa)
Water potential (MPa)
0
-1
-2
-3
-4
-5
-6
-7
-8
-8
-7
-6
-5
-4
-3
-2
-1
0

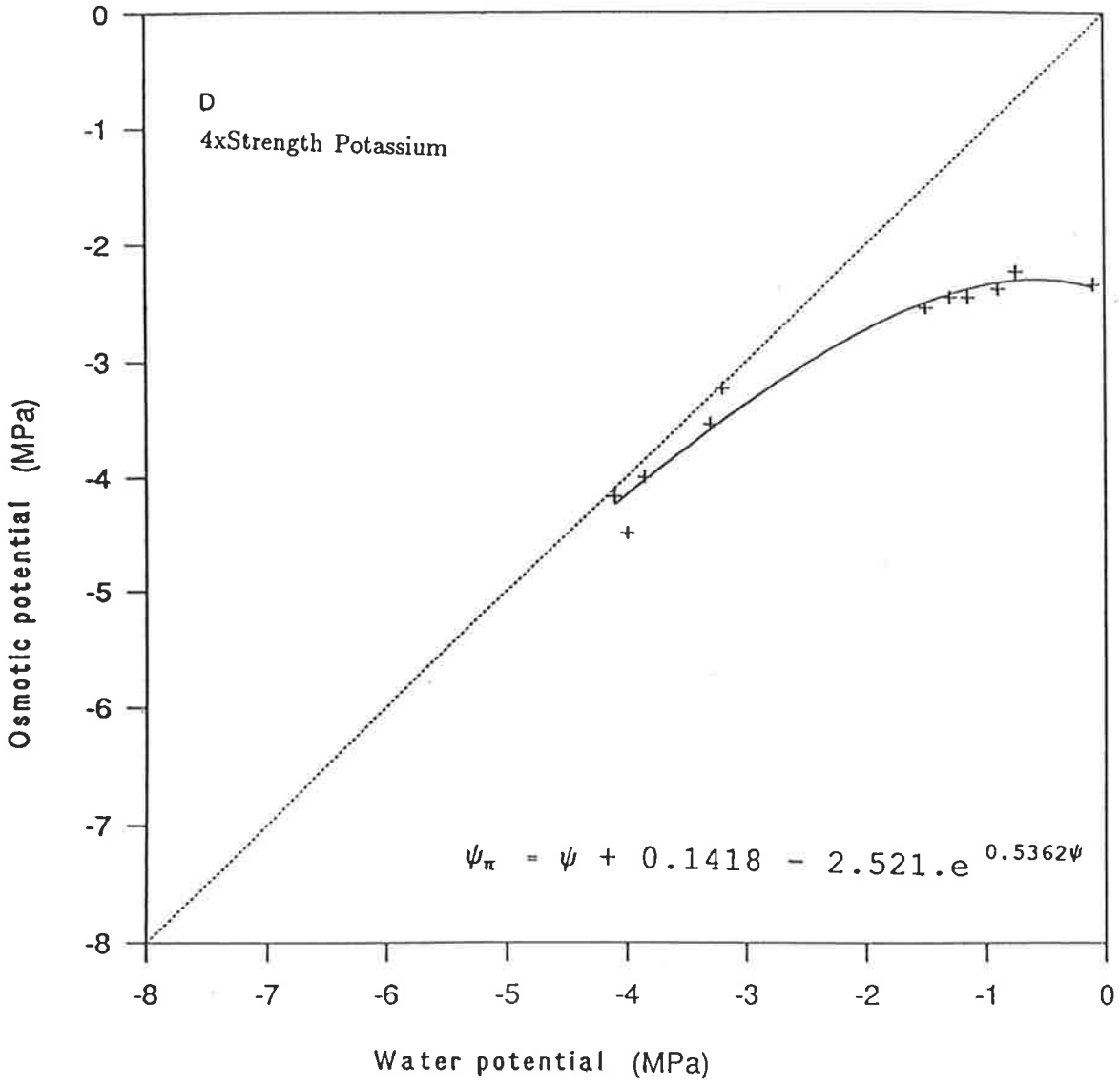

D
4xStrength Potassium
$\psi_\pi = \psi + 0.1418 - 2.521.e^{0.5362\psi}$
Osmotic potential (MPa)
Water potential (MPa)
0
-1
-2
-3
-4
-5
-6
-7
-8
-8
-7
-6
-5
-4
-3
-2
-1
0

Table 6.3

The coefficients of the exponential regression describing the relationship between water potential and osmotic potential of pot grown seedlings *of Acacia iteaphylla* grown under different nutrient treatments with special respect to potassium. The two variables were measured during a drying cycle. The equation of the regression has the form:

$$\Psi_\pi = \Psi - \beta_0 - \beta_1 e^{\gamma \Psi}$$

where Ψ is water potential, Ψ_π is osmotic potential and β_0, β_1 and γ are constants. Units are MPa. (*cf Table 3.9*).

Treatments	β_0	β_1	γ	SE(β_0)	SE(β_1)	SE(γ)	r^2
Complete Hoagland	-0.0656	2.467	0.6157	0.34802	0.33459	0.34142	0.9345
Minus potassium	-0.0253	2.080	0.6371	0.11125	0.17334	0.19810	0.9504
1/2 Strength potassium	-0.0801	2.457	0.7131	0.23987	0.30705	0.33798	0.9920
4 x Strength potassium	-0.14177	2.521	0.5362	0.23641	0.20309	0.13523	0.9692

The assumption underlying the fitting of the exponential regression model is the same as in Section 3.3, i.e. that as the water potential decreased, the turgor pressure decreased to some constant level (maintaining turgor above zero). To achieve this condition, the osmotic potential has to be lowered to a certain degree below water potential and follow its decline.

As has been emphasized previously, the value of β_o must be at least twice its standard error in order to qualify the linear part of the curve to be statistically different from the 1:1 line (Section 3.3). If this occurs, then osmotic adjustment is taking place. In fact, Table 6.3 clearly shows that all plants from the four nutrient treatments have not fulfilled the requirement, i.e. all the β_o values are *much smaller than* twice their standard error. Hence, no significant osmotic adjustment occurred in any of the four treatments.

6.2.2 *Effects of drying on K^+ and Na^+ accumulations*

Table 6.2 (Columns 1c to 4c) and Figure 6.1, show the fluctuations of K^+ following the gradual changes of water potential and osmotic potential. There is no clear relationship between potassium fluctuations and either water potential or osmotic potential, except in the 4x K^+ treatment. A regression analysis in Table 6.4.a shows that the K^+ concentrations are significantly different between treatments, that is the concentrations of K^+ in the tissues vary with the different concentrations in the nutrient solution. Also, there is evidence for the effect of decreasing osmotic potential on the K^+ concentrations. Thus K^+ changes as osmotic potential becomes more negative.

But further analysis (Table 6.4.b) suggests that the only treatment in which the increase in K^+ concentration with decreasing osmotic potential was significant is 4x K^+. Thus a linear regression line could be validly drawn through these data points.

Table 6.4.a

Regression analysis of ***potassium*** concentration in *A.iteaphylla* treated with four different potassium levels.

Source	df	Sum of Square	Mean Square	F
Treatments	3	2455290	818430	59.23
Osmotic potential within treatments	4	243357	60839	4.40
Residual	39	538883	13818	
Total	46	3237530		

Test for treatment effects:

$F = 59.23$ $F_{0.95}(3.39) = 2.85$

therefore ***retain*** hypothesis that potassium concentrations between treatments are significantly different.

Test for osmotic potential changes within treatments:

$F = 4.40$ $F_{0.95}(4.39) = 2.61$

therefore retain hypothesis that the level of potassium changes as osmotic potential changes.

Table 6.4.b

Test of regression coefficients for the effect of decreasing osmotic potential within treatments.

Treatment	β_o	SE	T
Complete Hoagland	-13.14	9.830	-1.337
Half strength K^+	-2.041	9.830	-0.2076
Minus K^+	-3.298	9.830	-0.3355
4x strength K^+	44.36	11.21	3.958

Therefore, the coefficient for 4 x extra strength potassium was the only one significantly different from zero.

Table 6.5

Regression analysis of ***sodium*** concentration in *A.iteaphylla* treated with four different potassium levels.

Source	df	Sum of Square	Mean Square	F
Treatments	3	42920	14307	5.57
Osmotic potential within treatments	4	1843	461	0.18
Residual	39	100118	2567	
Total	46	144881		

Test for treatment effects:

$F = 5.57$ $\qquad\qquad$ $F_{0.95}(3.39) = 2.85$

therefore retain hypothesis that sodium concentrations between treatments are significantly different.

Test for osmotic potential changes within treatments:

$F = 0.18$ $\qquad\qquad$ $F_{0.95}(4.39) = 2.61$

therefore, ***reject*** the hypothesis that the level of sodium changes as osmotic potential changes. Thus the concentration of Na^+ is different between treatments but does not change during the drying treatment.

However in spite of this relationship, the increase in K^+ concentration is not sufficient to produce significant osmotic adjustment even in this treatment, as shown in Figure 6.2.a-d and Table 6.3.

Table 6.5 presents evidence of significant differences of Na^+ concentration in the tissues between the four treatments. These differences seem likely to be due to the different concentrations of Na^+ in the stock solutions (see Table 6.1). However there are *no significant* changes of this ion as plant osmotic potential changes within treatments. Such a change might have been expected for the treatment without potassium, as Leigh and Johnston (1983) found a significant increase of Na^+ in dry matter of barley with poor K^+ supply.

6.3 *Discussion*

6.3.1 *Potassium concentrations - Osmotic adjustment*

It is clear that when these potted *A.iteaphylla* plants were fed with different potassium concentrations manipulated in Hoagland solution, the content of K^+ within the tissue was proportional to these different applications. The K^+ did not increase due to water stress treatments, except for the plants which received nutrient solution with 4 X K^+ strength. Nor did Na^+ increase during the drying cycle.

For *A.iteaphylla* seedlings in this experiment, it was expected that at least the plants which received extra potassium would have better osmotic adjustment. However, Figure 6.2 and Table 6.2 show that in fact extra potassium did not improve the level of osmotic adjustment in those seedlings. Indeed none of the treatment plant groups are statistically qualified to be considered as having osmotic adjustment. Figure 6.2 shows that in all treatments the points which describe the relationship between water potential and osmotic potential lie close to the 1:1 line when water potential is low (below -3 MPa). Therefore, no turgor maintenance occurred in low water potential.

On the other hand, potassium concentrations are significantly different between treatments, therefore better osmotic adjustment would be expected; unless something else changes to compensate for the increase in K^+. One possibility is that K was present as organic salts, and that as these increased the concentrations of sugars decreased due to the plant providing carbon for the organic salts (F.A. Smith, 1995, pers. comm.)

This poor adjustment in the pot grown seedlings of *A.iteaphylla* seems consistent with the previous pot experiment on osmotic adjustment in the glass house (see Chapter 3, Figure 3.3). In that case the β_o value for *A. iteaphylla* was also much smaller than twice the standard error.

Two questions emerge here. Does the plant use potassium as an osmotic agent? If it does, then could the plant adjust its osmotic potential at higher levels of potassium? In fact, when sufficient potassium is available, the adjustment still does not take place any more than in the other treatments. Therefore, the lack of potassium is probably not the reason for the absence of adjustment.

Changes of water potential not accompanied by increased potassium concentration in tissues, have also been reported by Cutler and Rains (1978) in pot grown seedlings of cotton. They found that even though water potential declined from -1.2 to -1.5, -2 and -2.5 MPa, potassium concentration did not change following that decline. They then suggested that potassium was apparently not an important solute in the osmotic adjustment of cotton seedlings.

Also, a report of Beringer et al (1992), found that different K-nutrition had no modifying effect on seed water relations of *Pisum sativum* ssp. *medullare* and ssp. *sativum*, i.e. osmotic potential and seed moisture.

In contrast however, Behboudian and Anderson (1990) reported on potassium deficient (-K) and sufficient (+K) tomato cv. Castlehye 1204. Well-watered -K and +K plants

had comparable rates of transpiration; +K plants had larger leaf area and lower water potential and RWC than -K ones. The -K plants had lower photosynthetic rates, were more sensitive to reduction in plant water potential, and showed more significant reduction in growth.

Jones et al (1980) also detected that potassium was the only inorganic ion which responded to water stress in pot grown (glass house) sorghum and sunflower seedlings. It was found that potassium in stressed pot grown seedlings of sorghum significantly increased 1.5 times over that in well-watered treatments, while sunflower was 1.2 times higher. In the sorghum leaves, the potassium salt of carboxylic acid contributed 15% of the osmotic potential, while potassium balanced by Cl^-, could be up to 24%. These values may be up to 30% more if the authors considered the symplastic water concentration.

Dhindsa et al (1975) detected a significant increase of K^+ which they considered an osmoregulatory solute in growing cotton fibre. Umar et al (*1992),* showed increased drought resistance capacity in groundnut i.e. increase in leaf proline content and stomatal resistance, decreased RWC and transpiration rates when they added potassium into the medium.

6.3.2 *Potassium concentration and its osmotic effect in conjunction with the symplastic water volume*

One result obtained from this experiment is that, although *A.iteaphylla* seedlings were enriched by extra potassium in the nutrient solution, there was no evidence for improving osmotic adjustment by those plants, compared to normal, half strength and no potassium in the nutrient compositions. On the other hand, there was evidence of accumulation of extra potassium in the 4 x K^+ treatment as stresses develop. Hence, the next Section examines how far the available potassium has a significant contribution to the osmotic values of *A.iteaphylla* tissues.

To find out the contribution of potassium to the osmotic potential of a particular tissue, its symplastic water volume must be known. Since in this experiment, unlike Chapter 5 (Section 5.1.1.4), pressure volume curves were drawn for each measurement of ψ_π, the corresponding symplastic water volume could be found by the method given in Section 2.4.1.2. The symplastic water volume can be combined with the potassium concentration to calculate the corresponding osmotic potential. With the assumption that all potassium ions occur in the cells, and that potassium is present as KCl, Table 6.6.a-d (column 8) presents the values of the contribution of potassium to the osmotic potential of all individual seedlings during the drying cycle. These values were obtained by calculating the concentration of potassium in the symplastic water volume ($C_K V_0$, column 5), from the equation:

$$C_K V_o = \frac{C_K \times 10^{-6} \times DW \times 10^{-3}}{V_o \times 10^{-6}} \quad \ldots\ldots \quad (13)$$

where $C_K V_o$ is the concentration of potassium in the symplastic water volume, C_K is the measured potassium concentration per unit dry weight, DW is the dry weight of sample phyllodes and V_o is the symplastic water volume. The derived value of potassium concentration is used to obtain the potassium osmotic potential for KCl in V_0 (column 6) from the curve (Figure 5.2) of potassium chloride osmotic potential values. By comparing those values with the measured osmotic potential of the sample (column 7), the percentage of potassium contribution to the plant's osmotic potential is obtained (column 8). The values of measured osmotic potential ($\Psi_{\pi m}$) are arranged in decreasing order in the Tables.

The contribution of K^+ to the total osmotic potential may also be estimated from measurements of fresh weight and dry weight of tissue, as was done in Chapter 5, Section

Table 6.6

The estimated contribution of potassium to the osmotic potentials of *A.iteaphylla* symplasm tissues with special respect to the symplastic water concentrations. The plants were grown in four different nutrient compositions. The contribution values were generated from interactions of several parameters as shown in the Columns.

Explanation of symbols in the Tables:

(1) Pot number

(2) V_0 , the values of symplastic water volume, μL sample^{-1}

(3) C_K, potassium concentration per unit dry weight of sample, $\mu mol\ g^{-1}\ DW$

(4) *DW*, dry weight of samples, *mg*

(5) $C_K V_0$, potassium concentration in symplastic water volume, *M*

(6) $\psi\pi_K V_0$, osmotic potential of potassium in symplastic water volume, *MPa*

(7) $\psi\pi_m$, the measured osmotic potential of plants, *MPa* and

(8) $\psi\pi_{Kcntb}$, the contribution of potassium to the osmotic potential of the symplastic water, % .

MN Means

For plants grown under Hoagland nutrient solution:

A. Complete
B. Without potassium
C. Half strength potassium
D. Extra strength (4x) potassium

NB: Pot numbers are not in order as the data have been arranged in order of decreasing osmotic potential.

A. Complete

No pot	V_0 (µL)	C_K (µmol g^{-1} DW)	DW (mg)	$C_K V_0$ (M)	$\psi\pi KV0$ (-MPa)	$\psi\pi_m$ (-MPa)	$\psi\pi_{K^+}$ cntb (%)
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
6	755.50	515.62	342.80	0.20	0.85	1.75	48
21	660.70	437.38	374.50	0.25	1.05	2.17	48
49	570.30	712.00	211.80	0.25	1.05	2.32	45
2	989.00	594.38	415.00	0.25	1.05	2.43	43
11	649.20	928.00	375.80	0.50	2.20	2.44	90
4	990.00	744.00	554.00	0.40	1.75	2.78	63
5	755.00	628.99	582.70	0.50	2.20	3.33	66
34	672.50	643.90	424.80	0.40	1.75	3.51	50
9	635.50	606.70	472.00	0.45	2.05	3.64	56
19	988.50	560.00	574.00	0.30	1.35	3.77	36
23	430.50	550.67	478.20	0.60	2.65	4.17	63
22	350.60	506.67	557.40	0.80	3.60	5.4	67
Mean	***703.94***	***618.99***	***446.92***	***0.40***	***1.80***	***3.21***	***56.25***

B. Without potassium

No pot	V_0 (µL)	C_K (µmol g^{-1} DW)	DW (mg)	$C_K V_0$ (M)	$\psi\pi_K v_0$ (-MPa)	$\psi\pi_m$ (-MPa)	$\psi\pi_{K^+}$ cntb (%)
(1)	**(2)**	**(3)**	**(4)**	**(5)**	**(6)**	**(7)**	**(8)**
1	665.00	360.90	560.00	0.30	1.35	1.85	73
44	535.20	367.19	249.50	0.15	0.60	2.10	28
37	1100.00	428.28	478.60	0.20	0.85	2.27	37
3	468.80	320.99	335.80	0.20	0.85	2.94	29
18	786.60	420.20	595.10	0.30	1.35	3.45	39
39	525.00	304.00	457.20	0.25	1.05	3.77	28
30	902.00	312.00	732.20	0.25	1.05	4.08	25
26	790.00	300.00	620.80	0.25	1.05	4.08	25
10	644.50	320.00	561.50	0.30	1.35	4.17	32
8	730.00	376.00	649.70	0.30	1.35	4.76	28
40	279.50	359.18	489.90	0.60	2.65	5.30	50
31	322.00	347.47	597.90	0.65	2.90	5.30	55
Mean	***640.72***	***351.35***	***527.35***	***0.31***	***1.37***	***3.84***	***37.42***

C. Half strength potassium

No pot	V_0 (µL)	C_K (µmol g^{-1} DW)	DW (mg)	$C_K V_0$ (M)	$\Psi\pi_K V_0$ (-MPa)	$\Psi\pi_m$ (-MPa)	$\Psi\pi_{K^+}$ cntb (%)
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
28	457.10	465.67	118.40	0.10	0.40	1.85	21
45	515.50	492.93	205.80	0.20	0.85	2.11	40
46	673.00	468.61	244.40	0.15	0.60	2.17	27
32	755.00	376.00	350.90	0.15	0.60	2.44	24
15	704.70	530.39	323.90	0.25	1.05	2.63	40
9	796.50	456.00	402.80	0.20	0.85	2.70	31
41	688.00	553.09	473.90	0.35	1.50	2.65	56
20	585.50	340.00	496.20	0.30	1.35	3.03	44
13	760.00	520.00	553.50	0.35	1.50	3.51	42
24	615.00	538.46	466.20	0.40	1.75	3.61	48
17	405.00	417.72	493.20	0.50	2.20	4.44	50
16	339.50	437.79	371.00	0.45	2.05	4.76	43
Mean	***607.90***	***466.39***	***375.02***	***0.28***	***1.22***	***2.99***	***38.83***

D. Four times extra strength potassium

No pot	V_0 (µL)	C_K (µmol g^{-1} DW)	DW (mg)	$C_K V_0$ (M)	$\psi\pi_K V_0$ (-MPa)	$\psi\pi_m$ (-MPa)	$\psi\pi_{K^+}$ cntb (%)
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
50	644.90	840.00	207.80	0.25	1.05	2.22	47
27	480.00	880.00	223.90	0.40	1.75	2.33	75
43	516.20	695.10	246.00	0.30	1.35	2.37	57
12	977.50	899.02	497.30	0.45	2.05	2.44	84
38	747.50	826.23	281.60	0.30	1.35	2.44	55
42	784.10	1253.30	263.00	0.40	1.75	2.53	69
33	521.00	616.39	363.70	0.40	1.75	3.23	54
14	931.00	1003.20	561.60	0.60	2.65	3.54	75
35	668.00	1114.75	430.90	0.70	3.10	4.00	77
25	911.00	1272.10	487.00	0.65	2.90	4.17	70
47	664.00	1250.80	466.00	0.85	3.70	4.49	82
Mean	***713.20***	***968.26***	***366.25***	***0.48***	***2.13***	***3.07***	***67.73***

5.1.1.4. In this experiment FW and DW were measured for each plant along with other measurements. Table 6.7 shows these calculated values based on means for each treatment, in the same form as Table 5.3. Column 9 in the Table shows, for comparison, the values of ψ_π for potassium as calculated for symplastic water volumes in Table 6.6. The two methods give very similar results, which is explained by the fact that the mean symplastic water volumes (V_0) are very similar to mean total water contents. Thus it is also shows that apoplastic water content in these phyllodes was very low.

Table 6.6 a-d, column 5 shows a gradual increase of potassium concentration in the symplastic water in all treatments following the decrease of osmotic potential due to the drying cycle. The question is whether or not this increase is due to net accumulation of potassium within the symplastic water, thus whether potassium is involved in the process of osmotic adjustment, or whether the increase is simply due to the symplastic volume of water decreasing.

In accordance with equation (1), if the cell behaves like a perfect osmometer i.e. no osmotic adjustment, the measured osmotic potential would be proportional to the reciprocal of the cell (symplastic water) volume, $1/V_0$, where the decrease of osmotic potential would be merely caused by the solute concentration, due to the loss of water from the cells. Therefore, if there was no net accumulation, the relationship between measured osmotic potential and the concentration of potassium in the symplastic water volume should be linear. Figure 6.3 confirms that potassium concentration is linearly related to the measured osmotic potential, which evidently shows that *no net* accumulation of potassium has occurred. This figure clearly supported the previous field experiment where potassium concentration did not increase within the stressed plants during the period of stress.

If the potassium did accumulate then it would be expected that the shape of the curves would become non-linear, concave upwards.

Table 6.7

Contribution of potassium to the osmotic potentials of the pot grown plants of *Acacia iteaphylla* treated with difference K^+ concentrations calculated using fresh weight and dry weights of tissue. Here it is assumed that the total water content, and all potassium ions, are symplastic.
C_K^+ : potassium concentrations; FW, DW : fresh weight, dry weight; WC: water content;
K^+cntb: potassium contributions to ψ_π The Table has the same form as Tables 5.3 and 5.7.

Treatment	C_{K+} (µmol g^{-1} DW)	FW/DW ratio	C_{K+} at FW (µmol g^{-1} FW)	Total WC (%)	C_K^+ (mM)	Total ψ_π Kcntb (-MPa) (%)	Actual measured ψ_π (-MPa)	Symplastic ψ_πKcntb (- MPa) (%)
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
Complete Hoagland	618.99	2.74	225.91	63.52	355.65	1.6 (50)	3.21	1.8 (56)
Minus K^+	351.35	2.32	151.44	56.93	266.01	1.2 (31)	3.84	1.37 (37)
½ K^+ strength	466.39	2.58	180.77	61.19	295.42	1.3 (43)	2.99	1.22 (39)
4 x K^+strength	968.26	2.97	326.01	66.38	491.13	2.2 (72)	3.07	2.13 (68)

***Note*: Values in Column:**

(2) Mean potassium concentrations on a DW basis, from column (3) Table 6.6.a-d. (µmol g^{-1} DW).
(3) Mean fresh weight : dry weight ratios from measurements made on all plants in each treatment.
(4) Mean potassium concentrations on a FW basis (column 2/column 3). (µmol g^{-1} FW)
(5) Mean total water content, % FW.
(6) Potassium concentrations (column 4/column 5), assuming all water, and all ions, are symplastic. i.e using total instead of symplastic water content. (mM).
(7) Osmotic potentials of these solutions assuming all K is present as KCl, and using Figure 5.2. (-MPa).*
(8) Mean measured osmotic potentials, from Table 6.6.a-d column (7). (-MPa).
(9) Mean osmotic potentials due to KCl, as calculated in Table 6.6.a-d column (6) i.e using symplastic water measurements. (-MPa).*

*Values in parentheses are % of the total due to KCl.

Figure 6.3

Concentration of potassium in symplastic water (M) assumed to be KCl, as a function of osmotic potential of shoot (MPa) measured by pressure-volume curve, for *A.iteaphylla* seedlings grown with four different concentrations of potassium.
Data from Table 6.6.

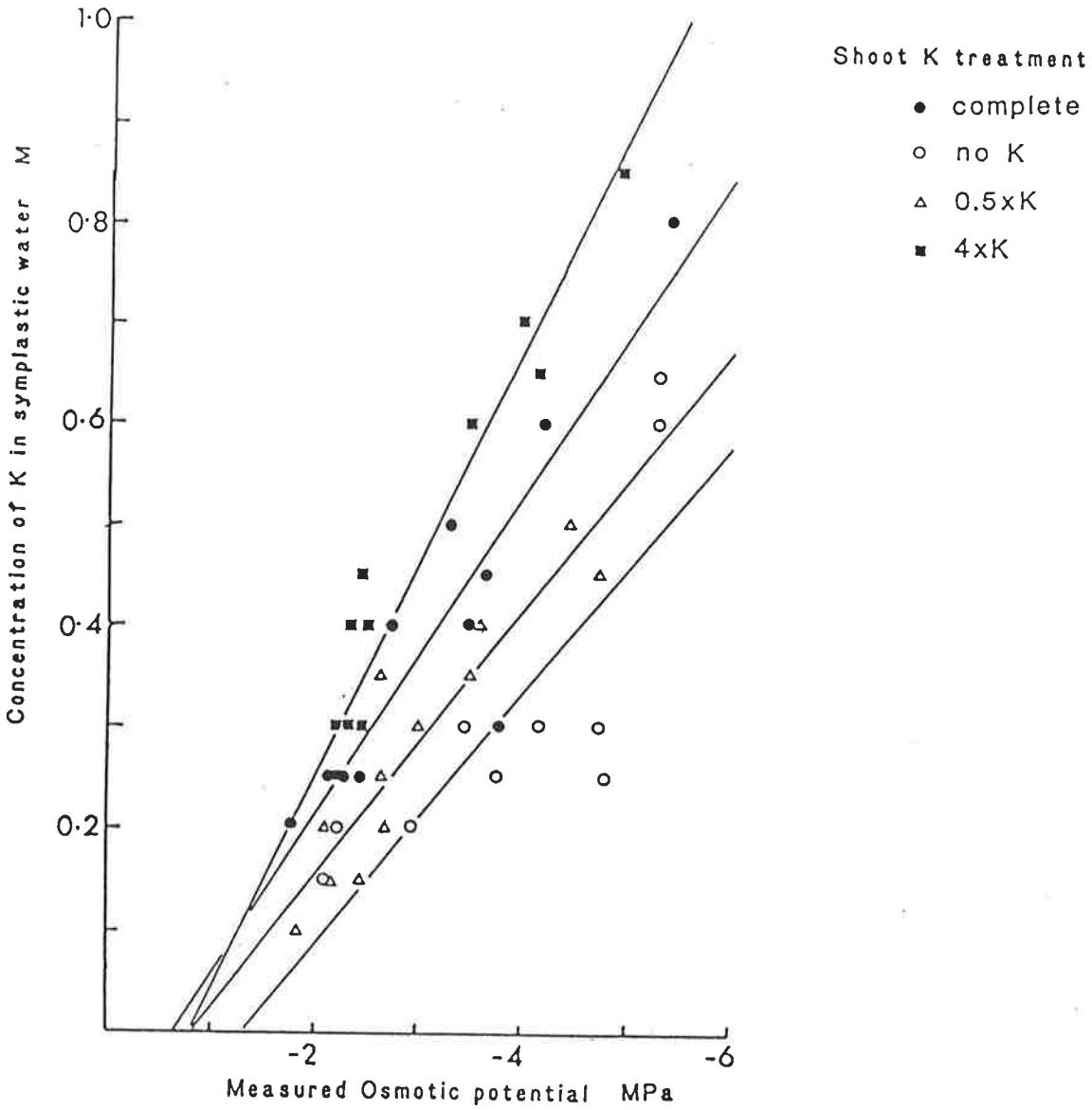

1·0
0·8
0·6
0·4
0·2
Concentration of K in symplastic water M
-2
-4
-6
Measured Osmotic potential MPa
Shoot K treatment
complete
no K
0.5xK
4xK

Table 6.8

The linear regression components describing the relationship between potassium concentrations (M) assumed to be KCl in the symplastic water (M) and the measured osmotic potentials (-MPa) for the 4 potassium treatments.

Treatments	Constant Terms (a)	Regression Coefficient (b)	Correlation Coefficient (r^2)
Complete Hoagland	-0.0427	-0.1436	0.7085
Minus K^+	-0.0356	-0.0948	0.5317
½ strength K^+	-0.1024	-0.1289	0.8484
4 x strength K^+	-0.1662	-0.2112	0.8742

Table 6.8 presents the linear regression equation of the relationship between potassium concentration in the symplastic water and the measured osmotic potential for all the four treatments. It reveals that the slopes (b) of the curves are different from each other due to the potassium treatment. The extra K^+ application had the greatest slope, followed by complete, half strength and no potassium treatment. The r^2 values provide evidence of good to fair relationship between the two variables with the no potassium treatment being quite low. Again, extra K^+ is the biggest r^2 while minus K^+ is the smallest.

Column 5 and 8 in Table 6.6.a-d show that potassium generated a significant contribution to the osmotic potential of the symplastic water. Although as shown in Column 8, a few values seem extremely high (one up to 90%, Complete Hoagland treatment), the values of osmotic contribution of potassium to the symplastic water seem acceptable. The average values (Column 8, bottom), are consistent with the gradual increase in the potassium concentration in the supplied nutrients. As shown, the average value of this contribution (%) was lowest in the treatment without potassium (37), followed by half strength (39), normal Hoagland (56) and extra strength potassium (68) respectively.

One reason which may contribute to these high percentages is that the osmotic effect of K^+ was calculated assuming it was all present as KCl. In fact, an unknown proportion would have been balanced by divalent or trivalent organic anions. This would reduce the osmotic effect of K^+, hence the osmotic values calculated are over-estimated, by an unknown amount.

Although there was no evidence of K^+ accumulation in response to the fall of water potential, K^+ levels in the plants did increase as the concentration in the supplied nutrient increased. Its presence would automatically affect the osmotic potentials of the tissues. As the ion may be quite toxic at high concentrations, tissue damage may have begun to appear had the experiment continued longer.

CHAPTER 7

PROLINE

7.1 *The influence of nutrient application, soil type and environmental factors on proline synthesis in pot-grown seedlings of Acacia iteaphylla.*

The result of the study of proline in the field grown plants (see section 5.2.1), showed that all species displayed a large and significant difference in proline content between well-watered and stressed plants, except *A.iteaphylla*, where differences between the two treatments were small (Figure 5.5).

Several environmental factors influence solute accumulation or synthesis within plants. Among these are nutrient availability and light intensity (Mott and Steward, 1972; Wyn-Jones and Gorham, 1983; L.G.Paleg and B.P.Naidu, pers. comm.).

A possible explanation for the small change in proline concentration between well-watered and stressed *A.iteaphylla* might be nutrient availability in the Black Hill soil. In order to test this idea, an experiment was conducted on *A. iteaphylla* to show whether soil type or nutrient level affected the synthesis of proline. In addition, two environmental treatments were given, namely inside and outside a glasshouse. It was thought that light and temperature are probably the main environmental factors directly involved in promoting proline synthesis.

7.1.1 *Materials and Methods*

Two soils were prepared. The first was BHNFC soil, dug from the field experimental site and collected from up to 50 cm depth. The second was a sandy loam

potting mixture, obtained from BHNFC Nursery. Twenty-four pots (with drainage holes) were filled with Black Hill soil, and another 24 with potting mixture.

Two months old seedlings of *A.iteaphylla,* grown in vermiculite by The South Australian Department of Woods and Forests Nursery, Murray Bridge, S.A., were transplanted to the pots, and watered as required.

Twelve pots of each soil type were put in the glass house, while the other 12 were placed outside in direct sunlight. (See Plate 4). Each 12-pot group, (both Black Hill soil and mixed soil, inside and outside glasshouse) received the following treatments:

- *six pots were fed with full strength Hoagland solution,*
- *the other six received no Hoagland solution at all.*

The nutrient treatment plants received 800 ml of Hoagland solution in 4 applications prior to harvesting. After 3 months, water was withheld from 3 pots i.e. three replications of each treatment (with and without nutrient, inside and outside glass-house). The other three pots were kept watered as controls.

Between 5 and 12 days were needed to allow the unwatered plants to develop significant stress. This difference was due to differences in size of the plants and hence the different capacity of leaves to hold water, as a consequence of different treatments, both soil and nutrient.

Water potentials were recorded with the pressure chamber from all plants. Samples of leaves were collected for proline analysis and estimated by the method described in section 5.2.1.1.

7.1.2 *Results*

Table 7.1.a and 7.1.b present the original values of proline concentrations in all treatments. Table 7.1.c summarizes the mean values of the results of the experiment. It is

Plate 4a Seedlings of *A. iteaphylla* grown in Black Hill soil with and without Hoagland's solution and positioned outside the glasshouse.

Plate 4b Seedlings of *A. iteaphylla* grown in potting mix soil with and without Hoagland's solution and positioned outside the glasshouse.

Table 7.1.a

The original values of proline concentrations (µmol g^{-1} DW) and corresponding water potential (-MPa) of potted seedlings of *Acacia iteaphylla* in nutrient experiment GROWN IN BLACKHILL SOIL, with and without nutrient (Hoagland) addition.

Column 1: Ntr-Trt, nutrient treatment; +/- Nut., plus or without nutrient; Column 2: In/Out, inside or outside glass house; Column 3: R, replications; Mn, mean values; Column 4a and 4b: water potential values (-MPa) of WW, well-watered, and STR, stressed treatments. Column 5a and 5b: proline concentrations (µmol g^{-1} DW) of WW and STR treatments.

Ntr-Trt (1)	Position (2)	R (3)	Water potential (4)		Proline concent (5)	
			a	b	a	b
			WW	*STR*	*WW*	*STR*
+Nut.	In	1	0.75	3.45	5.96	37.28
		2	0.65	3.76	3.14	46.54
		3	0.55	4.50	2.39	71.27
		Mn	***0.65***	***3.90***	***3.83***	***51.70***
	Out	1	0.65	3.7	11.37	93.76
		2	0.50	3.5	12.81	84.33
		3	0.70	5.8	16.44	109.03
		Mn	***0.62***	***4.33***	***13.54***	***95.71***
-Nut.	In	1	0.90	3.70	1.28	33.98
		2	1.25	4.50	2.02	36.35
		3	0.80	3.50	1.15	32.52
		Mn	***0.98***	***3.9***	***1.48***	***34.28***
	Out	1	0.85	3.50	4.36	53.75
		2	0.55	3.50	3.52	49.81
		3	0.75	3.20	4.01	48.29
		Mn	***0.72***	***3.40***	***3.96***	***50.62***

Table 7.1.b

The original values of proline concentrations (µmol g^{-1} DW) and corresponding water potential (-MPa) of potted seedlings of *Acacia iteaphylla* in nutrient experiment GROWN IN POTTING MIXTURE, with and without nutrient (Hoagland) addition.

Column 1: Ntr-Trt, nutrient treatment; +/- Nut., plus or without nutrient; Column 2: In/Out, inside or outside glass house; Column 3: R, replications; Mn, mean values. Column 4a and 4b: water potential values (-MPa) of WW, well-watered, and STR, stressed treatments. Column 5a and 5b: proline concentrations (µmol g^{-1} DW) of WW and STR treatments.

Ntr-Trt (1)	Position (2)	R (3)	Water potential (4)		Proline concent (5)	
			a	b	a	b
			WW	*STR*	*WW*	*STR*
+Nut.	In	1	0.60	3.80	5.22	50.16
		2	0.50	3.80	6.45	49.12
		3	0.65	4.00	6.02	57.24
		Mn	***0.58***	***3.87***	***5.90***	***52.17***
	Out	1	0.75	4.50	15.30	82.16
		2	0.50	4.25	11.05	78.21
		3	0.80	4.00	17.11	73.20
		Mn	***0.68***	***4.25***	***14.49***	***77.86***
-Nut.	In	1	1.00	3.00	2.53	2.47
		2	1.50	3.60	2.27	5.83
		3	1.45	3.00	2.66	3.26
		Mn	***1.32***	***3.20***	***2.49***	***3.85***
	Out	1	0.40	4.50	1.62	40.28
		2	0.35	3.50	1.04	16.45
		3	0.90	3.60	5.38	16.92
		Mn	***0.55***	***3.87***	***2.68***	***24.55***

Table 7.1.c

Summary from Table 7.1.a and 7.1.b.
The mean values (n=3) of water potential (-MPa), proline concentration (μmol g^{-1} DW), and the increase of proline concentration per unit decrease of water potential ($\Delta\Psi$) in potted *A.iteaphylla* seedlings grown in Black Hill soil and potting mixture with and without additional nutrient.

Note: WW= well-watered, STR= stressed treatments.

Location		INSIDE				OUTSIDE			
Soil type:	(1)	Black Hill		Mixed		Black Hill		Mixed	
Treatment: (+/- nutrient)	(2)	+	-	+	-	+	-	+	-
Ψ WW	(3a)	0.65	0.98	0.58	1.32	0.62	0.72	0.68	0.55
Ψ STR	(3b)	3.90	3.90	3.87	3.20	4.30	3.40	4.25	3.87
$\Delta\Psi$ (STR-WW)	(4)	3.25	2.92	2.32	1.88	3.68	2.68	3.57	3.32
Proline WW	(5a)	3.83	1.48	5.90	2.49	13.54	3.96	14.49	2.67
Proline STR	(5b)	51.70	34.28	52.17	3.85	95.71	50.62	77.86	24.55
Δ proline (STR-WW)	(6)	47.87	32.80	46.27	1.36	82.17	46.66	63.37	21.88
Proline increase per unit decrease of Ψ	(7)	14.73	11.23	19.94	0.72	22.33	17.41	17.75	6.59

clear that after the stress treatment, all plants showed a decrease in water potential. In Table 7.1.c, rows 3a and 3b present the values of water potential for well-watered and stressed plants, Row 4 the difference between them. Rows 5a and 5b present the concentration of proline in well-watered and stressed plants. The differences between the two values is given in row 6. Row 7 shows proline increase per unit decrease in water potential, which is generated by dividing the delta proline concentration by delta water potential. Figure 7.1 graphically illustrates the differences in proline concentrations.

A simple analysis of covariance presented in Table 7.2 (part a), reveals that regression lines which describe the relationship between water potential and proline concentration are highly significant ($P < 0.001$), and that soil, nutrient and environment (inside or outside the glass-house) all have significant effects on the proline concentration. The level of significance for environment and nutrient is at ($P < 0.001$) and soil is at ($0.001 < P < 0.01$). There are no overall significant interactions among these factors: environment and soil, environment and nutrient, soil and nutrient nor even the combination of all three.

Since the three factors had some effect on proline concentration, further analysis was necessary, as revealed in Table 7.2 (part b). This provides evidence of no significant differences in the y-intercepts between all regression lines of all treatments. However, the environment, soil and nutrient factors have significant effects on the slope of the regression curves ($P < 0.001$). The interaction of soil and nutrient provides a highly significant effect ($P < 0.001$). Other combinations of factors had no significant effect on the slope.

There are 8 regression lines which can be derived from the result of the experiment (Table 7.3). They describe the relationship between water potential and proline concentration in all treatments as

$$\mathbf{P = a\,\Psi + b} \quad \text{.....} \quad (14)$$

Figure 7.1

The mean values of phyllode proline concentrations (μmol g^{-1} DW) in the nutrient experiment with Black Hill soil and potting mixure.

+Nut-In	= plus nutrient, inside the glasshouse
+Nut-Out	= plus nutrient, outside the glasshouse
-Nut-In	= minus nutrient, inside the glasshouse
-Nut-Out	= minus nutrient, outside the glasshouse

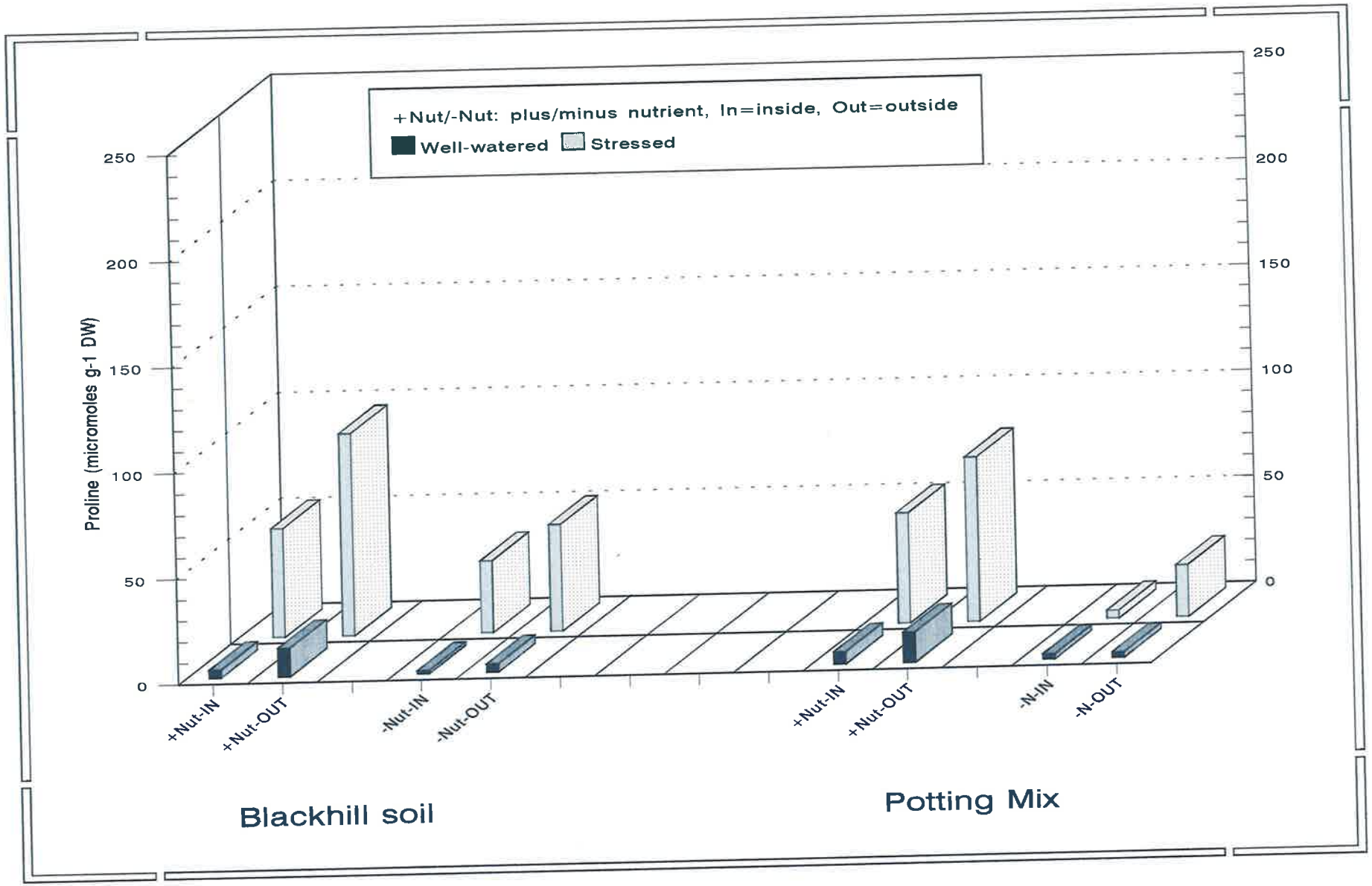

+Nut/-Nut: plus/minus nutrient, In=inside, Out=outside
Well-watered
Stressed
Proline (micromoles g-1 DW)
250
200
150
100
50
0
+Nut-IN
+Nut-OUT
-Nut-IN
-Nut-OUT
+Nut-IN
+Nut-OUT
-N-IN
-N-OUT
Blackhill soil
Potting Mix

Table 7.2

The ANCOVA of the relationship between water potential and proline concentration of *A.iteaphylla* seedlings as affected by different treatments of water, nutrient and environment.

A. Evidence of highly significant differences in the regression lines which describe the relationship between water potential and proline concentration, and the effects of environment, nutrient and soil.

B. Evidence of no significant differences of y-intercepts between the treatments, and highly significant effects of treatments on the slope of the curves.

*** $P \leq 0.001$
** $0.001 < P \leq 0.01$

A

Analysis of variance - Design 1

Test of significance for Proline

Source of variation	SS	DF	MS	F	P	
Within cells	4240.01	39	108.72			
Regression	25619.89	1	25619.89	235.65	.000	***
Environment	3048.26	1	3048.26	28.04	.000	***
Soil	878.36	1	878.36	8.08	.007	**
Nutrient	5901.04	1	5901.04	54.28	.000	***
Envir by Soil	149.30	1	149.30	1.37	.248	n.s
Envir by Nutr	99.68	1	99.68	.92	.344	n.s
Soil by Nutr	349.68	1	349.68	3.22	.081	n.s
Envir by Soil by Nutr	32.47	1	32.47	.30	.588	n.s

B

Analysis of variance - Design 2

Test of significant for Proline

Source of variation	SS	DF	MS	F	P	
Within+Residual	1094.55	36	30.40			
Envir	74.85	1	74.85	2.46	.125	n.s
Soil	44.93	1	44.93	1.48	.232	n.s
Nutr	86.91	1	86.91	2.86	.100	n.s
Water Potential (WP)	19064.81	1	19064.81	627.05	.000	***
Envir by WP	644.44	1	644.44	21.20	.000	***
Soil by WP	834.27	1	834.27	27.44	.000	***
Nutr by WP	1531.50	1	1531.50	50.37	.000	***
Envir by Soil by WP	123.62	1	123.62	4.07	.051	n.s
Envir by Nutr by WP	23.85	1	23.85	.78	.382	n.s
Soil by Nutr by WP	796.71	1	796.71	26.20	.000	***
Envir by Soil by Nutr by WP	12.03	1	12.03	.40	.533	n.s

Table 7.3

The linear regression equations for the relationship between water potential and proline concentrations of *A.iteaphylla* sedlings subjected to different treatments of water, nutrient and light intensity.

BH, Black Hill soil; MX, potting mix; NTR, nutrient; +, added nutrient; -, without nutrient; Ψ, water potential; P, proline.

	No.	Treatment	Equation	Coeff. regr. (r^2)
INSIDE				
	1.	BH +NTR	P = -15.347Ψ - 7.178	0.9557
	2.	BH -NTR	P = -10.849Ψ - 8.607	0.9749
	3.	MX +NTR	P = -14.121Ψ - 2.383	0.9938
	4.	MX -NTR	P = - 0.878Ψ - 1.187	0.4866
OUTSIDE				
	1.	BH +NTR	P= -20.367Ψ + 4.214	0.9521
	2.	BH -NTR	P= -17.292Ψ - 8.303	0.9940
	3.	MX +NTR	P= -17.772Ψ + 2.334	0.9999
	4.	MX -NTR	P= - 7.240Ψ - 2.374	0.7803

where P refers to proline concentration, and Ψ is water potential. To produce these curves, all data from both well-watered and stressed treatments have been plotted as in Figure 7.2.a-d. Based on the r^2 values (Table 7.3) it seems that linear regression provides a reasonable to very good empirical description of the relationship between the two variables, with one exception - potting mixture without nutrient treatment inside the glass-house. In this case proline concentration was generally low even under severe stress.

From these 8 curves, various plot combinations were made to compare the trends of proline synthesis as affected by water potential in each treatment (soil type, nutrient application and environment inside or outside the glasshouse).

Figure 7.2.a shows the relationships for plants grown *inside* the glasshouse, for each soil type and each nutrient treatment.

Figure 7.2.b presents the same relationships as above for plants grown *outside* the glasshouse. Figure 7.2.c summarizes the data again for plants grown in Black Hill soil, with or without nutrients, showing the effect of environment i.e. *inside* or *outside* the glasshouse. Figure 7.2.d summarizes the data for plants grown in potting mix, in the same combinations as in Figure 7.2.c.

7.1.3 *Discussion*

a. *The original question*

The original question behind this experiment was whether the nutrient status in Black Hill soil was so low that it failed to promote higher proline synthesis by *A.iteaphylla* undergoing water stress. Therefore, attention must be directed to the values of water potential and proline concentration of plants grown in Black Hill soil without extra

Figure 7.2.a

Regression lines describing the relationship between water potential (MPa) and proline concentration (μmol g^{-1} DW) generated from *A.iteaphylla* seedlings grown ***inside*** glasshouse in soil and with/ without added nutrient.

Soil type	Nutrients	Symbol
Black Hill	+	*
	-	⊛
Potting mix	+	●
	-	◉

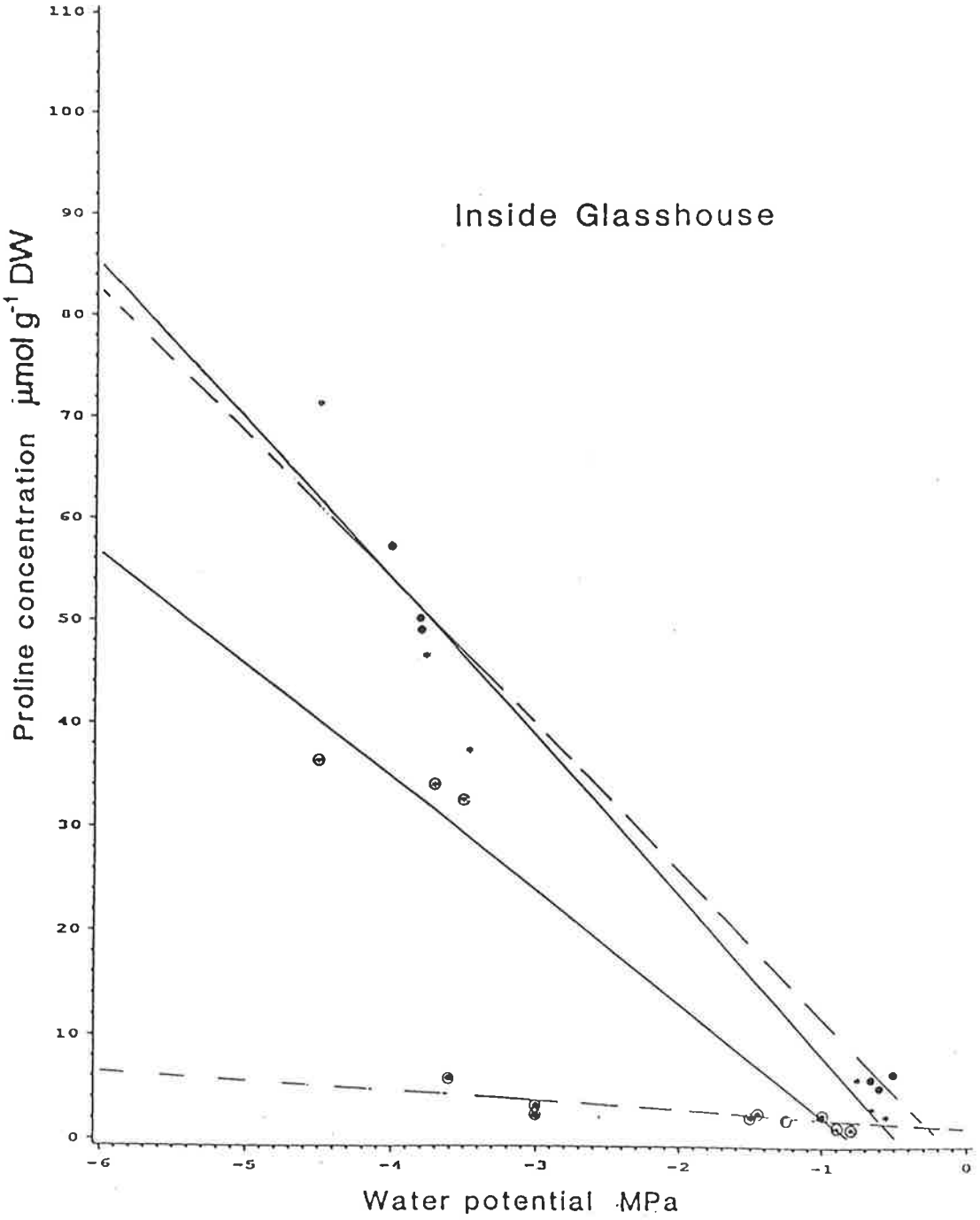

Inside Glasshouse
Proline concentration µmol g-1 DW
Water potential MPa
110
100
90
80
70
60
50
40
30
20
10
0
-6
-5
-4
-3
-2
-1
0

Figure 7.2.b

Relationship between water potential (MPa) and proline concentration (μmol g^{-1} DW) generated from *A.iteaphylla* seedlings grown ***outside*** the glasshouse.
Symbols as for Figure 7.2.a.

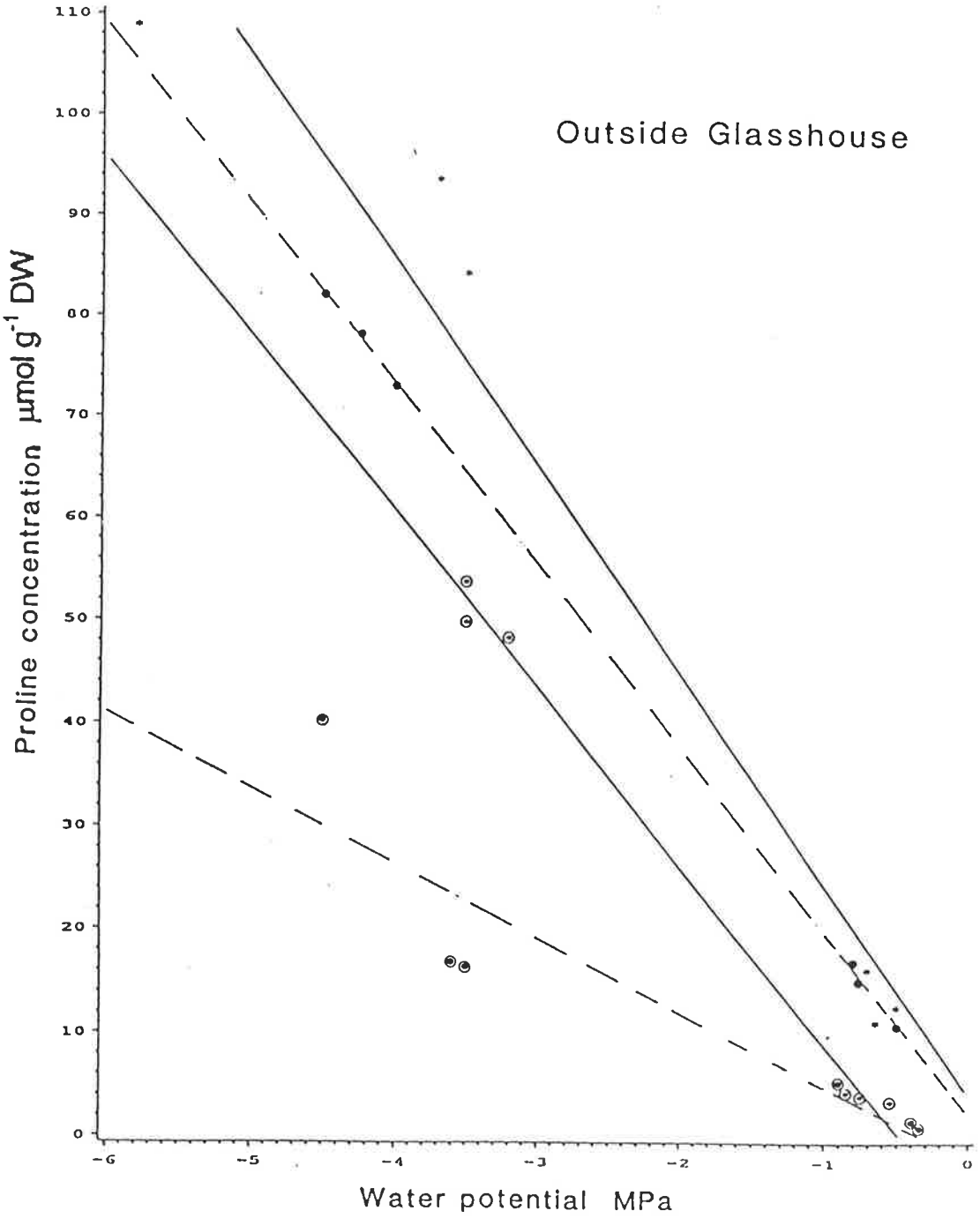

Outside Glasshouse
Proline concentration µmol g-1 DW
Water potential MPa
110
100
90
80
70
60
50
40
30
20
10
0
-6
-5
-4
-3
-2
-1
0

Figure 7.2.c

Relationship between water potential (MPa) and proline concentrations (umol g-1 DW), as in Figure 7.2.a and 7.2.b, but showing the influence of nutrient application and position of pots upon proline synthesis for seedlings grown in ***Black Hill soil***.

Position		Nutrients	Symbol
Outside	A	+	*
	B	-	⊛
Inside	C	+	●
	D	-	◒

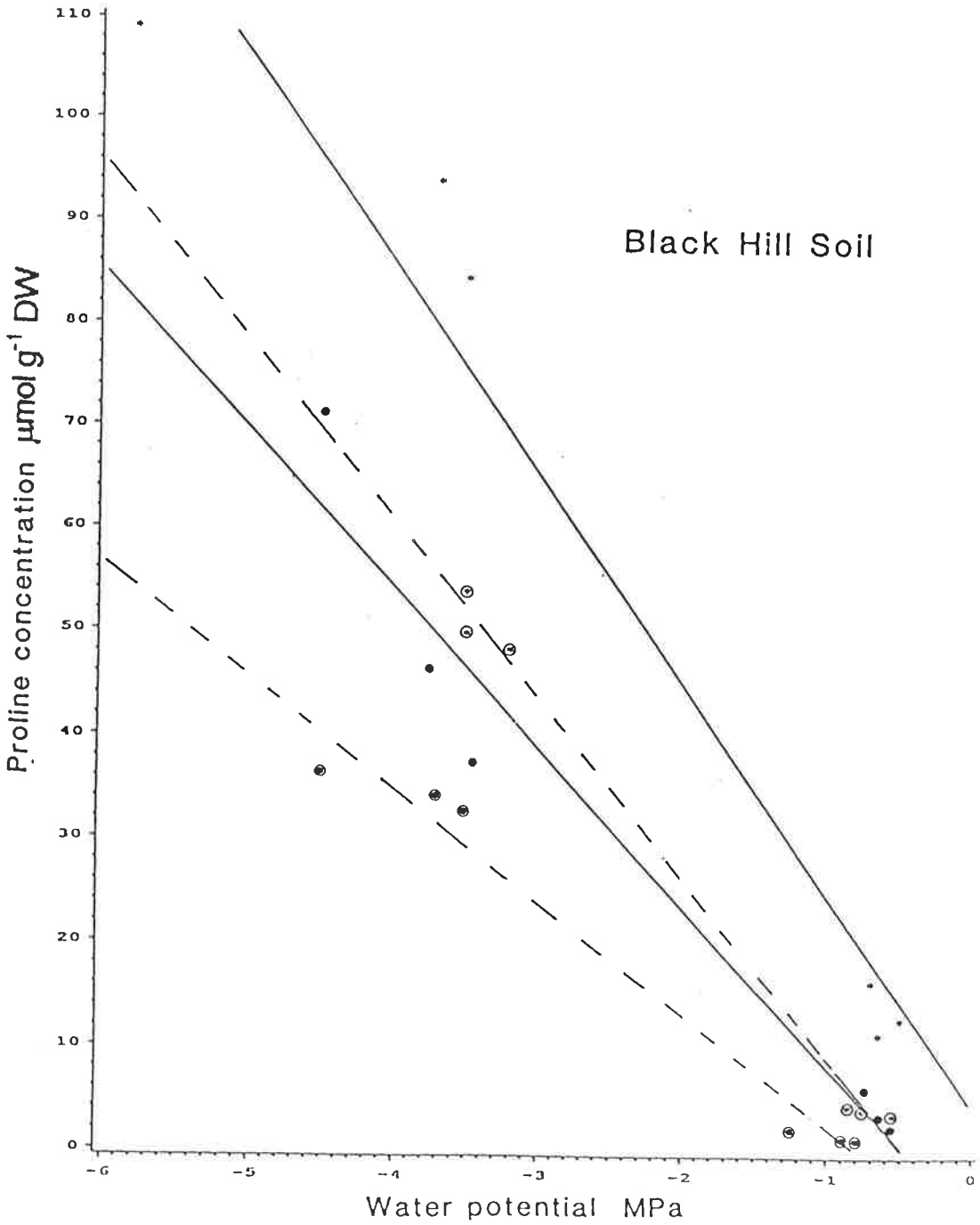
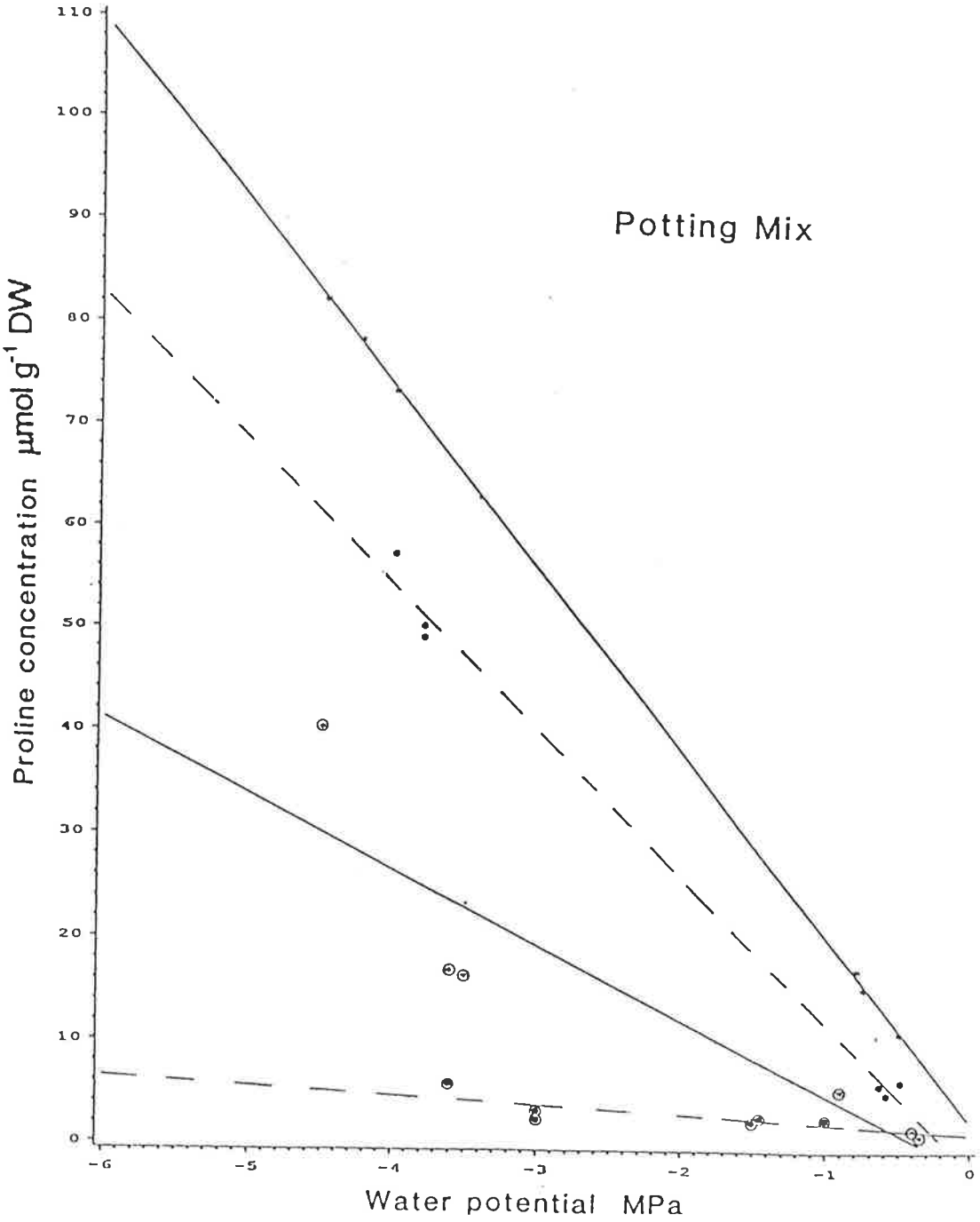

Black Hill Soil
Proline concentration µmol g-1 DW
Water potential MPa
110
100
90
80
70
60
50
40
30
20
10
0
-6
-5
-4
-3
-2
-1
0

Figure 7.2.d

Relationship between water potential (MPa) and proline concentrations (μmol g^{-1} DW), as in Figure 7.2.c, but for seedlings grown in ***potting mix***.
Symbols as for Figure 7.2.c.


Potting Mix
Proline concentration µmol g-1 DW
Water potential MPa
110
100
90
80
70
60
50
40
30
20
10
0
-6
-5
-4
-3
-2
-1
0

nutrient (Table 7.1). A simple *t*-test analysis provides evidence of significant differences of water potential and proline concentration between well-watered and stressed treatments.

Nutrient application increased the capability of plants to produce proline, however the answer to the original question was negative, as the plants without extra nutrient did produce proline under stress. These results suggest that nutrient level in Black Hill soil was not the reason for the similarity of the proline concentrations of well-watered and stressed field grown *A.iteaphylla*. Therefore, other factor(s) may be responsible for this phenomenon.

The oxidation process involved in the conversion of proline to glutamic acid is responsible for the low concentration of proline in turgid leaves. The high concentration in stressed plants could be due to the inhibition of proline oxidation. The presence of some carbohydrates or carbohydrate intermediates would inhibit the oxidation of proline (Stewart, 1972; Hanson and Hitz, 1982). Thus, the similarity of proline levels in well-watered and stressed field grown *A.iteaphylla* plants indicated that other factors may have been involved in the failure of proline oxidation in the turgid leaves. This of course needs further investigation. It is not at all clear why potted plants increased proline levels under water stress while field grown plants did not.

b. *Differences in proline concentration as affected by nutrient, soil and environment treatments*

All nutrient-treated plants whether grown in Black Hill or mixed soil, or positioned inside or outside the glass-house, revealed higher proline concentrations than those which did not receive nutrients (Table 7.1.c). The differences of proline accumulation in these plants between well-watered and stressed treatments varied with soil type. For example, the glasshouse plants in the Black Hill soil with added nutrient accumulated 1.5 times more proline than those without nutrient application. In comparison plants in potting mix with nutrient accumulated 34 times more proline than those without added nutrients.

The regression lines reveal the trend of the relationship between water potential and proline concentration within all individual plants. By looking at Figure 7.2.a (plants inside the glass-house) and Table 7.3, it is clear that the curves for treatments with extra nutrient supplies have steeper (negative) slopes. For Black Hill soil plus nutrient, the slope is -15.35 compared to -10.85 for plants grown without nutrient application. Thus the plants with extra nutrients could synthesize more proline.

The plants in Black Hill soil with added nutrient, have a slightly higher value of the slope (-15.35) than those in potting mix plus nutrients (-14.12). However, in the no nutrient treatment, the slope of Black Hill soil plants is -10.85, much higher than -0.88 for mixed soil. In fact, the first group accumulated 24 times more proline than the latter ones.

Proline concentration in plants in Black Hill soil without added nutrient is much higher than mixed soil in the outside treatment also (Figure 7.2.b). These results are as expected since a clay soil (Black Hill) would hold more water and nutrient than a sandy porous soil (potting mix). However, at this stage it is difficult to explain the actual reasons without additional experiments since other factors may be responsible as well.

Plants outside the glasshouse (Figure 7.2.b) showed a similar trend to glasshouse plants in that the addition of nutrient increased the accumulation of proline. Black Hill soil plants accumulated almost twice as much proline as no nutrient plants, while mixed soil plants were three times higher than the same series without nutrient (Table 7.1.c). Black Hill soil plus nutrient accumulated one and a half times the proline of mixed soil plus nutrient, while non-nutrient Black Hill soil accumulated 2 times the proline of mixed soil without nutrient. The slope for the plants in Black Hill soil with added nutrient is -20.37, steeper than the minus nutrient treatment (-17.29). Mixed soil plus nutrient plants slope is -17.77, steeper than minus nutrient plants (-7.24).

c. *Nutrient requirements for proline synthesis*

It is clear from the figures that plants have responded to the applied nutrient. Some nutrient elements are essential in the process of proline synthesis.

Mineral nutrition is involved in various ways. The lack of inorganic phosphate can affect the activation of g-glutamyl phosphate reductase, i.e. one of the five enzymes involved in the pathway of proline biosynthesis. Also, in the metabolic pathway of proline, 5-oxoprolinase needs Mg^{2+} or Mn^{2+} and K^{+} or NH_4^{+} to catalyze 5-oxo-L-proline to glutamic acid (Dashek and Erickson, 1981).

As one of the amino acids, proline synthesis requires a sufficient supply of nitrogen (N). Schobert (1977a) reported the source of N for proline synthesis to be NO_3^{-} and NH_4^{+} in diatoms. Elmore and McMichael (1981) found from pot grown seedlings of 3 cultivars of cotton, that under severe N-deficiency, the ability of plants to accumulate proline was reduced. In this case, cotyledons were 12 to 17 times lower than leaves in non N treated seedlings. An exogenous N source is important in proline synthesis. Schobert (1977b) did not find proline accumulation in diatoms unless they were supplied by exogenous N .

The source of N may come indirectly from photosynthates. Fukutoku and Yamada (1981) found that the decrease of water potential in soybean (*Glycine max*) is associated with a gradual decrease in the protein concentration. Total proline accumulation was stopped at severe water stress. The association of the occurrence of pipecolic acid and a-amino adipic led the authors to the conclusion that the N source for proline synthesis may be protein, since the occurrence of these two compounds normally is an indication of abnormality in proline synthesis.

However, this finding did not parallel the result by Tyankova (1980) in tobacco leaf disks which showed protein nitrogen content was not changed until after two days of drying. Unfortunately, Tyankova did not record the water potential of the samples as an indicator of

water stress. Later Fukutoku and Yamada (1984) confirmed their finding by the use of labelled ^{15}N. From 54% of the lost leaf protein ^{15}N, as much as 41% was found in proline and asparagine, showing that the N for proline synthesis during water stress originated from leaf protein. This finding may help to explain the Stewart et al (1966) report which found proline was still accumulating even without a N supply.

Interestingly, the source of N is not from seed protein as reported by Elmore and McMichael (1981) in cotton, which shows that seedlings from low seed protein cultivars accumulated higher proline than higher seed protein cultivars.

Potassium and sodium are inorganic ions responsible for the accumulation of proline. Mukherjee (1974) treated leaf disks of maize with and without KCl solution, showing the stressed samples incubated in the potassium solution contained more concentrated proline. Udayakumar et al (1976) reported that under water stress, cucumber cotyledons grown in KCl solution accumulated higher proline than the non-treated series. Proline concentration also followed the increase of KCl concentration in the nutrient solution.

In K^+ deficient finger millet, the leaves' ability to synthesize proline was reduced 78% under water stress conditions, compared to the K-sufficient plants (Nageswara et al, 1981a). The role of potassium chloride in this context is suggested as due to the inhibition of the enzyme pyrroline-5-carboxylic acid dehydrogenase (Bogges et al, 1975). In contrast, lower potassium content in groundnut leaves did not cause lower proline accumulation. However, feeding the leaves of K-deficient plants with potassium chloride and then with arginine (a precursor of proline), enhanced the plants' ability to convert arginine to proline. Therefore, with arginine, potassium synergistically increased the accumulation of proline. Also the addition of potassium to the K-deficient plants would increase the specific activity of the enzyme arginase in finger millet, thus increasing proline accumulation. This reveals that

potassium is involved in the conversion of precursor arginine to proline via enzymatic activity (Nageswara et al, 1981*b*).

Another suggestion for the relationship between potassium and proline accumulation, comes from Weimberg et al (1982) who found that proline was not accumulated in leaves of *Sorghum bicolor* unless the concentration of total monovalent cations in leaves reached a threshold level. Hence, synthesis of proline (a cytoplasmic solute) is necessary to counteract the build up of cations and anions in the vacuoles, proline acting as a balancing osmotic agent or compatible solute. If this is so, then a similar cation such as Na^+ should produce a similar effect. Interestingly, sodium did not promote proline accumulation in stressed maize leaf disks (Mukherjee, 1974). However this finding is in contrast to many reports showing that intact leaves of NaCl stressed plants would accumulate high proline concentrations (for example Cavalieri and Huang, 1979; Weimberg et al, 1982; see Section 8.3).

Carbon and hydrogen atoms are involved in proline synthesis through the carbohydrate pool. Stewart (1978) found that wilting increased the proline accumulation in non-starved (illuminated) barley leaves to 40 times that of starved plants. It is suggested that carbohydrate supplies the precursors i.e. carbon and hydrogen for proline synthesis.

These above reports provide some suggestions for interpreting this *Acacia* experiment. In well-watered conditions, those *A.iteaphylla* seedlings grown in sufficient available nutrient, may utilize the available nutrient sources for the synthesis of photosynthates such as protein, carbohydrates, glutamic acid etc. When the water potential approaches the stress threshold level, these nutrient-sufficient seedlings may start to synthesize proline, and would benefit from at least three sources. Firstly, from external sources as long as the transport process has not been stopped due to stomatal closure. The second source may come as a result of photosynthate breakdown especially for N requirement. The third possibility is the conversion of precursors such as glutamic acid or

arginine to proline. Potassium may be used in the activation of specific enzymes, however it has to be within the tolerance level of the cells.

In contrast, those seedlings which grew under nutrient deficiency whilst undergoing water stress would probably suffer from at least three effects in the process of proline synthesis. Firstly, the shortage of external sources of supply due to insufficient levels in the medium to synthesize photosynthates, which would lead to a reduced source (for example N) in the process of proline synthesis during water stress. Secondly, the available precursors may be at a limited level, and finally even though the external source (say nitrate for N) may be available at a limited level in the growth medium, nevertheless its supply may be inhibited by water stress.

The decrease of soil water content may create air spaces between soil particles and the root surface (Osonubi, 1984) and reduce the size of the water film among soil particles which is important in mineral transport. This may lead to the inhibition of nutrient transfer between soil particles and root surfaces. This will be true for all plants (both treatments) under stress. However, when external nutrients are low, this effect may be greater in the low nutrient plants.

d. *The influence of 'environmental factors' outside the glasshouse in proline synthesis*

Even though light intensity, temperature and other environmental factors were not recorded during the experiment, it is assumed that plants outside the glasshouse would have received higher light intensity and undergone higher temperature or a greater temperature range and the effect of other factors compared with those inside. In fact, the plants inside the glass-house were in such a position that the direct sunlight did not reach them until 10 to 11 a.m., and left them earlier than the actual sunset time. The plants outside

the glass-house would also have been shaded early and late in the day, however they still received more light than the inside.

To the best of my knowledge, two environmental factors namely light (Hanson and Tully, 1979) and temperature - relatively high (Chu et al, 1974) and -chilling (Yelenosky, 1979) have been reported as affecting proline synthesis. The involvement of light would be through the stimulation of the conversion of precursors to proline; higher temperature would promote the activation of enzymatic systems along the proline synthesis pathway. However, Chu et al (1974) found that at extra high temperature i.e. 35° C, no proline accumulation occurred if the water potential was kept at the least negative level. Cold would not be involved here since the experiment was carried out between November and February, thus summer in South Australia; therefore no chilling nights occurred. Wind was probably involved via rapid loss of water which leads to the more rapid lowering of water potential, but it seems it would not increase the proline concentration in terms of the conversion of precursors.

Proline concentrations of well-watered plants (Table 7.1.c, row 5a) are similar and statistically it was confirmed that there was no significant difference in y-intercept between all treatments (Table 7.2, part b). The similarities of proline concentration for both inside and outside treatments in well-watered conditions, indicated that environmental factors had very little influence on the proline concentration in the absence of stress. Also, the stressed plants were subjected to similar levels of water stress. Therefore, the differences in proline concentrations generated by the plants inside and outside the glasshouse after being subjected to stress, are assumed to be due to the two main environmental factors namely light and temperature.

All outside plants revealed higher proline concentration than plants with the same treatment inside the glasshouse. Table 7.1.c (row 5b), shows that plants grown in Black Hill

soil with extra nutrient outside the glasshouse accumulated almost twice as much proline under stress as those inside with the same treatment. Without nutrient application proline content of outside stressed plants grown in Black Hill soil is one and a half times as high as the inside ones. Mixed soil stressed plants outside with extra nutrient also accumulated about one and a half times as much proline as inside plants, while outside stressed plants without added nutrient accumulated 6 times the proline concentration of inside plants.

Figures 7.2.c and 7.2.d show the relationship between water potential and proline concentration for the combinations of inside and outside plants. It is clear that the slopes of all nutrient treatments outside are steeper than the corresponding inside treatments. This provides evidence for the effect of the environment, at least the two main factors i.e. light and temperature in stimulating more proline synthesis within water stressed plants.

The effect of light in proline synthesis has been reported by Hanson and Tully (1979). They showed that in water stressed conditions, illuminated excised leaf cuttings would have twice the proline concentration of darkened leaves. This is mainly due to the conversion of glutamate (precursor) to proline.

It should be pointed out that proline concentrations in these potted plants were frequently higher under stress than those in the field-grown plants (Figure 5.3, Table 5.4.b). Evidently the pot experiment did not reproduce field conditions.

Back to the original question which led to this experiment, namely whether or not soil type or nutrient level affected the synthesis of proline in *A.iteaphylla*. The results show that pot grown plants of this species did accumulate proline to high levels when water stressed. It is clear that the amount of accumulation was affected by soil nutrient levels and environment factors (particularly light intensity and temperature). It was also proved that proline accumulation may be suppressed at very low nutrient level. However, *A.iteaphylla*

did accumulate proline at high levels when growing in Black Hill soil, thus the nutrient levels in the soil can not be the reason for the lack of accumulation in the field.

One possible explanation may be that the root systems of pot grown plants occupy such a small space that their water potential falls very quickly without being recharged properly when they are exposed to stress. This may lead to higher solute synthesis, while the field grown plants had greater opportunity to recharge their water content due to the possible deeper penetration of their roots (cf. section 8.2.2, Chapter 8).

But this only a possibility, and it is suggested that there may be some other unknown factor(s) involved which remain unexplained.

CHAPTER 8

ADDITIONAL EXPERIMENTS ON ORGANIC SOLUTES

8.1 *Nutrient effect on proline synthesis in field grown plants of A.aneura, A.iteaphylla* and *A.myrtifolia*

Chapter 7 showed that for pot grown plants, nutrient application had an appreciable effect on proline synthesis. However, sometimes the behaviour of pot grown plants was different from the field grown plants as discussed in Chapter 5.

In order to obtain further information about the effect of nutrient on proline synthesis, a simple experiment was conducted to find out whether or not nutrient application would increase proline synthesis in the field grown plants. The plants, growing at the Black Hill experimental site, were the same as had been used in the earlier studies. It is necessary to emphasize that this was merely a preliminary observation with very limited samples and only one harvest. Therefore, the results may not be as expected. However, this simple experiment was intended to promote deeper investigations of this aspect in the future.

8.1.1 *Materials and Methods*

Three species were chosen for this test, i.e. *A. aneura, A.iteaphylla* and *A. myrtifolia.* Plants growing in the first and third blocks, both well watered and stressed, received nutrient treatment. The second and fourth blocks were kept as controls without nutrient.

Full strength Hoagland solution was applied three times over three and a half months i.e. 21st November 1988, 20th January 1989 and 24th February, 1989 respectively. Each application was 800 ml. For stressed-with-nutrient plants, some water was applied around

the stem base at the time of nutrient application to facilitate nutrient uptake by the plants. Each plant received the same amount.

At the end of summer 1989 (6th March) dawn water potential values were recorded with the pressure chamber as an indicator of stress level. Samples were stored for later proline analysis by the previous method (Section 5.2.1.1).

8.1.2 *Results and Discussion*

Table 8.1.a presents the original and the mean (n=2) values of only one harvest of the three species, while Figure 8.1 shows the mean proline results graphically. Column 5a and b in the Table shows how proline was accumulated by each treatment corresponding to their water potential in column 4a and 4b. Data were subjected to analysis of covariance, where species and nutrient status were considered as factors, water potential as covariate, and proline concentration as the quantity of interest.

Table 8.1.b presents the Ancova results. There was evidence that some factors are significant. Water potential has a significant relationship with proline concentration ($0.01 > P > 0.001$). The difference in proline concentrations between species was only significant at a lower level ($0.01 < P < 0.5$). The behaviour of each species in terms of water potential effect on proline concentration also showed a significant difference at the same (lower) level.

Interestingly, nutrient application *did not* cause a significant effect on proline synthesis. Thus plants with extra nutrient did not improve their proline pool.

Since water potential had a significant effect on the proline concentration, and the effect was also different in each species, it is therefore relevant to describe the relationship of these two variables by a linear regression. Table 8.1.c presents the figures for the linear relationship in each species, and Figure 8.2 displays the distribution of data points of all the treatments for all species.

Table 8.1.a

The original and mean (n=2) values of water potential and proline concentrations of field grown plants (**Black Hill**) of three species *Acacia aneura, A.iteaphylla and A.myrtifolia*, with/ without (+/-) additional nutrient (Hoagland).

Column 2: Ntr-Trt, nutrient treatment; +/- Nut., plus or without nutrient; Column 3: R, replications, Mn, mean values; Column 4a and 4b: water potential values (-MPa) of WW, well-watered, and STR, stressed treatments. Column 5a and 5b: proline concentrations (μmol g^{-1} DW) of WW and STR treatments.

Species (1)	Ntr-Trt (2)	R (3)	Water potential (4)		Proline concent (5)	
			a	b	a	b
			WW	*STR*	*WW*	*STR*
A.aneura	+Nut.	1	0.50	3.30	18.58	23.12
		2	1.00	4.10	12.47	28.78
		Mn	***0.75***	***3.70***	***15.52***	***25.95***
	-Nut.	1	1.00	2.20	7.53	17.38
		2	1.50	3.65	9.96	31.94
		Mn	***1.25***	***2.93***	***8.75***	***24.66***
A.iteaphylla	+Nut.	1	1.10	1.50	26.22	21.06
		2	0.80	3.70	18.30	30.06
		Mn	***0.95***	***2.60***	***22.26***	***25.56***
	-Nut.	1	1.20	1.50	10.88	24.62
		2	1.70	2.10	13.79	21.14
		Mn	***1.45***	***1.80***	***12.34***	***22.88***
A.myrtifolia	+Nut.	1	0.90	5.50	1.89	53.46
		2	0.70	3.60	3.80	17.78
		Mn	***0.80***	***4.55***	***2.85***	***35.62***
	-Nut.	1	1.50	3.60	3.54	18.24
		2	0.60	3.20	3.09	18.58
		Mn	***1.05***	***3.40***	***3.32***	***18.41***

Figure 8.1

Summary of proline concentrations (μmol g^{-1} DW) in phyllodes of three field grown plant species: *Acacia aneura*, *A.iteaphylla* and *A.myrtifolia,* after nutrient (Hoagland's solution) applications. For legends, see Figure.

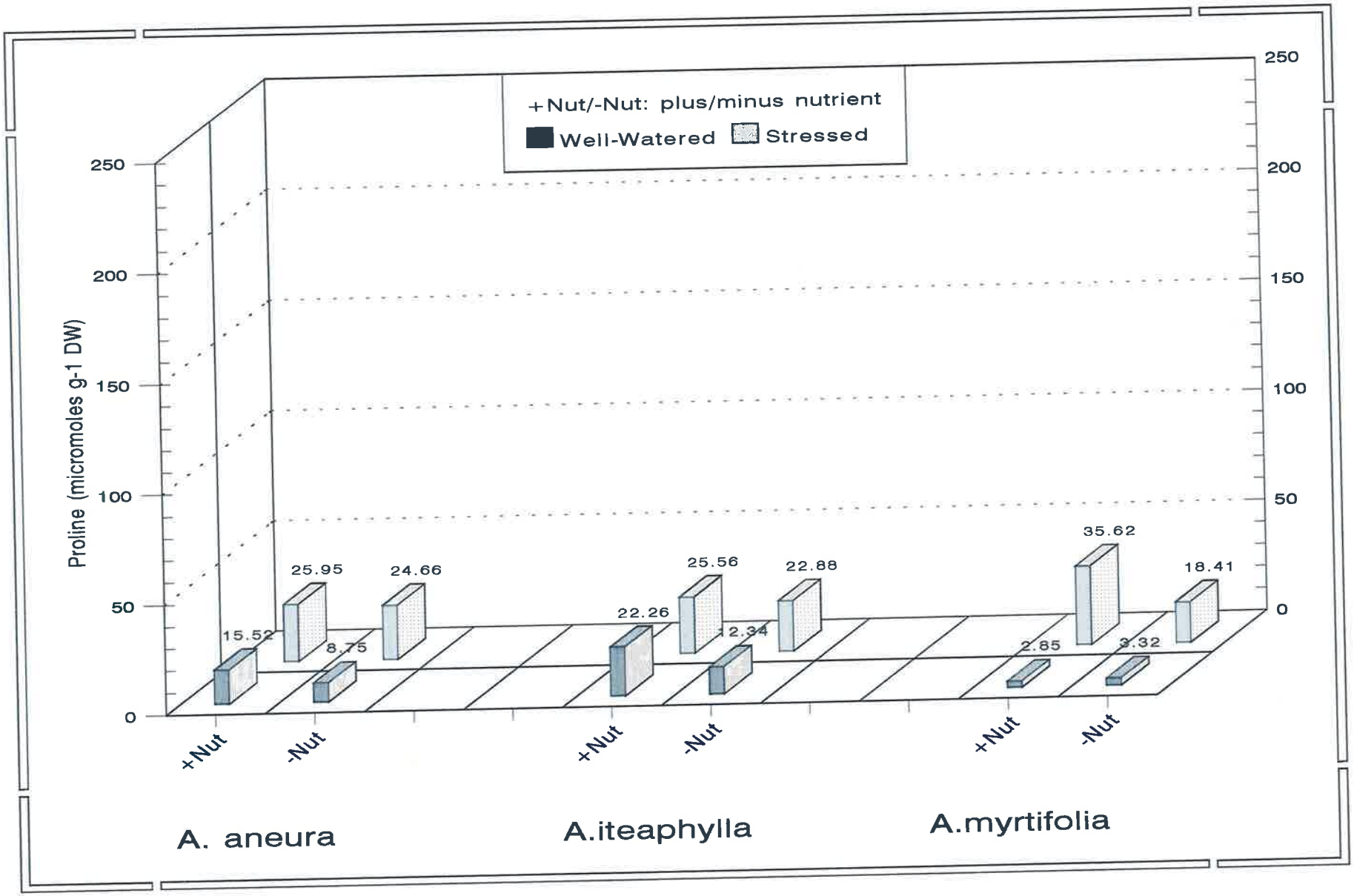

+Nut/-Nut: plus/minus nutrient
Well-Watered
Stressed
Proline (micromoles g-1 DW)
250
200
150
100
50
0
250
200
150
100
50
0
15.52
25.95
8.75
24.66
22.26
25.56
12.34
22.88
2.85
35.62
3.32
18.41
+Nut
-Nut
+Nut
-Nut
+Nut
-Nut
A. aneura
A.iteaphylla
A.myrtifolia

Table 8.1.b

ANCOVA of proline for three field grown *Acacia* species (*A.aneura, A.iteaphylla* and *A.myrtifolia*) with nutrient treatment.

Test of significance for proline (linear covariate)

Source	DF	SS	MS	F	P
WITHIN + RESIDUAL	12	391.40	32.62		
WP	1	332.38	332.38	10.19	0.008 **
SP	2	395.02	197.51	6.06	0.015 *
NUT	1	12.04	12.04	0.37	0.555 n.s
SP BY NUT	2	410.60	205.30	6.29	0.014 *
NUT BY WP	1	1.51	1.51	0.05	0.834 n.s
SP BY NUT	2	42.08	21.04	0.65	0.542 n.s
SP BY NUT BY WP	2	27.41	13.70	0.42	0.666 n.s

Note:

WP : Water potential
SP : Species
NUT : Nutrient

Table 8.1.c

The linear regression equations describing the relationship between water potential and proline concentrations derived from three field grown (Black Hill) *Acacia* species with special respect to nutrient application.

Note: +, added nutrient; -, without nutrient; Ψ, water potential; P, proline.

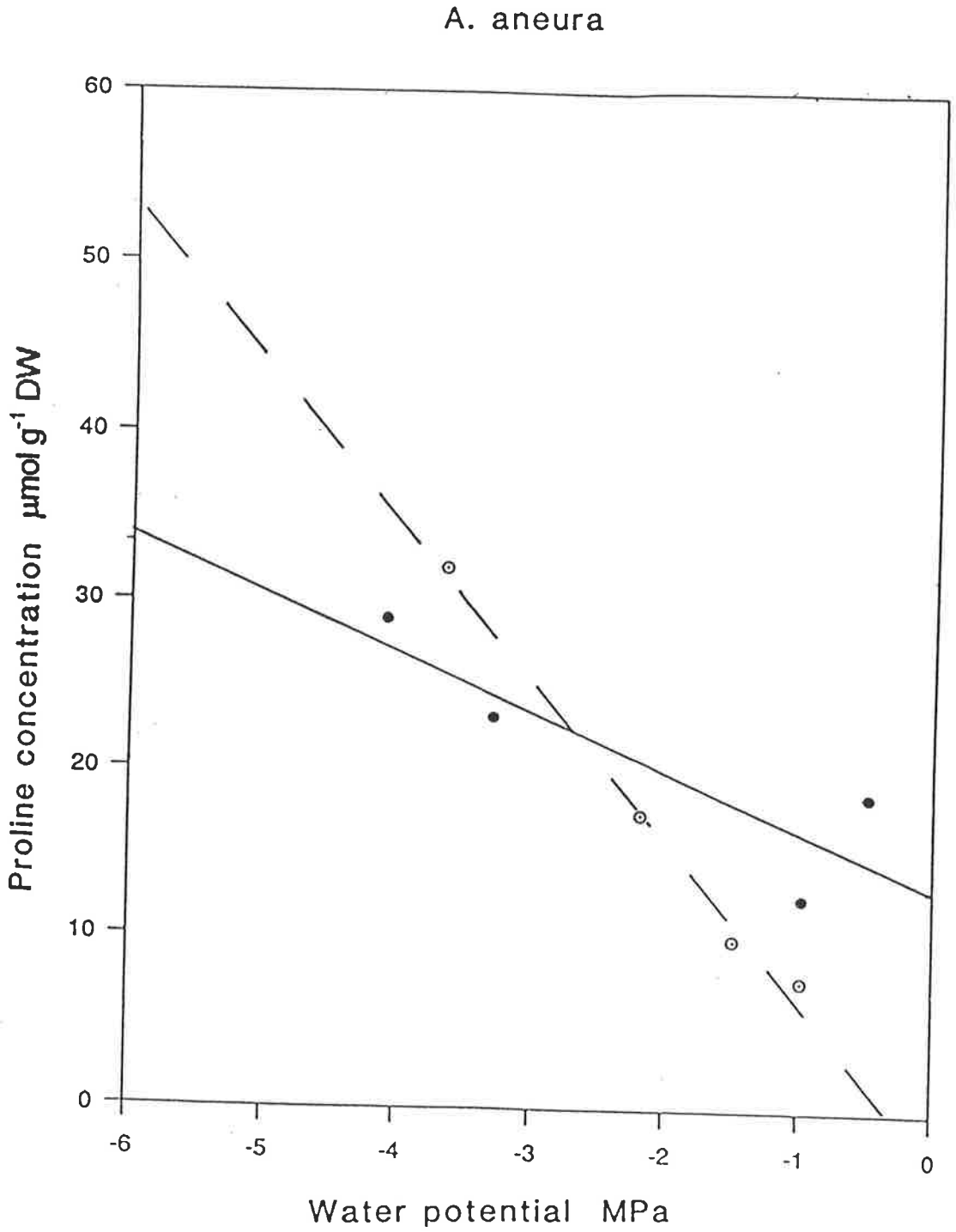
Species	Treatment	Equation	Coeff. regr. (r^2)
A.aneura	+ Nutrient	P = -3.44Ψ + 13.08	0.756
	- Nutrient	P = -9.50Ψ + 3.13	0.992
A.iteaphylla	+ Nutrient	P = -3.19Ψ + 18.25	0.637
	- Nutrient	P = -7.89Ψ + 4.78	0.219
A.myrtifolia	+ Nutrient	P= -9.86Ψ - 7.14	0.902
	- Nutrient	P= -5.93Ψ - 2.33	0.926

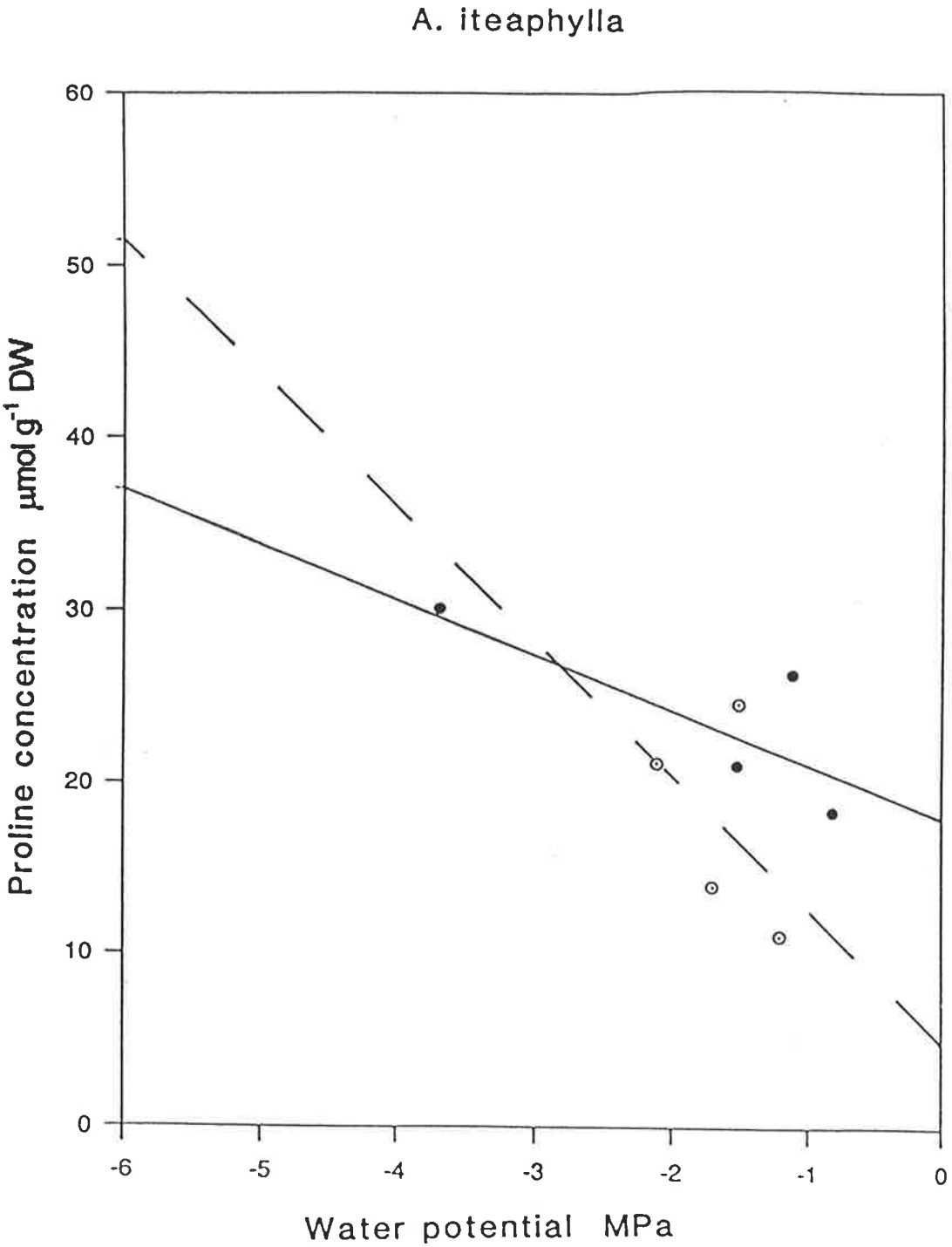
Figure 8.2

The distribution of points describing the relationship between proline concentration and water potential in three field grown *Acacia* species with and without nutrient treatment.

● : added nutrient

◎ : no added nutrient


A. aneura
Proline concentration µmol g-1 DW
60
50
40
30
20
10
0
-6
-5
-4
-3
-2
-1
0
Water potential MPa


A. iteaphylla
Proline concentration µmol g-1 DW
60
50
40
30
20
10
0
-6
-5
-4
-3
-2
-1
0
Water potential MPa

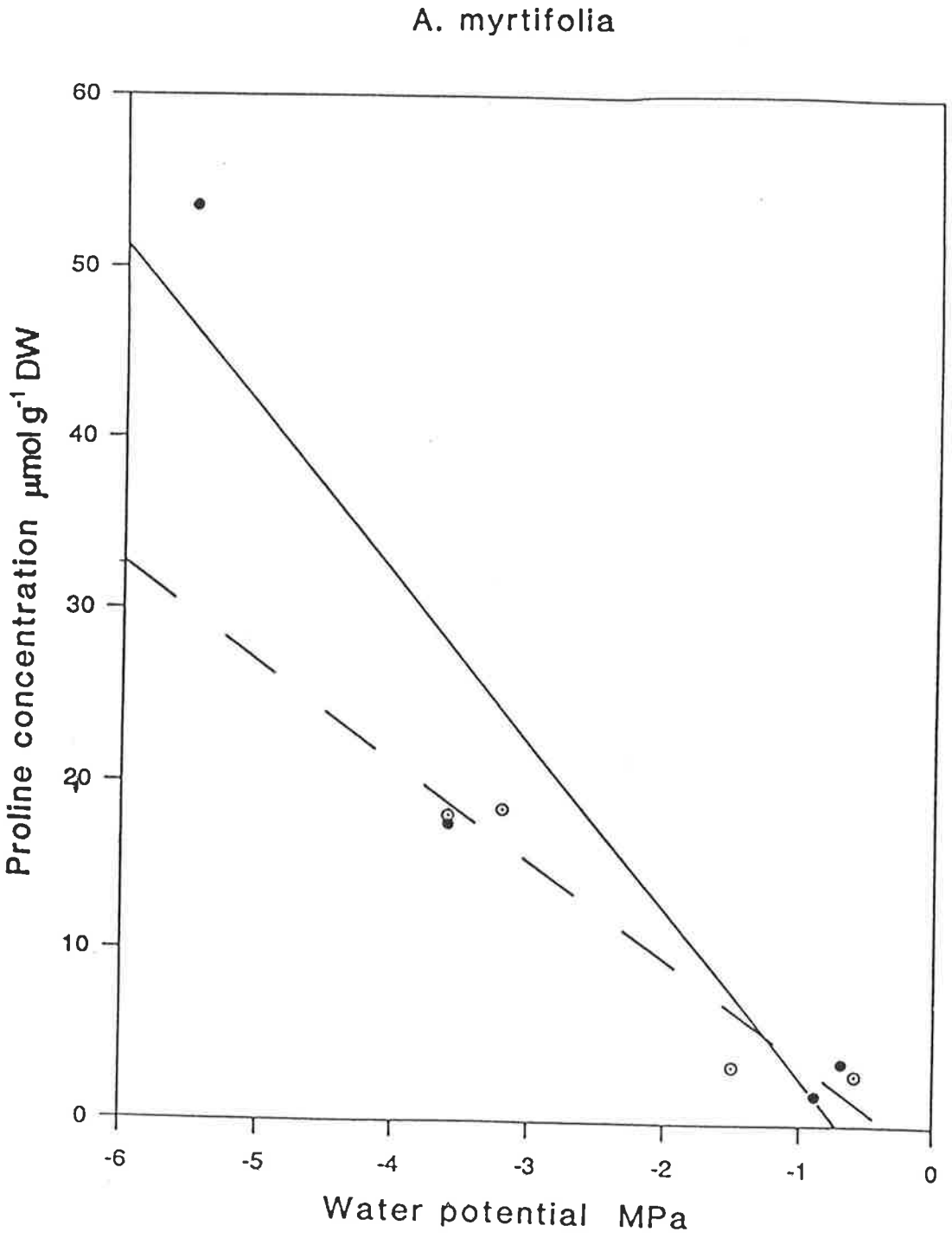

A. myrtifolia
Proline concentration µmol g-1 DW
60
50
40
30
20
10
0
-6
-5
-4
-3
-2
-1
0
Water potential MPa

Table 8.1.a reveals that the differences in proline concentrations between 'well-watered' and 'stressed' in added nutrient plants of *A. aneura* and *A. iteaphylla* were lower than in plants without added nutrient. The main reason was that the values of well-watered plants were relatively high (column 5a). These high values can not be explained as due to water stress, because the water potentials of these plants were less negative than the corresponding values for the well watered plants without nutrient (column 4a). In contrast, in well-watered *A. myrtifolia* similar water potential values produced very low proline concentration. The similar level of proline in both treatments of *A. iteaphylla* clearly repeated the phenomenon found in the field grown plants of this species during the first field experiment (see Chapter 5, section 5.2.1).

The non-significant effect of nutrient on proline synthesis by field grown plants (at least in this experiment) is difficult to explain, but may be caused by one or more of the possibilities below:

a. It was shown in Chapter 7 that proline levels were higher in Black Hill soil than in the potting mix, and the response to added nutrient was not so great. It may simply have been that the plants growing at the field site had sufficient nutrient and the extra supplied had minimal effect.

b. The plants were already quite large when this experiment was done. The nutrient applied may not have been sufficient to have a significant effect.

c. The number of replications was too low (n=2) so that variability of readings did not allow the differences to become significant.

8.2 *Proline synthesis by pot-grown seedlings of A.aneura, A.iteaphylla and A.myrtifolia*

Proline synthesis in the field grown plants (Section 5.2.1) increased significantly when water potential decreased. However, there has been a lack of information concerning proline

accumulation in field grown and glasshouse plants of *Acacia* species. The experiment below was conducted to provide some additional information on the behaviour of three *Acacia* pot grown seedlings in accumulating proline when they were subjected to water stress. The aim was to find out:

(i) whether or not the glass-house and field grown plants behave similarly, i.e. when a decrease in water potential would be followed by an increase in proline

(ii) the magnitude of the concentration in pot grown plants compared with the field

These magnitudes would be merely an approximation, since the potted plant data were obtained only within one drying cycle, while the field grown plants were harvested four times over a period of months.

This simple experiment *was not* intended to obtain strictly comparable data between these three species, since two species (*A.aneura* and *A.myrtifolia*) were much older (about 10 months) than *A.iteaphylla* (about 5 months). A comparative study between species should involve plants of similar ages, since a species might behave differently at different stages.

8.2.1 *Materials and Methods*

Approximately 10 month old pot grown seedlings of *A.aneura* (14 pots) and *A.myrtifolia* (11 pots) and 5 months old *A.iteaphylla* (11 pots) were used. The plants grown in potting mixture were well-watered in advance before being subjected to water stress. The surfaces of pots were covered with polystyrene balls to reduce rapid dehydration. Water

potential values were recorded every two days with the pressure chamber. Phyllodes were collected and stored for proline analysis by the previous methods. The drying cycle was between 8 and 12 days. Data were gathered each time from a different single plant.

8.2.2 *Results and Discussion*

Table 8.2 presents the original values of the gradual decrease of water potential and the increase of proline in the three species during the drying cycle. The results were subjected to a simple linear regression analysis. The regression lines are drawn in Figure 8.3, which shows a remarkable accumulation of proline. This enhancement is clearly a response to moisture stress as indicated by water potential.

The relationship between water potential and proline concentration was linear. The y-intercept was not significantly different from zero for any of the three species; thus the relationship between water potential and proline concentration is of the form

$$P = a\,\Psi \quad \ldots\ldots\ldots\ldots \quad (15)$$

where P is proline content, a is constant and ψ is water potential. Then, for each species the relationship is

		r^2	
A.aneura	$P = 11.6\,\psi$	0.7859	..... (15a)
A. iteaphylla	$P = 18.4\,\Psi$	0.8617	..... (15b)
A. myrtifolia	$P = 7.7\,\Psi$	0.8989	..... (15c)

Table 8.2

The original values of proline concentrations (µmol g^{-1} DW) with corresponding water potential (-MPa) measurements gathered during a drying cycle from pot grown seedlings of 3 species acacia i.e *A.aneura*, *A.iteaphylla* and *A.myrtifolia*.

Species	Water potential	Proline Concent.
A.aneura	1.45	28.99
	1.45	22.36
	1.45	23.30
	1.50	26.26
	1.50	18.09
	1.60	26.99
	1.60	27.25
	1.90	14.12
	1.90	17.20
	2.41	18.86
	3.28	41.53
	4.14	61.54
	5.00	41.73
	6.55	76.78
A.iteaphylla	1.21	12.32
	1.38	10.17
	1.55	30.10
	2.24	29.71
	2.59	44.57
	3.28	24.62
	5.86	157.35
	6.21	148.89
	8.28	139.79
	8.62	135.36
	8.79	159.19
A.myrtifolia	1.00	1.85
	1.00	0.32
	1.25	5.87
	1.40	4.21
	2.50	2.88
	2.30	9.87
	2.95	4.57
	3.15	27.51
	6.21	69.70
	8.61	69.62
	8.79	63.68

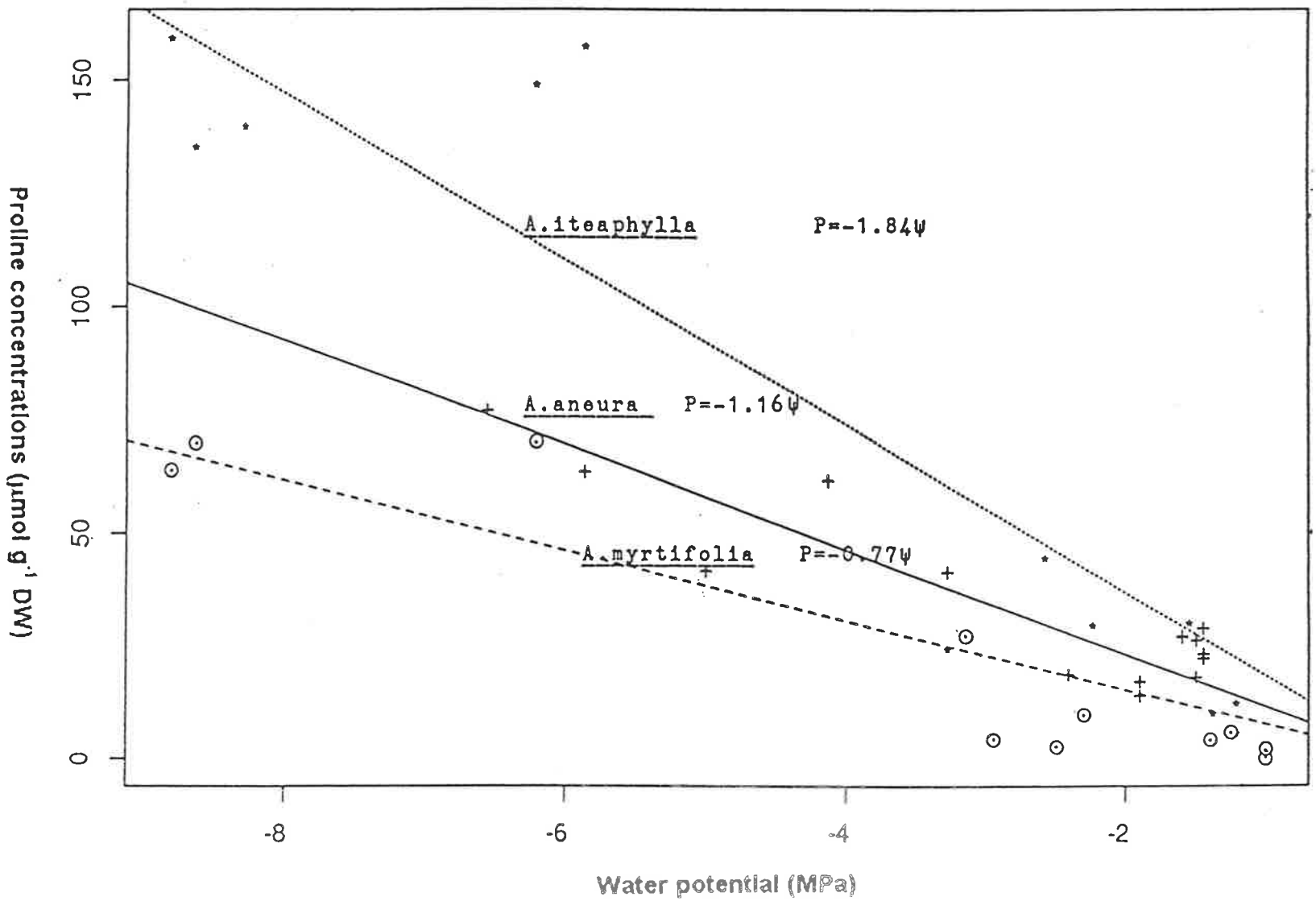
Figure 8.3

The regression lines for three *Acacia* species, showing the relationship between water potential and proline concentration in pot grown plants.

+ : *A.aneura*

* : *A.iteaphylla*

⊙ : *A.myrtifolia*


Proline concentrations (µmol g-1 DW)
150
100
50
0
-8
-6
-4
-2
Water potential (MPa)
A.iteaphylla P=-1.84ψ
A.aneura P=-1.16ψ
A.myrtifolia P=-0.77ψ

The regression lines produced a reasonable empirical description of the effect of water potential on the proline concentration as proved by the r^2 values.

Therefore, Figure 8.3 is evidence that the pot grown seedlings of these three *Acacia* species behave similarly to the field grown plants i.e. proline would be accumulated when water potential dropped. This pattern followed some previous reports of pot experiments in various species. For examples McMichael and Elmore (1977) in cotton, Blum and Ebercon (1976) in wheat, Jager and Meyer (1977), Waldren et al (1974) in soybean and Lawlor and Fock (1977) in sunflower.

The values of proline concentration were higher than those in the field grown plants. Additionally, Figure 8.3 revealed that the concentration of proline in stressed pot grown *A.iteaphylla* could become extremely different from the well-watered plants, while in field this difference was not so apparent.

In comparison between plants grown in the field (Figures 5.3 and 8.1) and glasshouse (Figures 7.1 and 8.3) it is clear that glass-house potted plants accumulated much higher proline than the field grown plants. This discrepancy may be a result of the interaction of various factors. The development of stress in the field was usually more gradual than in pots in the glasshouse and water potentials fell lower in the glasshouse. Even though environmental factors outside fluctuate more than in the glass-house (Rorison, 1981), field grown plants may regain their internal water balance during the night or cloudy times through better acces to soil moisture via an extensive root system. Root systems in pots may be more restricted and there is a much smaller volume of soil available for storing water.

Retransport of proline to the other parts of the plants may have taken place in the these two groups of plants (field and glass-house); however the difference in size due to different ages between field grown and glasshouse plants in this experiment may be another reason for the differences.

8.3 *The response of PEA to water, salinity and cold stresses in pot grown A.iteaphylla seedlings where proline is used as standard*

The purpose of this experiment was to find out whether or not pot grown plants of *A.iteaphylla* accumulated PEA as a response to three forms of stress as a comparison with the field grown plants.

Proline was also measured in the same samples as PEA to compare the behaviour of these two methylated compounds. Proline was chosen as a standard since in a large number of reports it has been shown that proline concentration increases remarkably during these types of stress (see for examples reviews by Aspinall and Paleg, 1981; Stewart and Hanson, 1980; Yelenosky, 1979).

8.3.1 *Materials and Methods*

About 8 months old pot grown seedlings of *A.iteaphylla* were used. Three treatments were applied i.e. water, salt and cold stresses. Each treatment consisted of three pots. All pots were well-watered prior to the application of treatments. Water potential values were recorded as a measure of stress. Phyllodes were collected as required, before the application of the stresses, and stored for analysis for PEA and proline following the steps as described in Chapter 5 Sections 5.2.1.1 and 5.2.2.1.

For the water stress treatment, water was withheld for 5 to 15 days. The three salt stressed plants were fed gradually with different concentrations of NaCl in one-fourth strength Hoagland solution. The application was on each second day. At each application, the concentration of NaCl was increased by 100 mM from an initial application of 100 mM NaCl until it reached 800 mM.

The three remaining pots were placed in a cold room (2 to 4° C), for 10 days. After stress treatments, phyllode water potentials were again recorded from all plants. Phyllodes were collected and prepared for colorimeter and NMR analysis.

8.3.2. *Results*

The original and mean (n=3) values of PEA and proline concentration as a result of the three stress treatments are presented in Table 8.3.a. Figure 8.4 shows the mean values before and after water, salt and cold stresses. In salt and cold treatments PEA concentration apparently became lower after stress was applied, this effect being greater for cold treatment plants. There was no significant change in PEA concentration after the water stress treatment. On the other hand, proline shows very contrasting results. There are big increases in concentration after stress within all treatments.

Regression analysis was used to test the effect of stress and water potential upon the PEA concentration. As presented in Table 8.3.b, it is revealed that by ignoring water potential information, there was sufficient evidence to retain the hypothesis that the type of stress (i.e. water, salt or cold) has no effect on the PEA concentration. Thus the level of PEA concentration was not significantly changed under any type of stress. By ignoring the stress type it was also possible to retain the hypothesis that water potential has no effect on PEA concentration.

In contrast, as presented in Table 8.3.c, there was sufficient evidence to reject the hypothesis that water potential has no effect on proline concentration. Therefore, the linear model was retained. However, by ignoring water potential information, it is shown that type of stress has no effect on the proline concentration. Thus the effects of each stress namely water, salt and cold are not significantly different.

Table 8.3.a

The original and mean values of PEA and proline concentrations (µmol g^{-1} DW) in pot grown seedlings of *A.iteaphylla* treated (n=3) with drought, salt and cold stresses.

R, Replications; Before and After application of stress treatments.
Mn, mean values.

Type of stress	R	Treatments			
		Before		After	
		PEA	Proline	PEA	Proline
Water	1	118.00	35.37	124.20	294.43
	2	129.80	35.08	81.80	199.11
	3	128.20	34.59	188.40	121.24
	Mn	***125.3***	***35.01***	***131.47***	***204.93***
Salt	1	189.60	46.30	154.10	207.52
	2	163.00	55.16	178.30	236.02
	3	204.50	57.39	47.60	195.18
	Mn	***185.70***	***52.95***	***126.67***	***212.91***
Cold	1	154.30	52.50	83.00	139.89
	2	151.50	66.63	69.80	196.50
	3	146.00	61.65	85.90	191.20
	Mn	***150.60***	***60.26***	***75.57***	***175.86***

Figure 8.4

The average values of PEA and proline (μmol g^{-1} DW) concentrations before and after stress (water, salt and cold), gathered from three individual seedling plants per treatment of *A.iteaphylla.*
For legends, see Figure.

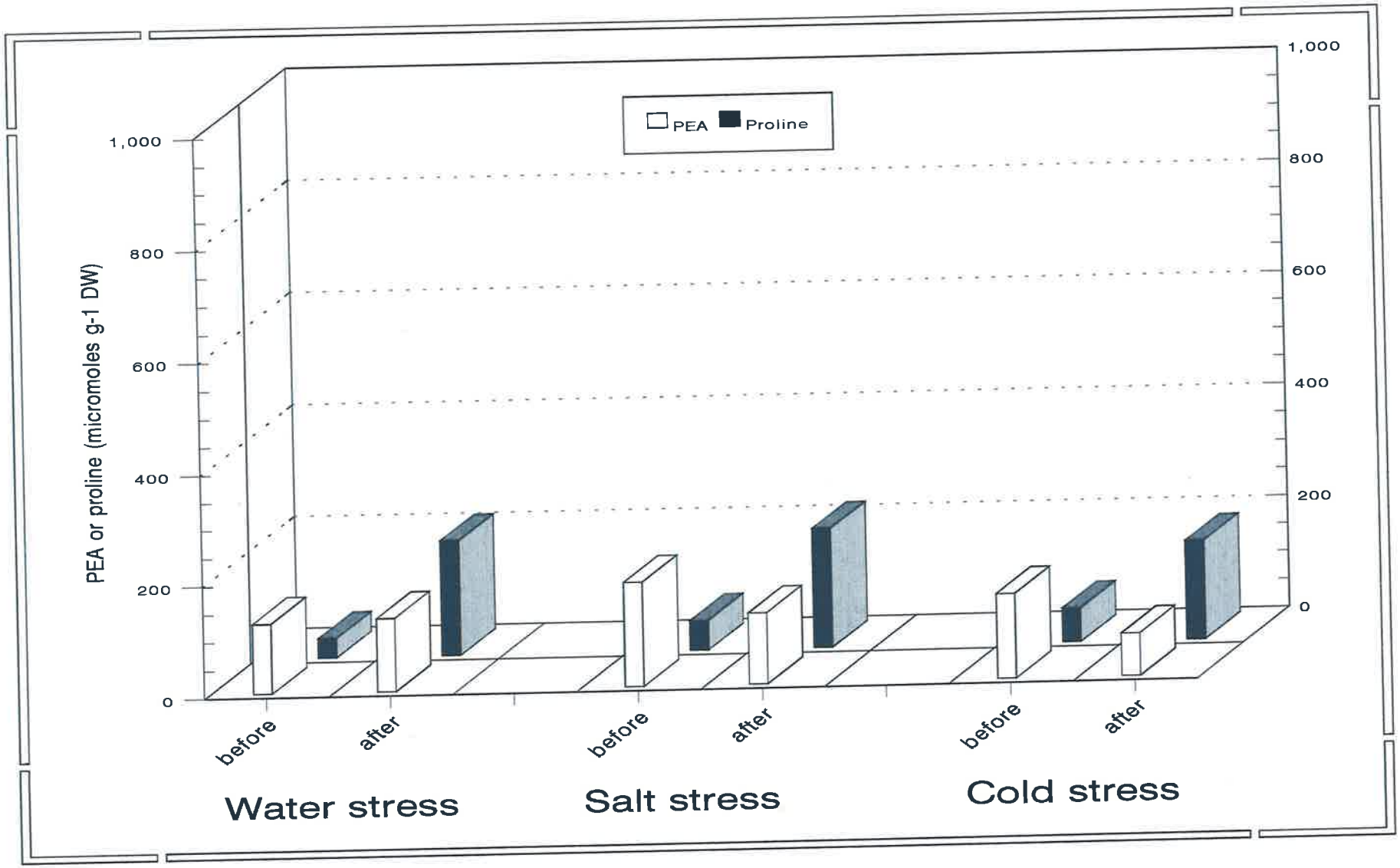

PEA
Proline
PEA or proline (micromoles g-1 DW)
1,000
800
600
400
200
0
before
after
before
after
before
after
Water stress
Salt stress
Cold stress

Table 8.3.b

Regression analysis of the response of PEA to water, salinity and cold stress.

Source	df	Sum of Squares	Mean Square	F
Stress ignoring water potential	2	5270	2635	1.35
Water potential ignoring stress	1	2077	2077	1.06
Residual	14	27341	1953	
Total	17	34688		

Test for stress type ignoring water potential:

$F = 1.35$ $\qquad$ $F_{0.95}(2, 14) = 3.74$

therefore ***retain*** hypothesis that the type of stress has no effect on the PEA concentration.
Test for water potential ignoring stress:

$F = 1.06$ $\qquad$ $F_{0.95}(1, 14) = 1.06$

therefore ***retain*** hypothesis that the level of water potential has no effect on PEA concentration.

Table 8.3.c

Regression analysis as in Table 8.3.b for proline.

Source	df	Sum of Squares	Mean Square	F
Stress, ignoring water potential	2	785	393	0.58
Water potential including stress	1	110259	110259	162.15
Residual	14	9525		
Total	17	120569		

Test for stress type ignoring water potential:

$F = 0.58$ $\qquad F_{0.95}(2, 14) = 3.74$

therefore ***retain*** hypothesis that the type of stress has no effect on the proline concentration.

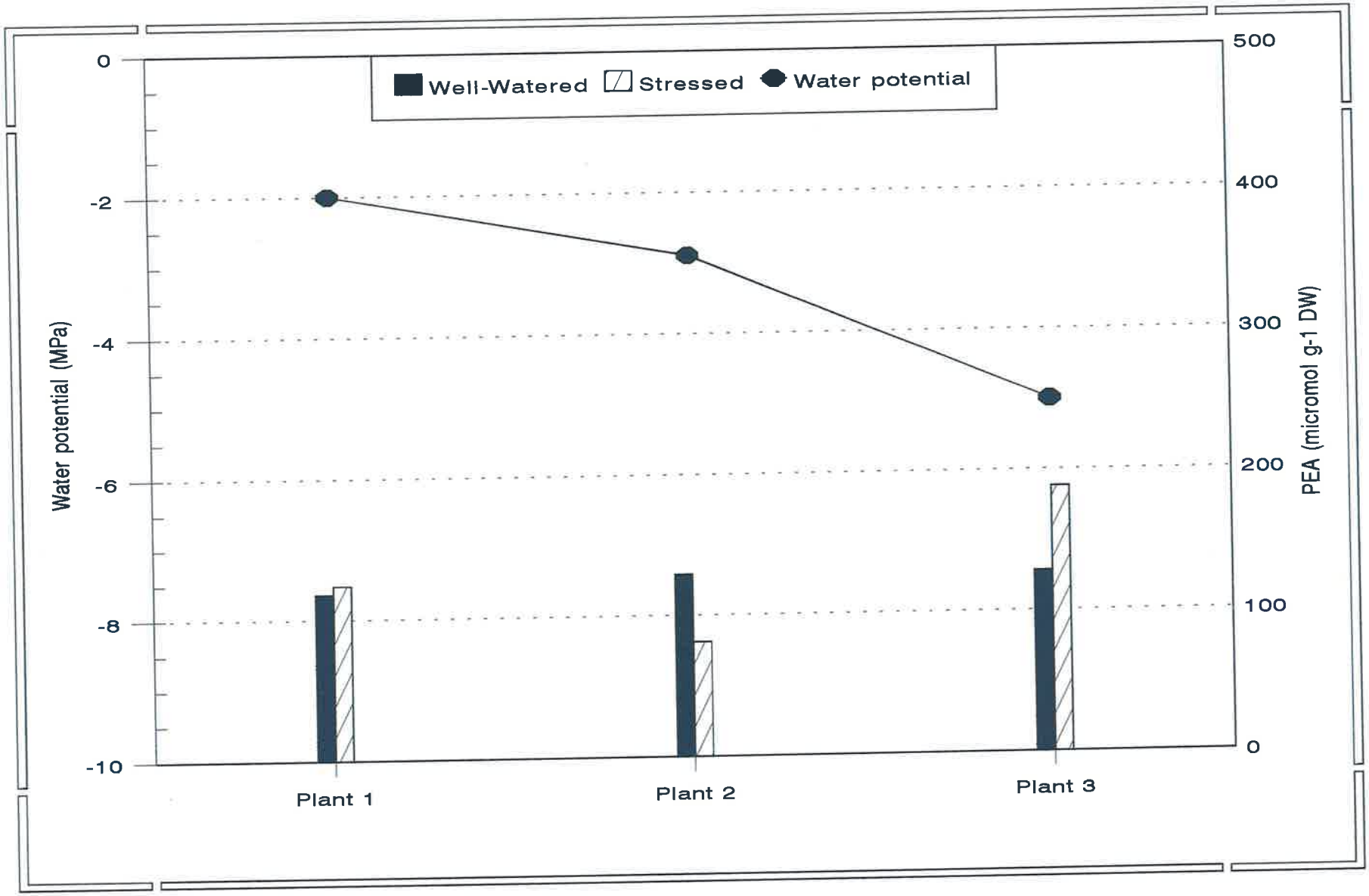
Test for water potential without including stress:

$F = 162.15$ $\qquad F_{0.95}(1, 14) = 1.06$

therefore ***reject*** hypothesis that the level of water potential has no effect on the proline concentration, and the linear model is retained.

Figure 8.5

PEA concentrations in three single plants of *A.iteaphylla*, measured before and after a period of water stress. Water potentials (-MPa) at the end of the stress period are shown above the columns.
For legends, see Figure.


Well-Watered
Stressed
Water potential
0
-2
-4
-6
-8
-10
Water potential (MPa)
500
400
300
200
100
0
PEA (micromol g-1 DW)
Plant 1
Plant 2
Plant 3

8.3.3. *The inconclusive result*

The data in Figure 8.4 are averages of three plants. However, due to environmental factors, at the end of stress each individual plant of the water stress treatment had reached a different value of water potential. This may have caused variability in the PEA concentration. In Figure 8.5 the single plant values are shown, together with the final water potential values. It can be seen that in the well-watered plants PEA concentration reached about 120 μmol g^{-1} DW. At moderate water stress, its concentration was lowered to approximately 80 μmol g^{-1} DW. At severe water stress, the concentration went up to 190 μmol. Thus the results are inconclusive. This fact led to the second experiment (Section 8.4) using more plant material (pot grown plants) to show whether or not this pattern is a typical PEA response to water stress.

8.4 Changes in PEA with time in *A.iteaphylla* during droughting

8.4.1 *Materials and Methods*

Eight month old potted seedlings were subjected to a drying cycle in the glasshouse. Twelve pots were separated into 4 groups. The plants were watered well before the treatment was applied. Each second day, water potential values were measured from 3 pots (namely three replications). Phyllodes were collected as required for further PEA analysis by NMR.

8.4.2 *Results and Discussion*

The original values of PEA concentrations as a result of drying cycle, are shown in Table 8.4.a. The results do show a trend. Under well-watered conditions (with water potentials above -3 MPa) the PEA concentration is high (200-300 μmol g^{-1} DW). Between moderate and severe stress, when water potential fell to approximately -6 MPa, the

Table 8.4.a

The original values of PEA concentrations (µmol g^{-1} DW) with corresponding water potentials (-MPa) during a drying cycle from pot grown seedlings of *A.iteaphylla*.

Water Potential	PEA Concentration
1.21	181.2
1.38	293.5
1.55	246.9
2.24	250.1
2.59	193.7
3.28	246.0
5.52	136.5
5.86	144.7
6.21	171.3
8.28	285.8
8.62	228.9
8.79	320.2

PEA concentration declined to lower levels, a mean of 150 μmol g^{-1} DW. When the water potential declined further to -8 MPa the concentration increased quite dramatically to about 300 μmol g^{-1} DW. Thus this figure seems to repeat the pattern of PEA fluctuations shown in Figure 8.4, at least in pot grown seedlings.

Table 8.4.b shows evidence from regression analysis to retain the hypothesis that a quadratic function fits the data. The relationship between the two parameters (water potential and PEA concentration) is expressed as

$$P = 347.41 - 7.85\Psi + 0.0839\Psi^2 \quad \ldots\ldots\ldots \quad (16)$$

where P refers to PEA concentration and Ψ is water potential. Figure 8.6 shows the quadratic relationship between PEA and water potential.

So far, there are no detailed physiological or ecological studies of the role of PEA, especially its response to environmental changes. Thus there was some difficulty in developing a comparative discussion.

In the comparison between the two compounds PEA and proline Figure 8.4 clearly shows that proline was highly responsive to all types of environmental stress namely water, salt and cold. The effect of the three types of stress was roughly the same. Proline concentrations in the pot grown plants under stress were several fold higher than in the field.

The increase of proline in response to all these environmental stresses is consistent with many previous reports. For water stress references have been given; for salinity, see Stewart and Lee (1974); Chu et al (1976); Dix and Pierce (1981); and for cold (chilling) stress, see for example Yelenosky (1979) and Purvis (1981).

In contrast, the pattern of PEA response to water stress was different. In the field grown plants, its concentration could be higher in well-watered than in stressed plants. There

Table 8.4.b

Regression analysis of the response of PEA in the *A.iteaphylla* pot grown seedlings to water stress.

Source	df	Sum of Squares	Mean Square	F
Quadratic	1	24182	24182	13.13
Linear	1	2102	2102	1.14
Residual	9	16576	1842	
Total	11	42860		

Test for linear function:

$F = 1.14$ $\qquad F_{0.95}(1, 9) = 5.12$

thus the linear function ***is rejected***.

However, by scrutinizing the scatter of the data, it is suggested to test for the quadratic function which is as follows:

$F = 13.13$ $\qquad F_{0.95}(1, 9) = 5.12$

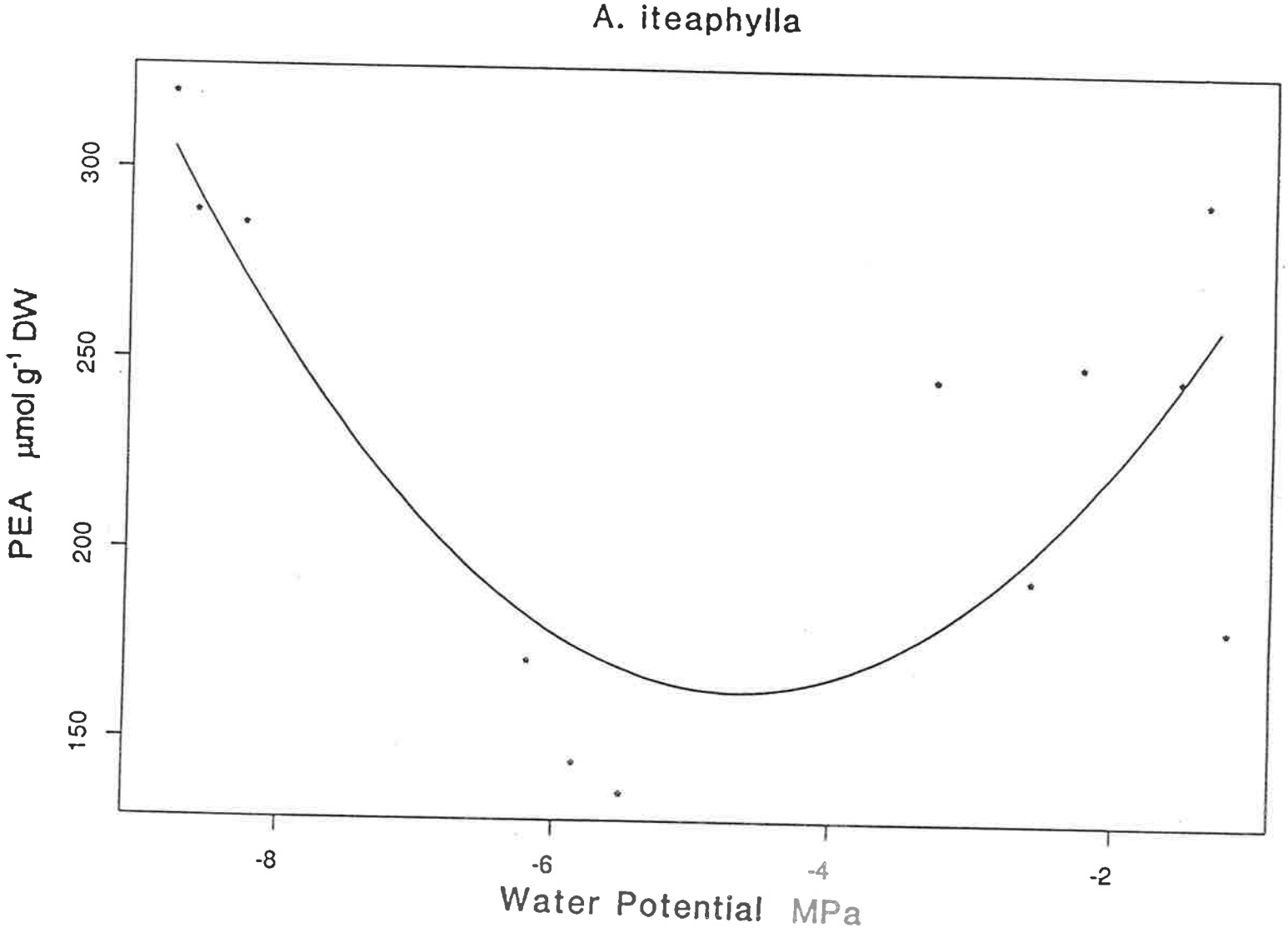
therefore ***retaining*** the hypothesis that the quadratic function fits the data. The relationship between water potential and PEA is described as:

$$P = 347.41 - 7.85\,\Psi + 0.0839\,\Psi^2$$

where P is proline, and Ψ is water potential.

Figure 8.6

The quadratic relationship between water potential (MPa) and PEA concentration (μmol g^{-1} DW) in *A.iteaphylla* seedlings during a drying cycle.


A. iteaphylla
PEA µmol g-1 DW
300
250
200
150
-8
-6
-4
-2
Water Potential MPa

was no relationship between the decline of water potential and the increase of PEA concentration, while proline concentration of field grown plants did show a linear relationship with water potential.

However, the lower concentration of PEA after salinity and cold stress suggests that the enzymes involved in the synthetic pathways of this compound may have been inhibited during these stresses. In cold stress, the water potential did not decrease to severe levels.

The inconclusive condition with the water treatment in the first experiment may have arisen from the fact that under well-watered conditions, PEA concentration was high; when water potential decreased to a moderate level the concentration appeared to decrease; under severe stress its concentration increased again. This phenomenon apparently was repeated in Section 8.4. Figure 8.6 shows that after water potential had reached -6 MPa and then moved to -8 MPa, there was about a twofold increase in the concentration of PEA. The significant fit of the quadratic expression supported the phenomenon. The amount of data was still limited and further work is needed to confirm these results.

However, whether or not the changes are ecologically, physiologically or biochemically important in terms of osmotically active solutes in osmoregulation is still far from clear. Further research is needed to determine whether the increase of PEA concentration is solely due to the loss of water during drying treatment, or to *de novo* synthesis. If it is a result of the latter, then its osmotic properties should be investigated in a more detailed sense.

CHAPTER 9

GENERAL DISCUSSION

It is clear from the available references that physiological studies, especially of water relations of Australian *Acacias* are limited. In the compilation of Ferrar and Vranjic (1988), a bibliography of studies (including university theses) in Plant Water Relations of Australian native plants, no detailed studies of *Acacia* were listed. At the same time a workshop on Australian acacias in developing countries (Turnbull, 1987) emphasized how big and important the role of the Australian acacias could be, yet little has been published in the last 3 years either.

Different *Acacia* species should display a wide range of characteristics in water relations due to their distribution in a wide variety of habitats throughout Australia. The study of inter- and intra specific variation then becomes important.

One of the consequences of this is the need for comparative studies between species in various aspects such as plant physiology. An ideal comparative study would require the use of different species of the same age, at the same time, location and environment, since a species may behave differently at different ages or times. However, this is almost impossible without sacrificing other principles. For example, the use of species from different ecological backgrounds in this comparative study and growing them in one set of environmental conditions (at Black Hill) may have unknown consequences. The "introduced" species may behave quite differently from when they grow in their original native sites.

Take *A. myrtifolia* as an example. It is a native of the Black Hill region, thus may have performed to its optimal growth capability in these studies. We might expect a "good" performance from *A.aneura* due to its wide distribution. But for the endemic species it is

difficult to predict whether or not they have performed to their optimal capacity. It is still not clear whether the endemic status of the three species is due to their inability to control their internal water balance.

A comparative study involving native stands of different species may be the most ideal. However, difficulties may arise in finding populations of different species with the same age within an accessible distance.

This study covered only a small portion within the large and complex physiological frame work required in developing Australian acacias for any purposes. The study concentrated on osmotic adjustment, and was designed to demonstrate the existence of osmotic adjustment, its variability among species, and which solutes may have been involved in the adjustment process.

In order to study osmotic adjustment, water potential components have to be measured. In this study some assumptions were made. When measuring water potential using shoots or leaf blades with a pressure chamber, the quantity measured is actually considered as xylem water potential (Ψ_{xylem}). As explained in Section 2.4.1.1, this study did not measure water potential simultaneously with pressure chamber and thermocouple psychrometer to test for the possible discrepancy between Ψ_{xylem} (xylem water potential) and Ψ_{leell} (leaf cell water potential). However, the information that was available on this question showed that the differences between these two measurements, due to non zero osmotic potential of xylem sap were usually very small (not more than 0.2 bar). Thus the values generated from pressure chamber alone were thought to be reliable.

All measurements of Ψ_{π} with the P-V curve were on samples which had not been pre-rehydrated. Consequently, incomplete P-V curves were produced. Hence, several items of information could not be generated, such as $\Psi_{\pi\,(100)}$ (osmotic potential at full turgor), and RWC, since the Ψ_I (initial water potential) was less than zero. However, since the main

interest of the study was in $\Psi_{\pi I}$ (initial osmotic potential), the conclusions were not invalidated by this method.

In concentrating on osmotic adjustment, the glasshouse study data were approached by two methods of analysis i.e. anova followed by *lsd* test at the end of the drying cycle, and a non-linear curve-fitting to data obtained during the course of the drying cycle. The *lsd* test can only show that differences occur between species and treatments in their water potentials and osmotic potentials. The analysis did not proceed for turgor pressure since the results of this pot experiment were considered as merely suggestive for further field experiments.

Table 9.1 summarizes the final (most negative) values of Ψ and Ψ_{π} reached by ***potted plants*** when soil water content had fallen to between 2 to 4 % (Table 3.4), and proline concentrations of stressed plants of the 5 ***field grown*** *Acacia* species (Table 5.4.b) corresponding to the rainfall in their area of distribution. It might be expected that the response of these *Acacia* species to water stress would be related to the environment in which they occur naturally. The Ψ values of potted plants did seem to correspond to some extent with the rainfall of their areas of distribution. The South Australian endemic species occupied the top of the list separated by the *lsd* test at 5% level of confidence (Table 3.6.a), i.e *A.rivalis* (-6.44 MPa) occurs naturally in an area with 150-300 mm annual rainfall (Table 3.1), *A.iteaphylla* (-5.23 MPa, 200-500 mm y^{-1}), *A.gillii* (-4.47 MPa, 500 mm y^{-1}) and *A.leiophylla* (-4.54 MPa, 500-800 mm y^{-1}) respectively. The "pan australia" species (*A.aneura*) was less negative (-3.16 MPa, 150-250 mm y^{-1}) but still below *A.saligna* (-2.61 MPa, 300-700 mm y^{-1}), a "saline species" which is endemic to Western Australia. However, the other species do not fit the pattern, so that for all 10 species there is no clear relationship between final water potential level and climate of origin.

In the case of osmotic potential, three of the endemic species occupied the top level of the *lsd* list (Table 3.6.b), i.e. *A.rivalis* (-6.53 MPa), *A.anceps* (-5.43 MPa), and

A.iteaphylla (-5.42 MPa). However, once again the species with wide distribution did not show a strong relationship with their area of distribution.

Table 9.1

The relationship between final Ψ (-MPa), Ψπ (-MPa) of stressed potted glasshouse grown seedlings of 10 *Acacia* species, and proline concentration (umol g^{-1} DW) of 5 stressed field grown species with the rainfall (mm y^{-1}) of their area of natural distribution.

Species	Ψ	Ψπ	Proline	Rainfall
A. anceps	3.62	5.43	2.78	200-500
A.aneura	3.16	5.09	26.60	150-250
A.cyclops	3.87	4.34	-	200-500
A.gillii	4.47	4.81	7.97	500
A.iteaphylla	5.23	5.42	14.57	200-500
A.leiophylla	4.54	4.57	-	500-800
A.longifolia	3.40	4.27	-	500-1000
A.myrtifolia	4.36	4.65	18.35	500-1200
A.rivalis	6.44	6.38	-	150-300
A.saligna	2.61	3.63	-	300-700

To express the evidence of osmotic adjustment, the non linear curve fitting was applied. As explained theoretically in Figure 3.1and shown experimentally in Figure 3.4 and 4.4, the osmotic potential/water potential relationship was nonlinear, and the curve-fitting analysis clearly separated the species which did have osmotic adjustment from those which did not. These results did not show any strong relationship between the osmotic adjustment performed by a species and the area of distribution of that species in terms of rainfall.

However, glasshouse figures are not always repeatable in field grown plants. Therefore it was necessary to do further tests in the field, although due to limitations of time and space the number of species had to be reduced from ten to five. The study of the field grown *Acacia* species recorded quite clear seasonal fluctuations in the seedlings' water relations. The well-watered treatment might represent the behaviour during a wet spring and

summer. Statistical analysis of the results suggested that there were variations in Ψ and Ψ_π over time but while at any time, a watered plant differed from a stressed plant of the same species, these differences were the same for each species. Also, at any one time, one species differed from another given the same treatment, however these differences were the same whether the plants were watered or stressed. For Ψ_p (turgor pressure) there were differences between watered and stressed plants, and these differences did differ among species (Tables 4.3 to 4.5).

Curvilinear analysis of the field results showed evidence that the 5 selected species developed stronger osmotic adjustment than the same species in the pot experiment (Table 4.6). The species which showed no significant adjustment in the pot experiment (*A.iteaphylla*), in fact produced a good adjustment of 1.07 MPa in the field, while three of the other four adjuster species showed greater osmotic adjustment. *A.anceps* retained the same magnitude of adjustment as in the glasshouse, *A.aneura* gained 0.23 MPa, *A.gillii*, 0.33 MPa and *A.myrtifolia*, 0.85 MPa. Hence, it is suggested that the study of osmotic adjustment by pot experiment needs to be extended in field grown plants since the results from the latter would be more realistic in representing the behaviour of the species. The glasshouse trial is still important as a preliminary approach i.e. suggestive output for further field experiment.

Some explanations have been produced as to why in many cases the glasshouse grown plants do behave differently from field grown plants (for example in osmotic adjustment and proline concentrations). One reason is that the glass-house plants are grown in a limited soil volume. When the stress is applied they can rely only on a small reserve, while the field plants would benefit from larger resources in a larger volume of soil. Turner et al (1978) noted that the differences in water relations between these two kinds of plants may be the result of the differences in fluctuations of environmental factors outside and inside.

Field plants would experience higher light intensity, a larger gradient of diurnal water potential, and a more gradual decrease of plant water deficit.

To obtain the values of osmotic potential in the field experiment, two different pieces of equipment were used. The calibration of the psychrometer against the pressure chamber (Section 4.4.2) produced values of osmotic potential which were comparable to other previous reports. The estimation of maximum turgor at 3 MPa after this calibration still lies within the range reported for some drought-tolerant species. The pressure chamber was used to calibrate the thermocouple psychrometer in order to avoid "negative turgor" values by taking account of the dilution of symplastic by apoplastic water when tissue is frozen for the psychrometer readings.

Negative turgor remains a controversial issue - thus the opportunity for further research is still wide open. Oertli (1986) provides some basic arguments on this issue while retaining the concept as existing in nature. There is a common impression that negative turgor pressure usually does not appear if the values are obtained by pressure chamber. However, in Figure 3.3, where the values were generated without previous rehydration several points indicated apparent negative turgor pressure i.e. those above 1:1 line. Further investigations should re-examine whether or not the phenomenon is a result of error or is a true negative turgor pressure.

If the process of osmotic adjustment requires osmotically active solutes (Hsiao, 1973; Turner and Jones, 1980; Morgan, 1984; Myers and Neales, 1986), and solute accumulation is a matter of metabolic energy consumption, then species that alter their osmotic potential as little as possible whilst retaining their positive turgor values, should have a metabolic advantage. This depends on the type of solutes involved. Solutes which originate from retransport will require less metabolic cost than ones which must be synthesized (Wyn-Jones and Gorham, 1983; Boyer, 1985; Marschner, 1986).

The field data also show how big the differences are between Ψ of well-watered and stressed plants, which give the impression of how vulnerable the young plants are while they rely on rainfall to wet surface soil. However, besides osmotic adjustment, other mechanisms also may have played parts in their survival. Available reports suggested that several factors such as changes in bulk modulus of elasticity, stomatal regulation and smaller cell formation may have taken place as well.

The study of solutes in these *Acacia* species provided some exceptional results such as the concentrations of K^+ and Na^+ (Table 5.1.a and 5.1.b). Even though K^+ and Na^+ did not fluctuate with changing of water stress conditions, i.e. their concentrations in the phyllodes were not controlled by Ψ fluctuations, their roles should not be simply considered as unimportant osmotically (cf. Cutler and Rains, 1978; Ford, 1984 who considered that unchanging K^+ and Na^+ concentration in water stressed samples indicated a less important osmotic role for these ions). This has to be scrutinized in relation to symplastic water volumes. As presented in Table 5.3, estimation of the contribution of these two inorganic ions to osmotic potential (with several assumptions), suggested that their presence was not merely as "less important ions" but was actually very important osmotically. Their total ranges of contribution to the cellular osmotic potential lay between 29% (*A.gillii*) and 52% (*A.aneura*).

Increasing K^+ concentrations in the nutrient solution supplied to potted *A.iteaphylla* seedlings (Chapter 6), did not always affect the degree of osmotic adjustment, but the calculation of contributions of this ion to the osmotic potential of the plant, also showed its major osmotic role.

The fact that the two inorganic ions were generally high in the five species studied, and did not change in well-watered and stressed plants, supports the suggestion of Hsiao et al (1976) that high solute content is typical of arid zone plants. Therefore another suggestion

emerges here that potassium and sodium could be the major solutes for these drought adapted *Acacia*.

For proline behaviour, there are five conclusions that can be drawn from the study. First was the evidence of a marked increase of its concentration between well-watered and stressed field grown plants. The increase differed among species and did not occur at all in *A.iteaphylla*. However, clearly these increases did not correlate with a role in the overall osmotic properties of the cells. Table 5.7 shows that the contribution in all species was less than 0.3%.

The second conclusion concerned potted plants. In some cases, potted plants may have higher accumulations than field-grown ones. Take *A.iteaphylla* as an example. Well-watered field grown *A. iteaphylla* accumulated a similar concentration of proline to the stressed plants, while in glasshouse grown plants large differences developed. As shown in Table 8.3.a, proline concentrations detected in individual potted plants ranged between 35 (well-watered) to 294 (stressed) μmol g^{-1} DW. Unfortunately, no osmotic potentials were measured in this experiment, so the per cent contribution of proline could not be calculated. However, if the values of Ψ_π, fresh weight and dry weight had been the same as for the field grown *A.iteaphylla* plant shown in Table 5.7, the contribution of proline might have been up to about 27% of total Ψ_π!. Are there any factors present, or absent in the pot environment, which promote higher proline synthesis?. The opportunity for further research remains open to find out which factor(s) are actually involved.

Thirdly, proline synthesis was in fact increased in potted plants with better nutrient supplies (Chapter 7). In the case of potted *A.iteaphylla,* proline synthesis was shown to have a positive relationship with increased nutrient availability. So, if proline has a specific role in the survival of a plant, those with better nutrition would benefit more in water stress conditions. However, application of Hoagland's nutrient to the field grown plants of 3

species did not significantly increase their proline concentrations. Insufficient volume of nutrient applied was one possibility. But this still needs an answer in the future.

As shown in Chapter 5, the lowest proline concentration per unit dry weight of stressed plants was in *A. anceps* which suggests that this species did not use much proline for any purposes; or there may be other compounds that replace it. However, like the other four, *A.anceps* did not accumulate betaine under water stress. Available literature shows that proline did not accumulate in the pasture legume siratro (*Macroptilium atropurpureum*), either well-watered or stressed, but *d-pinitol* was accumulated in stressed samples (Ford and Wilson, 1981). Would the application of nutrient change the habit of proline synthesis of the field grown *A. anceps* ? Or what other unknown factors may have been involved as well ?.

Fourthly, the role of proline in plant survival is a topic of controversy. In the early years since it was discovered to accumulate within stressed plants, proline received much applause as an osmotic constituent. Further research suggested its role as a protector for enzymatic activities, a source of nitrogen, protein solvent, etc. Its position as an osmotic agent would be clear if it could be determined exactly whether proline is located in cytoplasm or vacuoles. If it is in the cytoplasm, then its role as a compatible solute would be more acceptable. At the moment at least two main opinions of its role are held: (a), as suggested by Weimberg et al (1982) proline is involved in the charge balance of the potassium build up in salt stressed plants; but (b), Nageswara et al (1981*a*) preferred to believe that the increase of proline in salt stress is due to the influence of potassium on the activation of the enzyme P5C which is involved in the pathway of proline synthesis. While a satisfactory resolution has not been produced, a suggestion of a "new role" for proline has been made by Klein and Itai (1989). They believe the possibility of proline being involved in stomatal regulation after the plant recovers from salt stress. Here is another area which needs clarification. But by

looking at the concentrations of K^+ and Na^+ in stressed plants it seems that the role of proline in the *field grown plants* is more likely to be involved in balancing charges.

Fifthly, at this stage due to the limited number of harvests (one season, 4 harvests), it is not possible to correlate the proline concentration with species drought resistance. However, as shown in Table 9.1, there were at least indications that the proline concentrations (P, $\mu mol\ g^{-1}$ DW) of stressed plants during 4 harvests taken from Table 5.4.b did not follow the pattern of their area of distribution as in Table 3.1, based on rainfall patterns of distribution area.

PEA is another interesting topic. Poor linear relationship was found between its concentrations and water potential fluctuations. There was some indication that the scatter of this relationship tended to follow a quadratic model. This pattern was repeated twice in the glass-house plants. The indication of PEA being synthesized at severe water stress needs to be supported by further experiments. The function of PEA in *A. iteaphylla* is not yet clear. Its discovery was a surprise. Surveys of other plant groups may lead to the discovery of other such interesting compounds.

This study was finalized leaving some still unsolved questions in water relations of *Acacia*. Topics include negative turgor, the balance of water between symplastic and apoplastic, measuring the dilution factor when the thermocouple psychrometer is used to measure osmotic potential, the role of proline, the role of K^+ and Na^+, PEA and other compounds; what solutes caused the observed osmotic adjustment, since neither K^+, Na^+, proline nor PEA increased enough to explain the changes in osmotic potential; nutrient and osmotic adjustment in woody perennials, and the relation between distribution patterns of species and their mechanisms of drought resistance.

As has been mentioned previously, the samples in this experiment were selected from a small range of tube-grown seedlings available in the Black Hill nursery. Further study

seems needed with a greater variation of species including ones from truly wetter areas (North Eastern Australia). Moreover, the variation within a species should be looked into. For example, *A. aneura* is distributed over a large range of areas from north to south throughout the mainland - from the tropics through arid to temperate zones. This species might be expected to exhibit high diversity in osmotic adjustment.

The genetic variability of osmotic adjustment has been investigated by Morgan (1977; 1983) in wheat. He pioneered the study of detailed differences in productivity of wheat cultivars with different ability to osmoregulate. Such a comparative study as Morgan's needs a careful approach since productivity itself is a result of so many processes which interact during the growth period. However, a recent study by Basnayake et al (1993) in 21 inbred sorghum lines was able to show the benefit of osmotic adjustment to dessication tolerance. There was a linear relationship between the increase of lethal RWC, lethal leaf water potential and maximum osmotic adjustment. Lines with high osmotic adjustment would live longer compared to lines with low adjustment.

If osmotic adjustment is genetically controlled, a wide range of Australian *Acacia* may display different ability in this respect due to their wide distribution within a large variety of habitats throughout Australia. Biodiversity richness in the response of *Acacia* species through osmotic adjustment to drought may be seen as genetic potential. The biodiversity of Australian *Acacia* varies from high to low economic importance. From the perspective of Plant Genetic Resources (germplasm), they may possibly act as a source of drought resistance inheritance characters for developing new strains. Knowledge of plant response to water (and salinity) stress, including osmotic adjustment, is the kind of input needed for a multidisciplinary approach to plant improvement (Pitman, 1986). In fact high variation within *Acacia* species would be very relevant to such a programme, in assessing the value of the genus in fulfilling the need for firewood, building materials, fodder, wayside

trees, nitrogen fixation, reclamation biology etc. The enormous effects of drought (and salinity) on agricultural productivity mean we need to understand the plants' response to these conditions in order to expedite programmes aimed at breeding for drought and salt tolerance (Rains and Valentine, 1980; Flower and Yeo, 1986). This may give some answers to the difficult question thrown up by Munns (1988): "Why measure osmotic adjustment?".

Appendix 1.

Steps in ninhydrin solution preparation:

a. For each sample, put 125 mg of ninhydrin into a mixture of 3 ml glacial acetic acid, and 2 ml of 6 M orthophosphoric acid

b. dissolve it by heating at 70^{0} C.

***Appendix* 2.**

Steps in the preparation of a standard proline solution:

a. Five mg of L-proline (Sigma, USA) was diluted with 25 ml methanol/water 30:70 and shaken well to dissolve.Hence, each 5 µl of the solution would contain 1 µg proline.

b. Eight boiling tubes were prepared for the proline concentration series: 0, 2.5, 5, 10, 20, 40, 60 and 80 µg respectively by adding the correct volume of standard solution.

c. 10 ml of distilled water was then added to each tube, followed by 5 ml of ninhydrin solution and 5 ml of glacial acetic acid, etc (see text for following steps.)

Appendix 3.

The molecular structure is presented in Fig. 1 and Fig. 2 shows the molecular packing.

Related literature. This study represents the first in a series of acridines containing 9-alkyl or aryl substituents which we are studying. These acridines are being linked to oligonucleotides in hopes of synthesizing new anti-AIDS or anti-cancer agents. The crystal studies provide important information which will form the basis for molecular modeling studies. Of special interest is the fact that the absolute values of the torsion angles in the two molecules are 98·32, 63·20° and 97·22, 62·11° (for C8*a*—C9—C11—C12 and C9—C11—C12—O12) and are virtually identical, showing the similarity in the conformation of the two molecules per asymmetric unit. Few 9-alkyl or 9-aryl acridines have been studied crystallographically. Pett, Rossi, Glusker, Stezowski & Bogucka-Ledochowska (1982) have reported the structure of 9-methyl-1-nitroacridine. Berman & Neidle (1979) have reviewed the structural studies of acridine intercalators.

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Structure of Phenethylamine Hydrochloride

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Abstract. Phenethylammonium chloride, $C_8H_{12}N^+.Cl^-$, $M_r = 157·6$, orthorhombic, $P2_12_12_1$, $a = 4·603$ (1), $b = 5·906$ (1), $c = 32·360$ (2) Å, $V = 880$ (1) Å^3, $D_x = 1·190$ Mg m^{-3} for $Z = 4$, Mo $K\bar{\alpha}$ radiation, $\lambda = 0·7107$ Å, $\mu = 0·318$ mm^{-1}, $F(000) = 336$, $T = 293$ (2) K, $R = 0·036$ for 724 observed reflections. The crystal structure determination of the title compound shows that the ethylamine side chain is fully extended and the C(6)—C(1)—C(7)—C(8) torsion angle is $-70°$. Each of the three ammonium H atoms forms significant intermolecular contacts with symmetry-related chloride anions such that Cl···H are 2·35, 2·27 and 2·20 Å.

Experimental. Phenethylamine was isolated from *Acacia iteaphylla*, a tall spreading shrub endemic to South Australia. Leaf tissue was extracted with methanol/water (70:30 v/v) and after removal of the methanol component (reduced pressure) the extract was subjected to ion-exchange chromatography on a column containing DOWEX 50W (H^+ form) resin. After washing the column with water phenethylamine was eluted with 4*M* HCl. The product was identified from both its ^{1}H [90 MHz, D_2O, pH 1·5; δ 7·56 (5H), 3·24 (2H) and 3·06 (2H), ref. *tert*-BuOH δ 1·245] and ^{13}C (22·5 MHz, D_2O, pH 1·5, ^{1}H decoupled, δ 139·45, 131·86, 131·70, 130·18, 43·39 and 35·54, ref. *tert*-BuOH δ 32·45) NMR spectra. Suitable crystals for X-ray study obtained from the slow evaporation of a methanol/diethyl ether solution of the compound; colourless needles, m.p. 493–494 K. Enraf–Nonius CAD-4F diffractometer controlled by a PDP8/A computer, graphite-monochromated Mo $K\bar{\alpha}$ radia-

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Table 1. *Fractional atomic coordinates* ($\times 10^4$) *and* B_{eq} *values* (Å^2)

$B_{eq} = 8\pi^2/3(U_{11} + U_{22} + U_{33})$

	x	y	z	B_{eq}
Cl(1)	3722 (3)	5692 (2)	2112 (1)	3·77
C(1)	−1042 (10)	−70 (7)	1048 (1)	4·04
C(2)	−1424 (13)	1555 (9)	747 (2)	5·05
C(3)	−3212 (14)	1169 (11)	407 (2)	6·01
C(4)	−4625 (13)	−851 (14)	369 (2)	6·79
C(5)	−4297 (15)	−2486 (12)	662 (2)	6·12
C(6)	−2471 (11)	−2107 (9)	1001 (2)	5·17
C(7)	897 (11)	369 (10)	1414 (2)	4·44
C(8)	−853 (9)	602 (9)	1802 (1)	3·62
N(1)	1062 (8)	749 (7)	2171 (1)	3·50

Table 2. *Selected interatomic distances* (Å) *and bond angles* (°)

C(1)—C(2)	1·380 (6)	C(1)—C(6)	1·379 (6)
C(1)—C(7)	1·505 (6)	C(2)—C(3)	1·391 (8)
C(3)—C(4)	1·364 (9)	C(4)—C(5)	1·364 (9)
C(5)—C(6)	1·399 (8)	C(7)—C(8)	1·499 (6)
C(8)—N(1)	1·486 (5)		

C(2)—C(1)—C(6)	117·9 (5)	C(2)—C(1)—C(7)	120·8 (5)
C(6)—C(1)—C(7)	121·3 (5)	C(1)—C(7)—C(8)	110·9 (4)
C(7)—C(8)—N(1)	111·1 (3)		

tion; $\omega/2\theta$ scan technique. Cell parameters on crystal 0·15 × 0·20 × 0·60 mm from a least-squares procedure on 25 reflections ($10 \le \theta \le 13°$). No absorption correction applied (Sheldrick, 1976). Total of 1047 reflections ($1 \le \theta \le 25·0°$) measured in the range $0 \le h \le 5$, $0 \le k \le 7$, $0 \le l \le 38$; some Friedel pairs also included. No significant variation in the net intensities of two reference reflections (2,1,13 and 129) measured every 7200 s. 960 unique reflections (R_{amal} 0·019)* and 724 satisfied $I \ge 2·5\sigma(I)$. Structure solved by direct methods (Sheldrick, 1986), full-matrix least-squares refinement of 139 parameters based on F (Sheldrick, 1976). Anisotropic thermal parameters for non-H atoms and H atoms refined with isotropic thermal parameters. At convergence R = 0·036 (preferred chirality), wR = 0·035, $w = 2·71/[\sigma^2(F) + 0·0002F^2]$, S = 2·5, $(\Delta/\sigma)_{max} \le 0·001$, $(\Delta\rho)_{max}$ = 0·18, $(\Delta\rho)_{min}$ = −0·18 e Å^{-3}; no extinction correction. Scattering factors for all atoms given in *SHELX*76 (Sheldrick, 1976). All calculations on SUN4/280 computer system. Atomic parameters given in Table 1, selected bond distances and angles in Table 2,† the numbering scheme used is shown in Fig. 1, and a unit-cell diagram is shown in Fig. 2.

* $R_{amal} = (\Sigma\{N\Sigma[W(F_{mean} - F)^2]\}/\Sigma[(N-1)\Sigma(WF^2)])^{1/2}$; the inner summations are over N equivalent reflections and are averaged to give F_{mean}, the outer summations are over all unique reflections and the weight W is taken as $[\sigma(F)]^{-2}$.

† Lists of structure factors, H-atom parameters, thermal parameters and all bond distances and angles have been deposited with the British Library Document Supply Centre as Supplementary Publication No. SUP 52827 (7 pp.). Copies may be obtained through The Technical Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

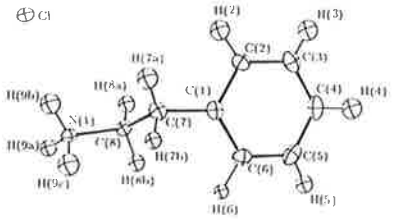

Fig. 1. Molecular structure and numbering scheme for phenethylamine hydrochloride drawn at 25% probability levels (Johnson, 1971).

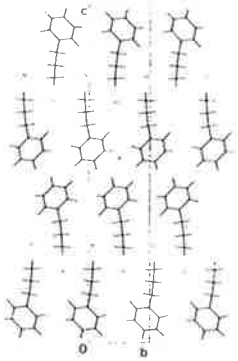

Fig. 2. Unit-cell contents for phenethylamine hydrochloride viewed down the *a* axis (Motherwell, 1976).

Related literature. This report represents a redetermination of the title compound by contemporary methods (Tsoucaris, 1961). Phenethylamine occurs widely in algae, fungi and higher plants (Smith, 1977) and relatively high concentrations of the compound have been reported to occur in Acacias (White, 1944). These high concentrations are associated with a distinct morphological group of *Acacia* (White, 1944) and in *Acacia iteaphylla* phenethylamine concentrations of up to 7% have been observed (Naiola, Jones & Paleg, 1989).

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Appendix 4

A Analysis of Repeated Measures without time changing covariates.

The two methods for the analysis of repeated measures, without time changing covariates, are Split-plot designs and a multivariate analysis of variance approach.

A.1 Split- plot approach to repeated measures

The split-plot approach can best be understood by considering a table in which the observations at each time on a plant form the rows and all the observations from one time form the columns. Considering one column/time in isolation we can apply an ordinary anova to these observations and obtain an estimate of variance and treatment means etc. Doing this for each time, we could obtain four sets of variance and means. If there was no connection between the original observations we could pool these estimates. Unfortunately, there is a connection between these estimates - the plants upon which observations are made over time. So to attempt allow for this connection a split-plot model can be used.

In this approach the times are considered a factor and each plant has also associated with it an error term, denoting the kth plant in treatment group i by Y_{ik}, e_{ik}. Also, with each observation y_{ikt} (the kth observation in treatment group i at time t) there is associated the usual error term e_{ikt}. It is assumed that the e_{ik} and e_{ikt} are mutually independent and $e_{ik} \sim N(0, \sigma_P^2)$ and $e_{ikt} \sim N(0, \sigma_T^2)$. Our model is that for the kth observation at time t in treatment group j,

$$y_{ikt} = \eta_{ikt} + e_{ikt} + e_{ik}$$

where η_{ikt} is the expected value of y_{ikt}. Thus, the variance at each time is $\sigma_P^2 + \sigma_T^2$ and the covariance between observations is σ_P^2.

A transformation of the data then allows the problem to be reduced to two ordinary analyses of variances, where σ_T^2 and the treatment effects are estimated in one analysis and $p * \sigma_P^2 + \sigma_T^2$ (p is the number of time points), the time effect and time - treatment interactions are estimated in the other. (cf. [Mor76a]).

A.2 Multivariate approach to repeated measures

The split-plot analysis can fail. It assumes that observations are equally correlated, no matter how long the time between them, that observations 10 days apart are as correlated as ones 2 days apart. While this is a reasonable approximation in many cases, it sometimes fails.

When a split-plot approach fails, a multivariate approach can be used. The multivariate model is that

$$y_{ijtk} = \eta_{ijt} + e_{ijtk}$$

where η_{ijt} is the expected value of y_{ijtk} and e_{ijtk} is the error component, $i = 1, ...g, j = 1, ..q, k = 1, ...f, t = 1, ...p$ It is assumed that if

$$\vec{e}_{ijk}$$

is the vector whose lth component is e_{ijlk}, then

$$\vec{e}_{ijk} \sim \mathrm{N}(\vec{0}, \Sigma)$$

where Σ is the $p \times p$ symmetric matrix - the variance matrix. It is also assumed that for differing k, k',

$$\mathrm{Cov}(e_{ijtk}, e_{ijtk'}) = 0$$

- that is, observations on different plants are independent. Let $\mathbf{Y}$ be the $qgf \times p$ matrix of observations, $\mathbf{X}$ the $qgf \times m$ design matrix and $\mathbf{B}$ the $m \times p$ matrix of model parameters, then our model is

$$\mathbf{Y} = \mathbf{XB} + \mathbf{E}$$

where $\mathbf{E}$ is a random matrix distributed as

$$\mathrm{N}(\vec{0}_{qgf \times p}, \mathrm{I}_{qgf} \otimes \Sigma)$$

, $\otimes$ is the kronecker product, I_x is the identity matrix of order x and $\vec{0}_{qgf \times p}$ is a $qgf \times p$ matrix of zeroes. We now rearrange all matrices by stacking their rows one under the other - thus

$$\begin{bmatrix} 1 & 2 \\ 3 & 4 \end{bmatrix}$$

becomes

$$\begin{bmatrix} 1 \\ 2 \\ 3 \\ 4 \end{bmatrix}$$

Thus the data matrix becomes a $qgfp \times 1$ vector y and our model reduces to a weighted multiple linear regression

$$y = (X \otimes I_p)\beta + e$$

where β is obtained from B by stacking the rows of B one under the other, and e is random $qgfp \times 1$ vector , distributed as

$$N(\vec{0}_{qgfp\times 1}, I_{qgf} \otimes \Sigma)$$

Note that this model would be an ordinary anova if e was distributed as

$$N(\vec{0}_{qgfp\times 1}, \sigma^2 I_{qgfp})$$

The model parameters, usually a grand mean plus treatment effects, and the variance matrix can be estimated from this model, and due of the matrices involved the process simplifies to regressing each of the columns of the data matrix on X separately to obtain the mean and treatment effects estimates (the columns of the matrix of parameters) and using the residuals from these regressions to estimate the variance matrix. For more detail see [Mor76b] and [Gra81a].

B Analysis of repeated measures with time changing covariates

B.1 Split- plot analysis

This only differs from the previous split-plot analysis in being an analysis of covariance. Unfortunately, the estimates of the gradient occur in both linear models. The usual method of testing hypotheses is to perform the tests with sums of squares with the greatest degrees of freedom.

B.2 Multivariate analysis of repeated measures with time changing covariates

Here our model is as in p. 61. This can be written as

$$Y = XB + \left[A_1\gamma \mid A_2\gamma \mid A_3\gamma \mid A_4\gamma \right] + E$$

where Y,X,E and B are as before, the columns of the A_i specify the water effect and γ is the vector conisting of the $\delta, \epsilon_i, \kappa_j$ and λ_{ij}. (In the case where the gradient varies over time the model can be written as

$$Y = XB + \left[A_1\gamma_1 \mid A_2\gamma_2 \mid A_3\gamma_3 \mid A_4\gamma_4 \right] + E$$

for details in this case see [Ver88] and [VV88] .) Again, stacking the rows of the matrices under each other our model becomes

$$y = (X \otimes I_n)\beta + U \begin{bmatrix} A_1 \\ A_2 \\ A_3 \\ A_4 \end{bmatrix} \gamma + e$$

where U is a permutation matrix (see [Gra81b]). If

$$e \sim N(\vec{0}, \sigma^2 I_{qqfp})$$

then this would be simply an analysis of covariance. However, the assumed covariance matrix complicates the estimation procedure. For sodium, proline, potassium and PEA data estimates were calculated using a generalised least squares procedure. The generalised likelihood ratio statistic was used to test the hypotheses. If the estimate of Σ from the outer hypothesis was S and the estimate from the null hypothesis was S_0 then for the proline, sodium and potassium data, the log of the generalised likelihood ratio was

$$40 * \log(\frac{\det(S_0)}{\det(S)})$$

and for the PEA data it was

$$7 * \log(\frac{\det(S_0)}{\det(S)})$$

These statistics are asymptotically chi-squared with degrees of freedom depending on the hypothesis under test. For further details see [Mor76c].

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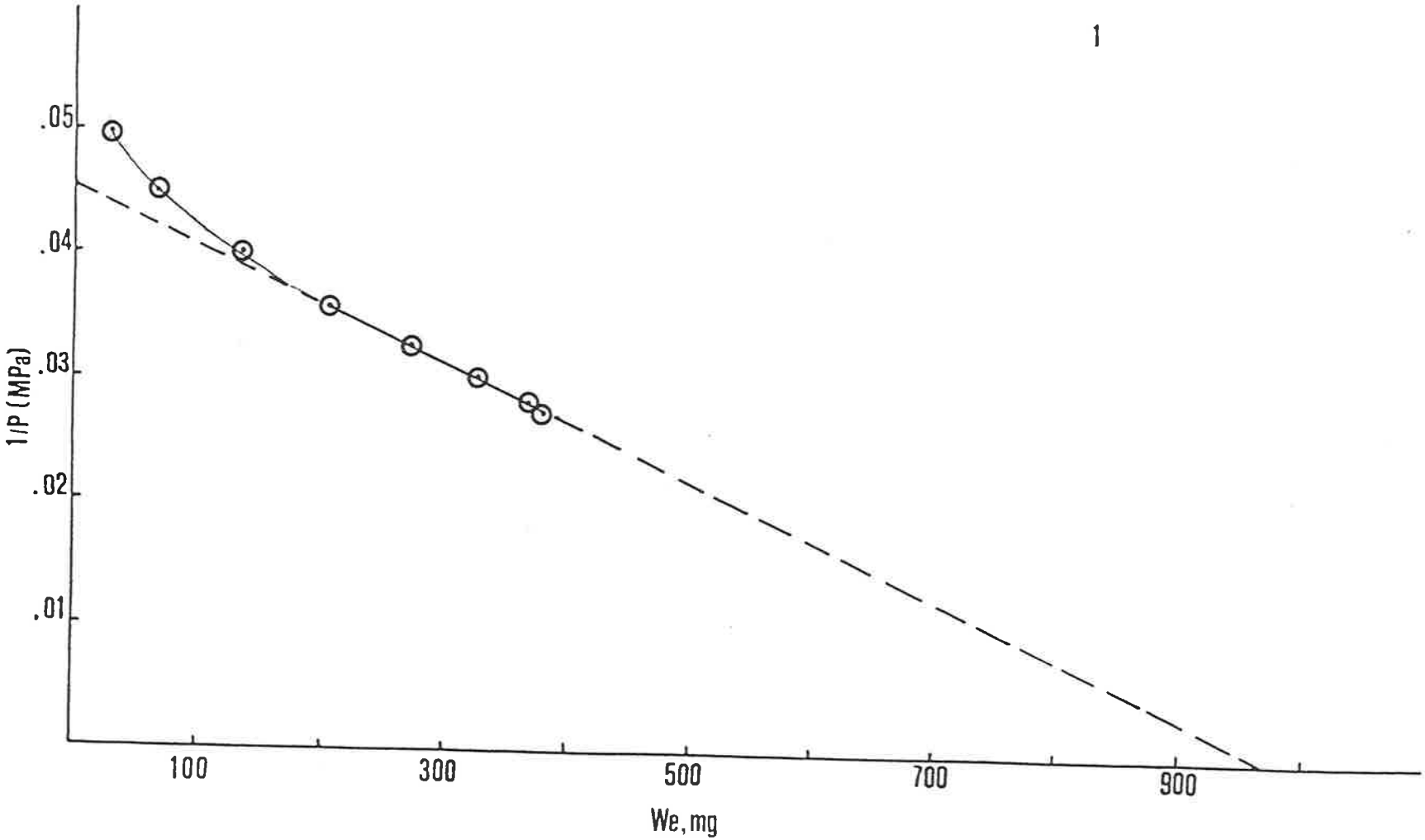
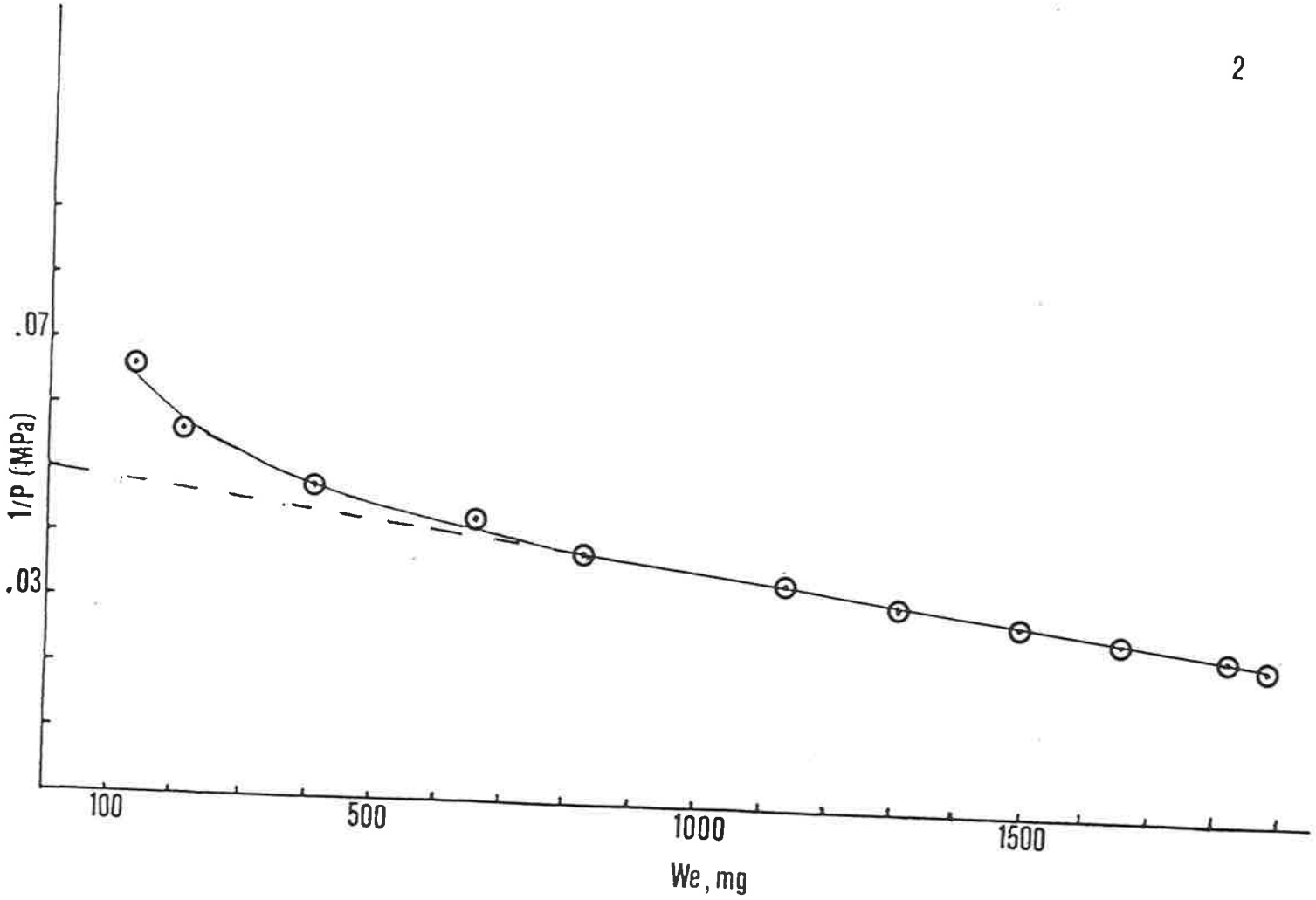
Appendix 5.a

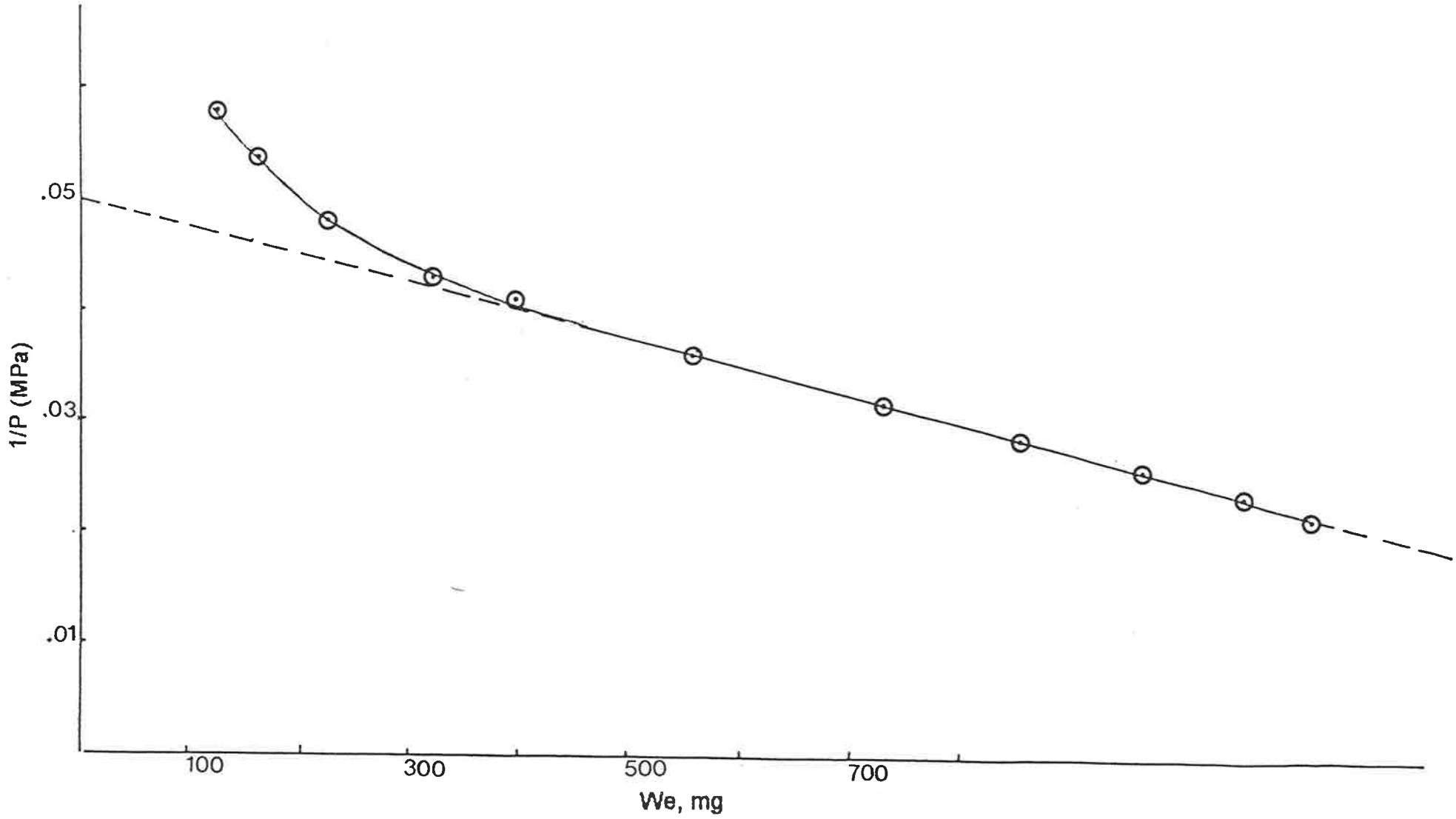
The 10 P-V curves selected from the glass house *Acacia* experiment in Chapter 3, to search for the possible error in determining osmotic potential values from lines fitted by eye. These species and treatments (WW, well-watered, STR, stressed) are as follows:

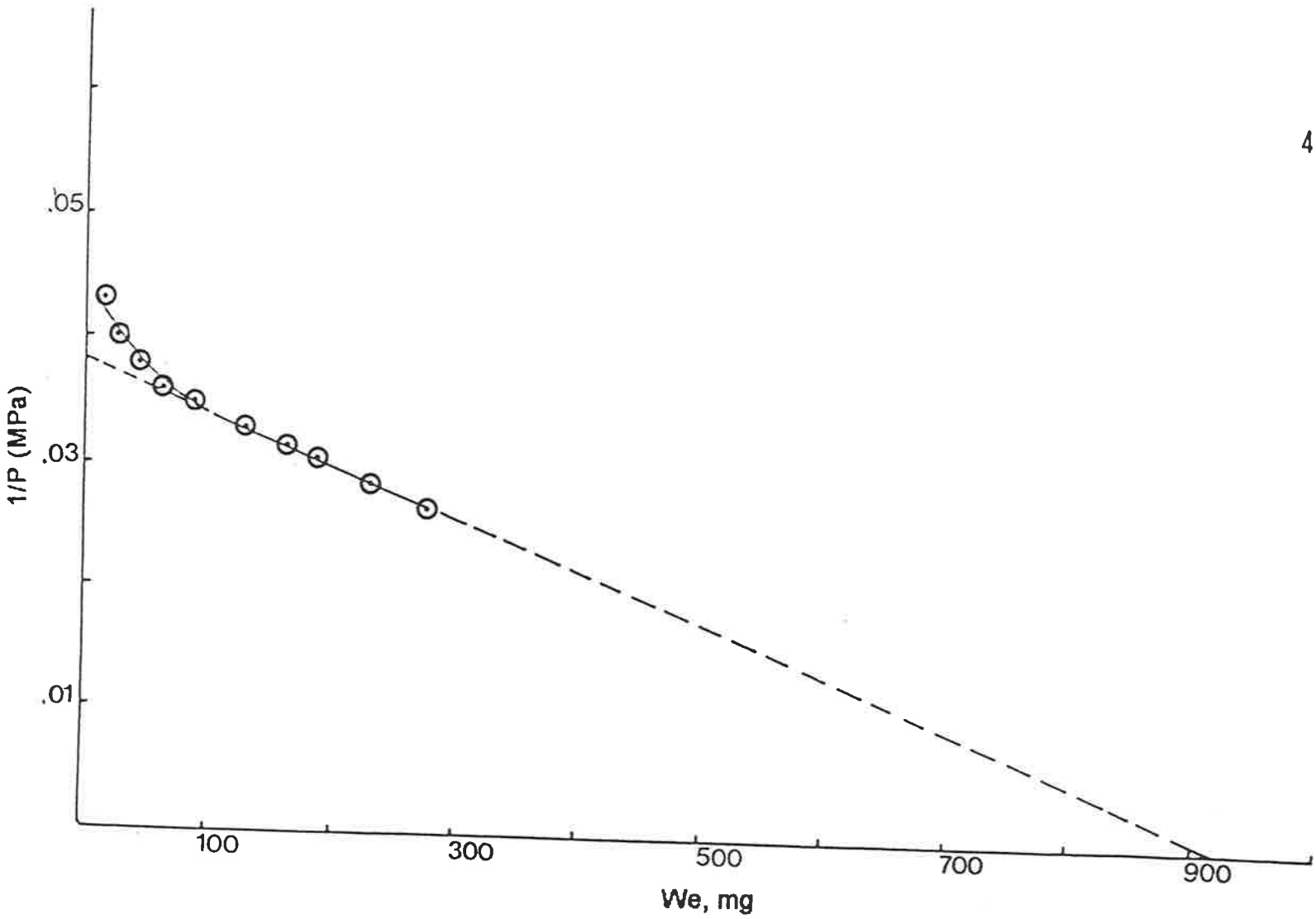
Graph no.	*Species*	*Treatment*	*Date (1986)*
1	*A.aneura*	STR	24-10
2	*A.leiophylla*	STR	24-11
3	*A.myrtifolia*	WW	14-11
4	*A.myrtifolia*	STR	14-11
5	*A.rivalis*	WW	27-11
6	*A.rivalis*	STR	27-11
7	*A.cyclops*	STR	11-10
8	*A.longifolia*	STR	18-10
9	*A.iteaphylla*	STR	12-11
10	*A.gillii*	STR	19-11

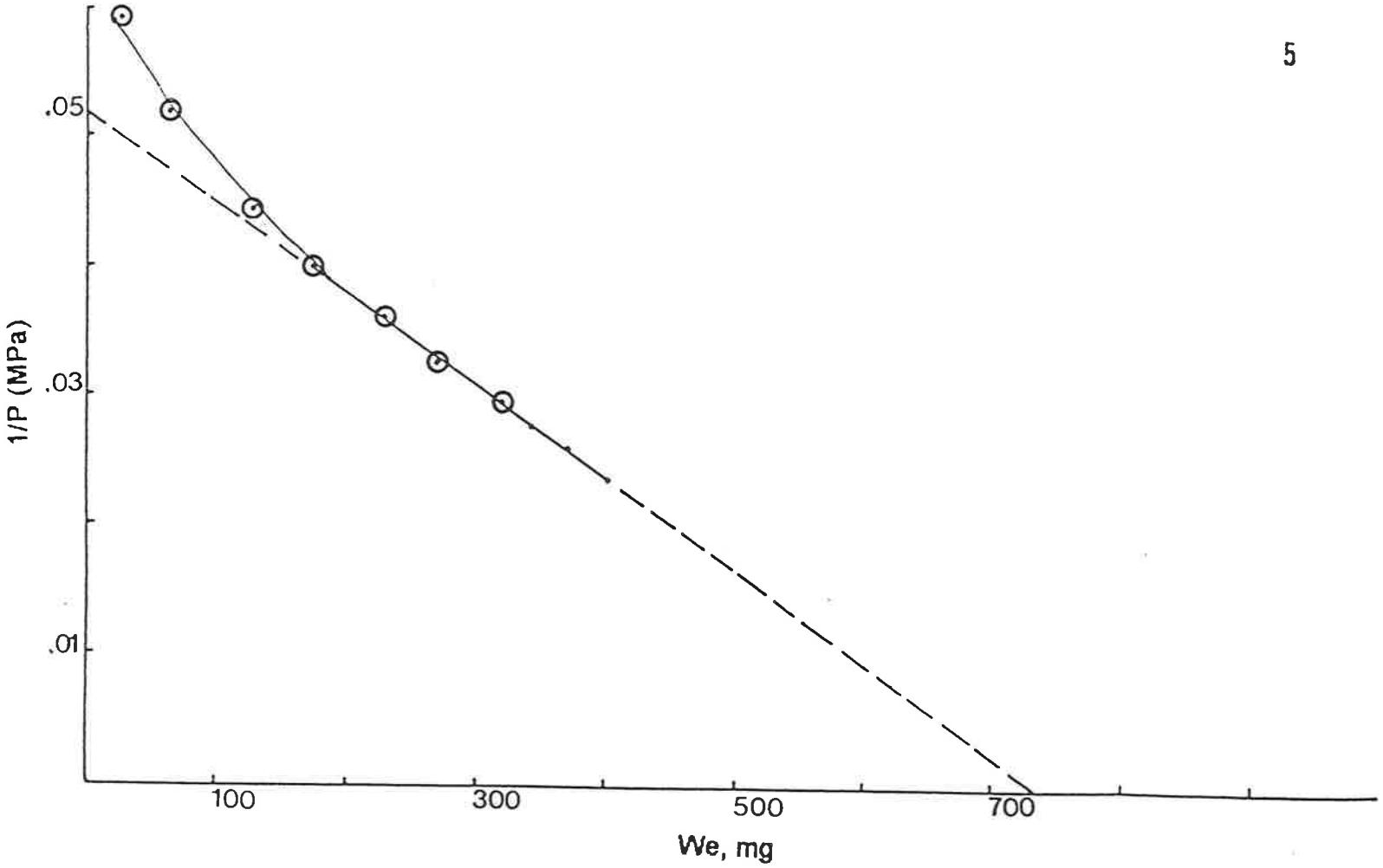
Note: For detailed explanation, please refer to Appendix 5.b.

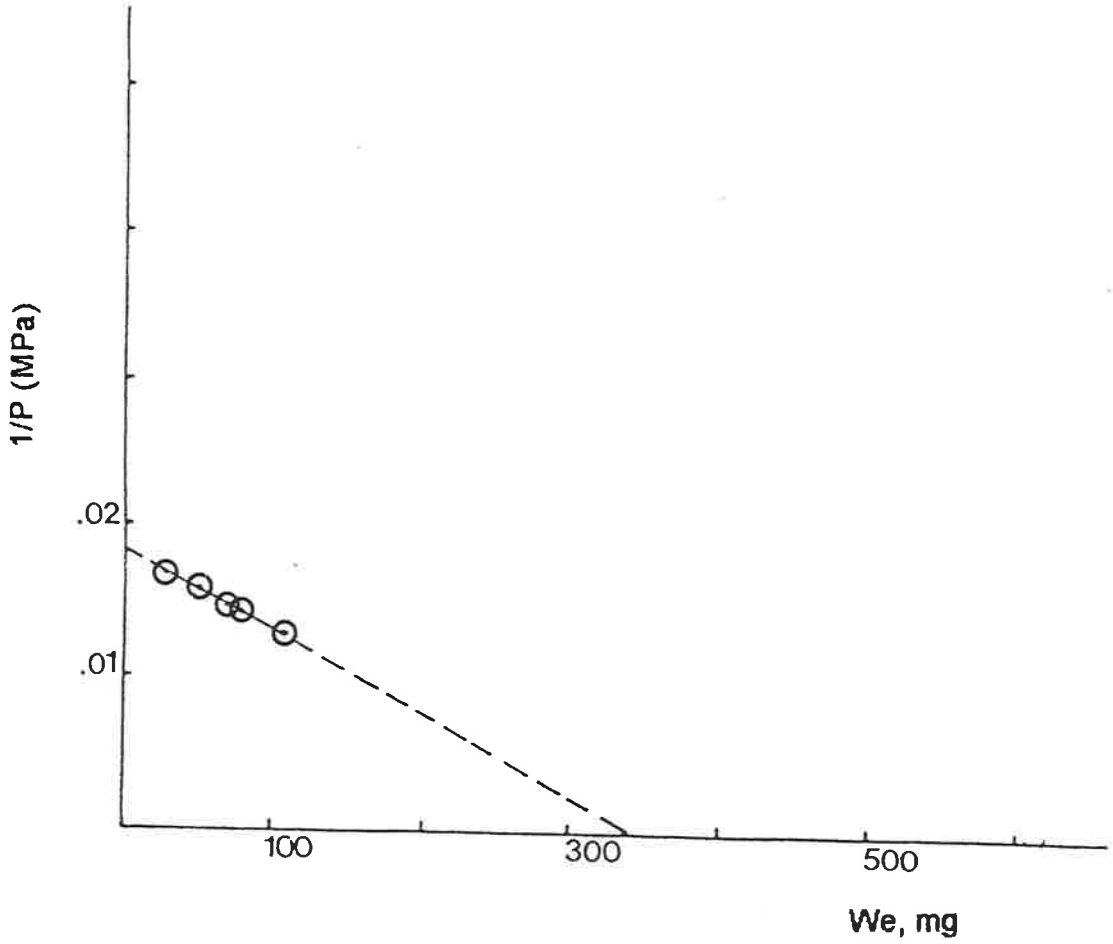

.05
.04
.03
.02
.01
1/P (MPa)
100
300
500
700
900
We, mg

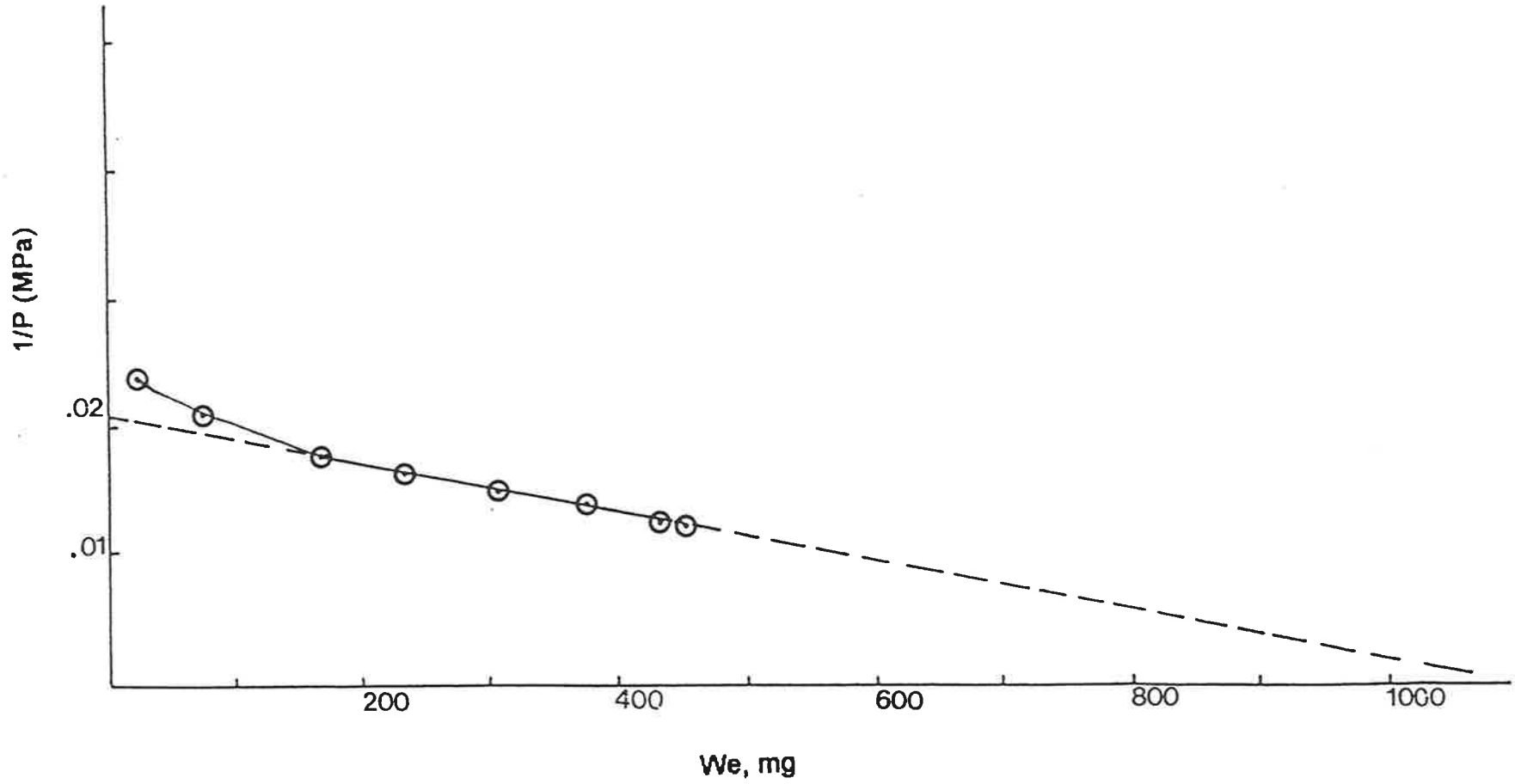

.07
.03
1/P (MPa)
100
500
1000
1500
We, mg

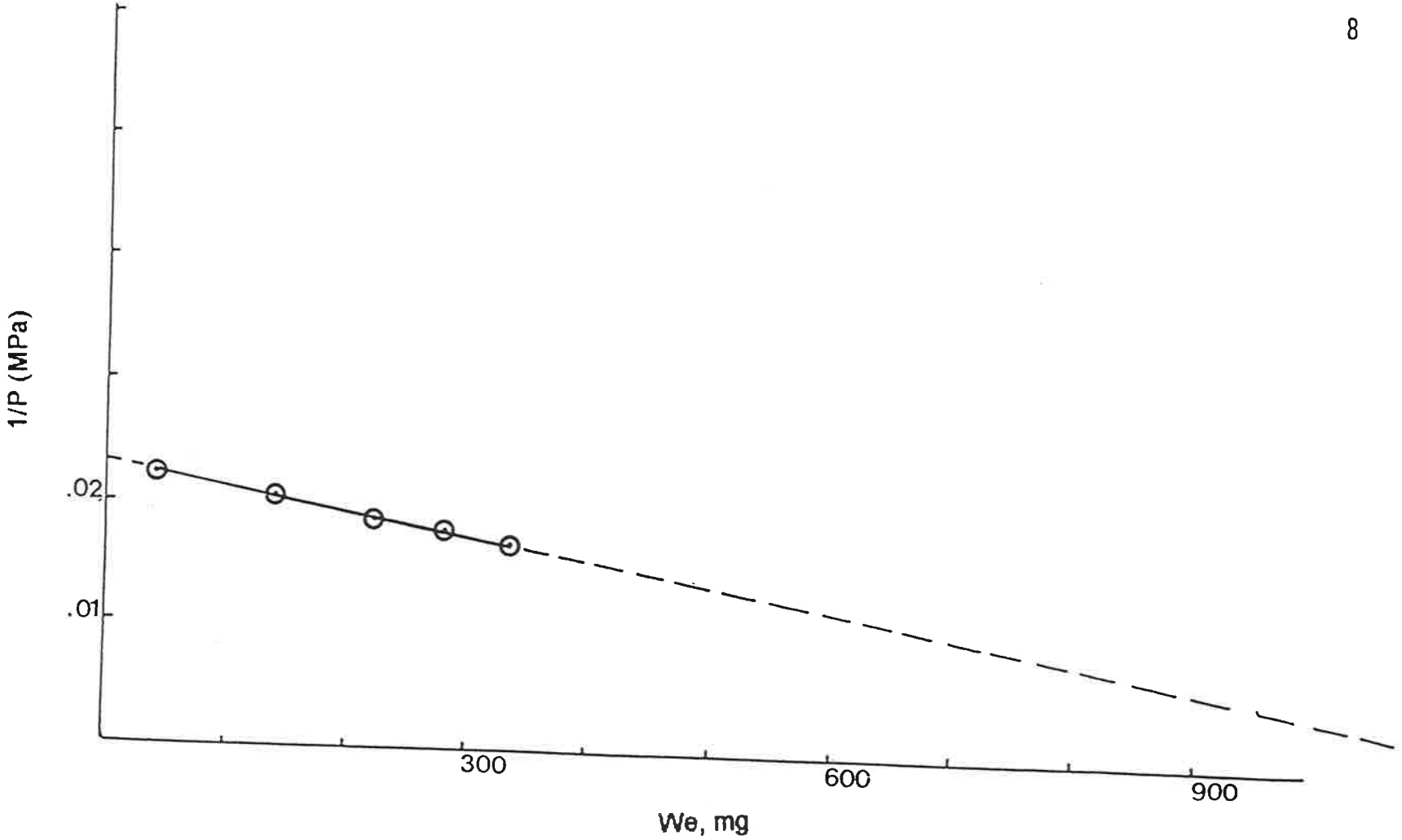

.05
.03
.01
1/P (MPa)
100
300
500
700
We, mg

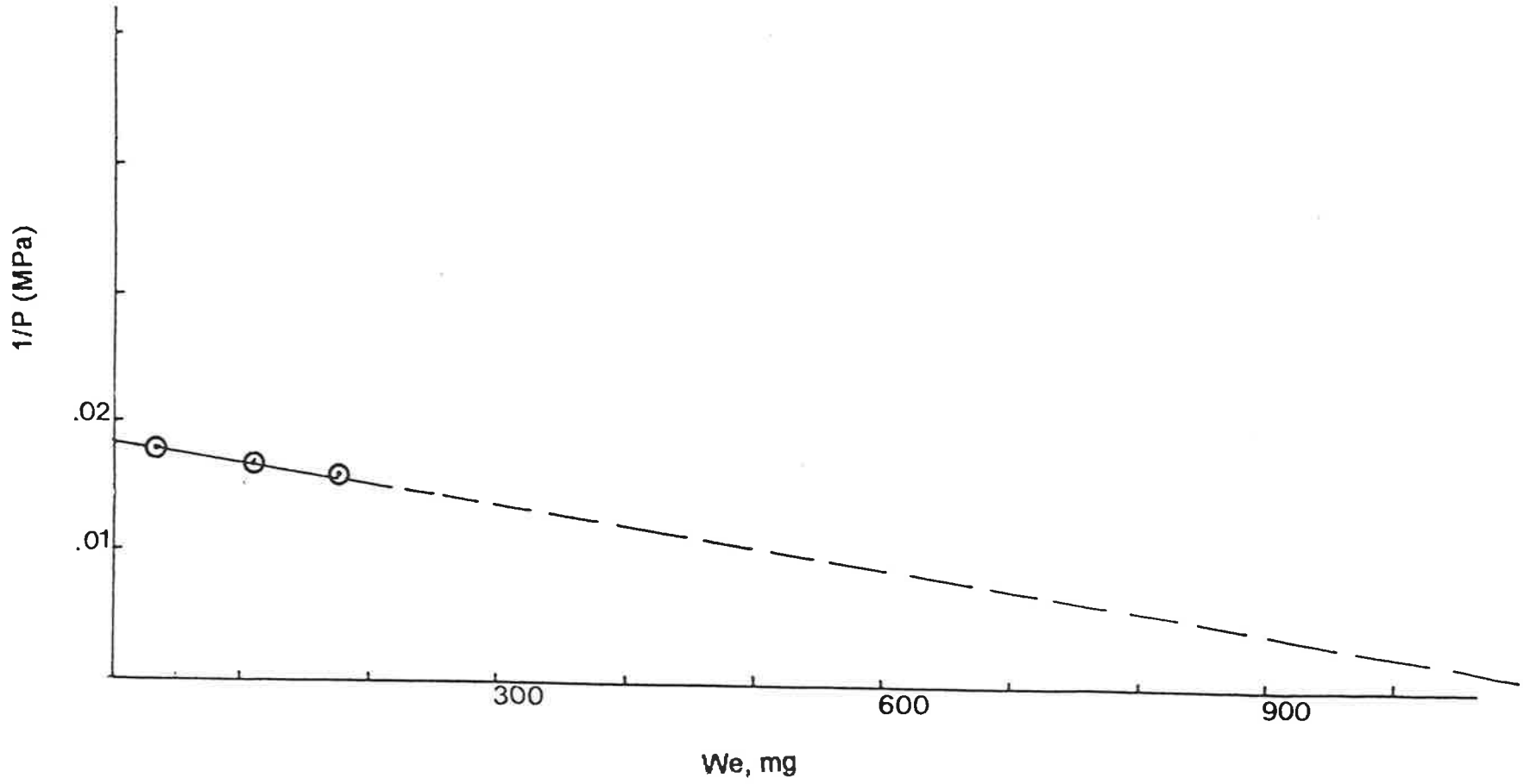

.05
.03
.01
1/P (MPa)
100
300
500
700
900
We, mg

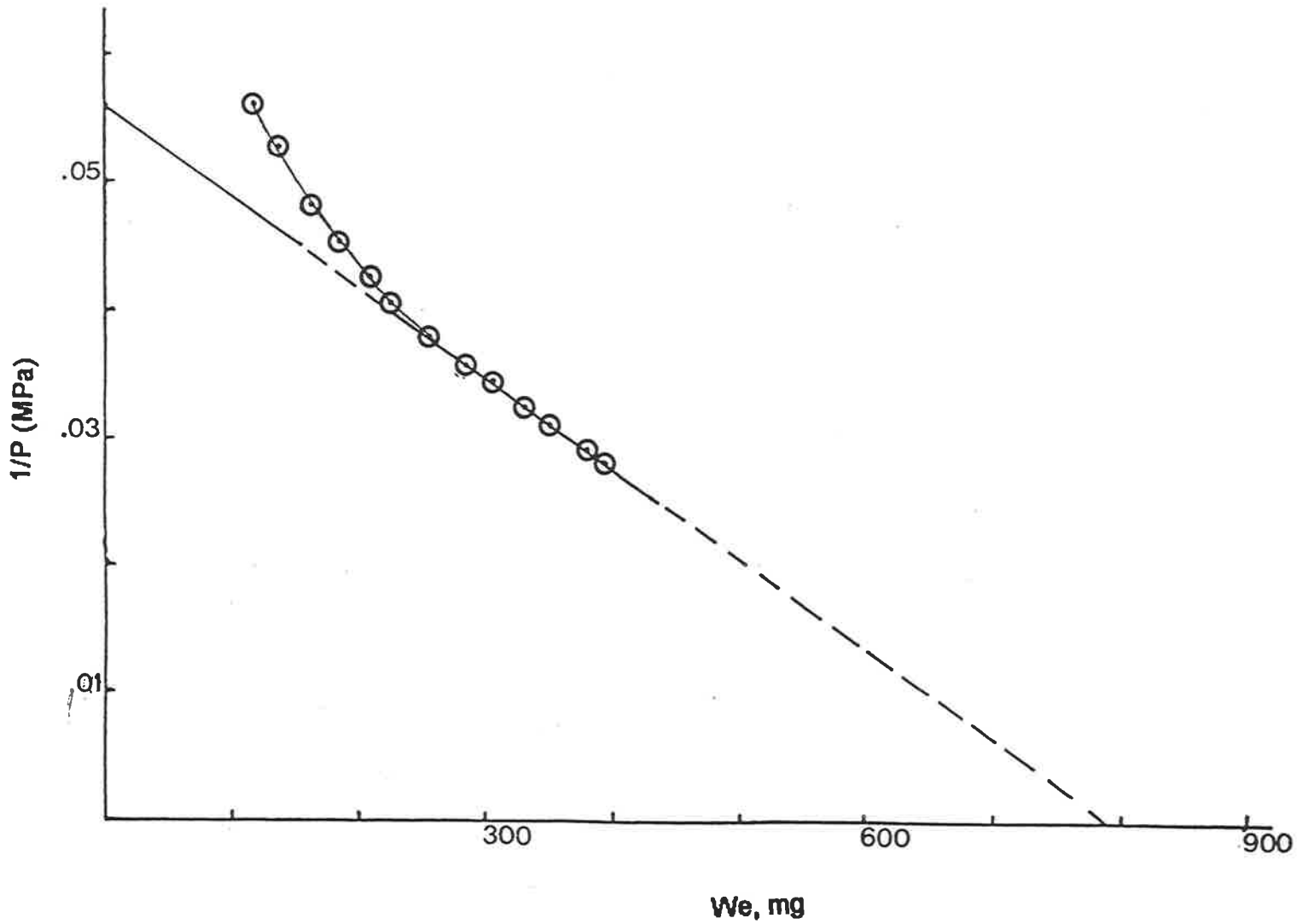

.05
.03
.01
1/P (MPa)
100
300
500
700
We, mg


1/P (MPa)
.02
.01
100
300
500
We, mg


1/P (MPa)
.02
.01
200
400
600
800
1000
We, mg


1/P (MPa)
.02
.01
300
600
900
We, mg


1/P (MPa)
.02
.01
300
600
900
We, mg


1/P (MPa)
.05
.03
.01
300
600
900
We, mg

Appendix 5.b

The values (-MPa) of osmotic potential (Ψ_π) from 10 P-V curves (Appendix 5.a), taken from the glass-house *Acacia* experiment in Chapter 3. Osmotic potentials were estimated by projecting to the *Y*-axis the lines drawn "by eye" ($\Psi_{\pi e}$, column 2), and calculated from linear regression for the same points ($\Psi_{\pi lr}$, column 3).

The difference between "eye mode" and "LR mode" ($\Psi_{\pi d}$) is also shown (Column 4), while *r* values are in Column 5.

No of curve	$\Psi_{\pi e}$	$\Psi_{\pi lr}$	$\Psi_{\pi d}$	r^2
(1)	(2)	(3)	(4)	(5)
1	2.22	2.17	0.05	0.9945
2	2.00	1.95	0.05	0.9997
3	1.99	2.00	-0.01	0.9991
4	2.65	2.73	-0.08	0.9119
5	1.93	1.90	0.03	0.9970
6	5.39	5.44	-0.05	0.9981
7	4.79	4.57	0.03	0.9910
8	4.34	4.31	0.03	0.9823
9	5.56	5.65	-0.09	0.7707
10	1.78	1.84	-0.06	0.9988

Explanations:

The range of deviation of osmotic potential generated by "eye mode" (Column 2) against "LR mode" (Column 3) is between 0.01 MPa and 0.09 MPa (Column 4). The mean value of this deviation was 0.05 $\pm$ 0.02. The *r* values (Column 5) show that apart from no. 9, which had only 3 points, the linear regressions fitted the data very well.

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