THE CARBON BALANCE OF *ATRIPLEX VESICARIA*

by

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A thesis submitted to the University of Adelaide in fulfillment of the requirements for the degree of Doctor of Philosophy.

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March, 1982

"In its right hand was a blade like a stabbing tongue of fire; in its left it held a whip of many thongs.... 'A Balrog,' muttered Gandalf. 'Now I understand.' He faltered and leaned heavily on his staff. 'What an evil fortune! And I am already weary.'"

Lord of the Rings

J. R. R. Tolkien

CONTENTS

SUMMARY

The work reported in this thesis was done to define the times of accumulation and depletion, as well as the location and magnitude, of stores of total non-structural carbohydrate (TNC) in *Atriplex vesicaria* (bladder saltbush), a chenopod shrub common in arid and semi-arid regions of southern Australia. The shrub is grazed by sheep and cattle when herbage is in short supply but, unlike some arid zone chenopods, cannot withstand heavy grazing. The reasons for the poor survival of heavily grazed saltbush are not known, but other researchers have suggested that the concentration of non-structural carbohydrate may be too low to support adequate regrowth. Seasonal records of TNC concentration were made in this study to provide a basis for the planning of future experiments on the relationships between defoliation, carbohydrate concentration and regrowth. Such relationships have been used to formulate suggestions for the management of perennial species in grazing systems elsewhere.

Between October, 1977 and January, 1980, monthly field trips were made to monitor TNC concentration, water potential and phenology of individuals in a small population of shrubs protected from grazing at Koonamore Station, an area with a mean annual rainfall of 214 mm in the north-east pastoral district of South Australia. TNC concentrations were measured in up to seven plant fractions. Shoot growth and leaf production were followed by means of a series of photographs of tagged shoots, while the timing and extent of root growth were assessed from measurements of the elongation of roots visible through perspex windows installed in covered pits.

The highest concentrations of TNC (70-145 mg/g dry weight of tissue) were found in the leaf and young stem fractions. Old stem and woody root had lower maximum TNC concentrations (50-65 mg/g) but because of

their greater dry weight contained the second and third largest stores of TNC, respectively. The largest store of TNC was contained in the leaves. Between April, 1979 and January, 1980, over 50 per cent of the dry weight of TNC was found in the leaf and young stem fractions which are accessible to grazing animals. These fractions also showed the greatest fluctuations in TNC concentration following rain and subsequent growth, both in summer and winter. The fluctuations were superimposed on a pronounced seasonal pattern. TNC concentrations were highest in summer and autumn and lowest in winter and early spring.

Root and shoot growth were most rapid in winter and spring, although some growth followed heavy rains at any time of the year. A peak of root and shoot growth was observed from late winter to mid-spring in each year. In summer and autumn some contraction of shoots was observed when water potentials were low, but rarely was the contraction in length due to loss of tissue from the shoot apex.

To further define the likely times of carbohydrate accumulation, CO_2 exchange and growth (leaf expansion, shoot and root elongation) of field-grown cuttings and glasshouse-grown seedlings were measured during drying cycles in the laboratory. The gas sampling and conditioning system used had to be assembled and built for this purpose. Studies on the CO_2 exchange of field-grown cuttings showed that during a drying phase, after shrubs had been rehydrated in the laboratory, terminal shoots were capable of positive net CO_2 uptake at a water potential of -9 MPa. Small positive rates were recorded for some shoots with field-grown leaves at water potentials less than -10 MPa and in one case less than -11 MPa. Plants which dehydrated to the point where net CO_2 uptake was zero responded to watering within one day and regained 90 per cent of their photosynthetic capacity in about five days.

Growth of glasshouse-grown seedlings, as measured by leaf expansion and shoot extension stopped or was substantially reduced at water potentials lower than -3 MPa.

In the field dawn water potentials as low as, or lower than, -11 MPa were recorded in summer for some individuals but in general, water potentials were higher than the hydration compensation point for net CO_2 uptake measured in the laboratory.

The data on TNC accumulation and water potential in the field and gas exchange at low water potential in the laboratory suggest that net CO_2 uptake by shoots of *A. vesicaria* is reduced to zero only in very dry years. The fluctuations in TNC concentration after heavy summer rains and the data on CO_2 exchange during and after rehydration in the laboratory indicate that TNC is needed to support summer growth.

The results of this study are also discussed in relation to the effects of climate and grazing on the vigour and survival of *A. vesicaria*.

STATEMENT

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university and, to the best of my knowledge and belief, it contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

Desmond F. Coleman

ACKNOWLEDGEMENTS

I am grateful for the encouragement and guidance from my supervisor, Dr R. Sinclair during the course of this study. I also thank Dr J. Silsbury of the Waite Agricultural Research Institute for his advice on infrared gas analysis techniques and Brian Rowland and Richard Norrish for their help and technical advice during the construction of the gas sampling and conditioning system. I appreciate the helpful discussions on my work with Joan Gibbs-Clema and her help with fieldwork and typing parts of this thesis. Cecilia Marcelline also assisted with the typing of two chapters. For the greater part of the typing, I am grateful to Joan Davison for her excellent and patient work.

Thanks are due to Mr A. P. McLachlan, the then owner of Koonamore, and the manager, Les Gardiner, for allowing a field site to be constructed on the property.

This project was carried out in the Department of Botany, University of Adelaide, with financial assistance provided by an Adelaide University Research Grant.

Finally, I thank my wife, Leona, for her understanding throughout and for her help with field work, editing and the final preparation of this thesis.

CHAPTER 1

1 *Atriplex vesicaria*, its biology and use by the pastoral industry

1.1 Biology

1.11 Distribution and general description

The perennial shrub, *Atriplex vesicaria* (bladder saltbush) is a widespread dominant or co-dominant component of chenopod shrublands, which occupy approximately 6 per cent of the area of Australia (Leigh, 1972).

It also occurs as a major component in the understorey of low, open wood-lands dominated by *Casuarina cristata*, *Myoporum platycarpum*, *Acacia aneura*, *Heterodendrum oleifolium* and *Eucalyptus* spp. (Hall *et al.*, 1964; Oxley, 1979) and has been recorded outside those areas described as chenopod shrublands on published maps. It is likely that these records are from small pockets of unmapped chenopod shrubland or of individuals from plant communities dominated by other species. In the central part of its range the species extends northwards to latitude 23°S (Hall *et al.*, 1964) a distribution which corresponds with that of the mapped areas of chenopod shrubland, which lie mainly below the Tropic of Capricorn (lat. 23.5°S) in areas with a mean annual rainfall of 150-500 mm (Leigh, 1972).

A. vesicaria, a dioecious, multi-stemmed shrub up to 1 metre tall, is a C4 species belonging to the family Chenopodiaceae. The leaves are flat with dimensions of the order of 5-25 mm. An account of leaf size and shape for a number of geographic and edaphic variants can be found in Parr-Smith and Calder (1979). The stems are brittle and usually erect, although in the eastern part of the species' range individuals growing on stony ridges may have decumbent stems (Parr-Smith and Calder, 1979).

The root system is shallow and fibrous with the greatest concentration of roots immediately under the canopy. Laterals extend to between one and two metres from the plant and the bulk of the dry matter (80-90%) is res-

tricted to the top 30 cm of the soil profile (Osborn *et al.*, 1932; Carrodus, 1962; Jones and Hodgkinson, 1970; Sharma, 1976). Lateral roots produce numerous groups of fine drought-deciduous roots which are renewed in wet periods (Osborn *et al.*, 1932).

The leaves are also drought-deciduous, the extent of defoliation depending on the severity of the drought. The lamina is covered with numerous bladder-like epidermal hairs which are rich in salt (Wood, 1925; Black, 1954). A number of functions have been ascribed to these vesicles, among them water storage and absorption of water vapour from the atmosphere, but their most likely function is that suggested by Osmond *et al.* (1969). They found some evidence that the vesicular hairs of *Atriplex spongiosa* play a role in regulating ion levels in the leaves. The eventual rupture of the vesicles spreads a layer of crystalline sodium chloride over the leaf surface. Sinclair and Thomas (1970) measured a high coefficient of reflection ($R \simeq 50\%$) for leaves of *A. vesicaria* and attributed this to the layer of salt and collapsed vesicles. The high reflectance was sufficient to significantly reduce the absorption coefficient and hence the heat load on the leaf. Of the species they examined only in *A. vesicaria* and *A. stipitata* were the leaf optical properties sufficiently modified to be considered a significant adaption to the high solar radiation encountered in warm arid regions. The matted layer of collapsed bladders probably serves to increase the boundary layer resistance to water vapour movement away from the leaf and since the epidermis has only a thin cuticule (Wood, 1925) this may also be an important adaption to arid conditions. The leaves of *A. vesicaria* are also likely to exhibit the high water use efficiency characteristic of those species with fix CO_2 via the C4 pathway.

The water potential of soils supporting stands of perennial saltbush is often very low and consequently plant water potential must be lower to maintain water flow from soil to plant. Anderson *et al.* (1972) have stated

that individuals of *A. vesicaria* can tolerate internal water potentials of about -13 MPa for long periods between rains. If at these times stomata are open during the day, a gradient along which water can flow from leaf to air will exist because of the low water vapour pressure of the atmosphere. Under these conditions, if the rate of water absorption from the soil is less than the transpiration rate, the leaf cells may lose water. When stomates close some water will be taken up, because water loss has lowered the osmotic potential of the mesophyll, but further rehydration can occur only if the cells are capable of osmotic adjustment.

Although there is no direct evidence of high concentrations of inorganic ions within the lamina of field grown plants of *A. vesicaria* it is probable that as leaf water potential falls turgor is maintained through active ion uptake by mesophyll cells. It is known that the leaf salt content is high even for plants growing on non-saline soils (Wood, 1925; Beadle *et al.*, 1957). Active ion uptake by mesophyll cells of another member of the genus *(A. spongiosa)* has been demonstrated by Luttge *et al.* (1970) and high ion concentrations have been found in both lamina and bladders of *A. vesicaria* grown on saline culture solution (Osmond *et al.*, 1969). The salt content of leaves of field grown plants is highest in summer and lowest in winter (Sharma *et al.*, 1972) a result consistent with osmotic adjustment during the dry season and one which provides some evidence of high ion concentration in the mesophyll as the summer values remained high despite heavy rains which would have washed some of the salt from the leaf surface. Charley (1959) showed that up to half the total salt content of the plant could be leached from the leaves by rain, so presumably there was a high concentration of ions in the mesophyll of those plants examined by Sharma and co-workers. The ability to maintain turgor by osmotic adjustment would explain the wide distribution of *A. vesicaria* in the arid and semi-arid zones despite the fact that

individual shrubs have a relatively small root system strongly concentrated near the soil surface where water potentials often fall to very low values.

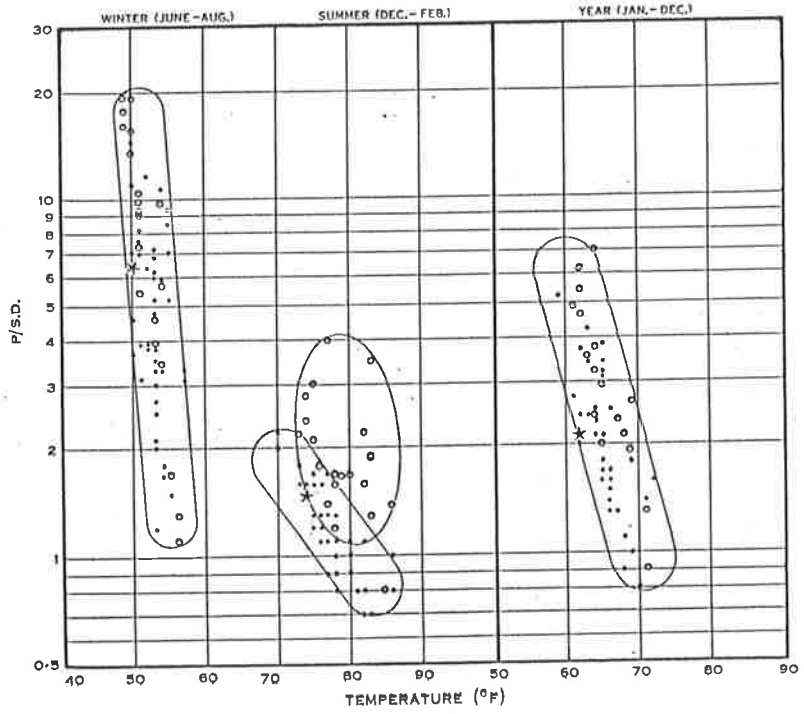
The distribution of *A. vesicaria* is shown in fig. 1 (from Hall *et al.* 1964). The field work reported in the following chapters was done at Koonamore Station, marked on the map by a star. From the diagram accompanying the distribution map it can be seen that Koonamore represents the cooler end of the distribution of *A. vesicaria*. It is also among the drier sites, as measured by mean monthly rainfall efficiency (precipitation/saturation deficit), occupied by the species but apparently receives more summer rain than many sites in the winter rainfall zone. Further information on conditions there can be found in Osborn *et al.* (1935) and in later chapters of this thesis.

1.12 Variation within the species

A knowledge of the conditions at Koonamore allows the work described here to be put into some perspective but any extrapolation from one area to another ought to be done with the understanding that *A. vesicaria* is a very variable species. Wood (1936) and Jessup (1951) described a number of ectotypes associated with different soil types. More recently Parr-Smith and Calder (1979) defined nine forms including the previously described ectotypes. The various forms were separated on the basis of characters of the female inflorescence and bracteoles surrounding the fruit, and with some reference to habit and leaf form. The single most useful character was the form of the dorsal appendage, or bladder, on the fruiting bracteoles. The true fruit is a nut encased in a membranous pericarp. Anatomical and micro-morphological features were not used as few appeared taxonomically useful. Parr-Smith and Calder intend to define these forms as subspecies of *A. vesicaria* in a future paper.

Figure 1.1 Diagram from Hall *et al.* (1964) showing the distribution of *Atriplex vesicaria* and climatic conditions for the localities occupied by the species. In the distribution map, closed symbols represent sites from which herbarium specimens have been collected and open symbols show localities for which reliable observations have been recorded in the literature. The position of Koonamore is indicated by a star.

The accompanying chart (precipitation/saturation deficit vs. temperature) shows mean winter, mean summer and mean monthly climatic conditions for sites occupied by the species. The small closed symbols are for sites in the winter rainfall zone and the open symbols are for sites in the summer rainfall zone. The climatic conditions for Koonamore are indicated by a star. Note that temperatures are in ^{o}F.


WINTER (JUNE–AUG.)
SUMMER (DEC.–FEB.)
YEAR (JAN.–DEC.)
P/S.D.
30
20
10
9
8
7
6
5
4
3
2
1
0·5
40
50
60
70
80
90
50
60
70
80
90
TEMPERATURE (°F)

The form of *A. vesicaria* adjacent to the Koonamore vegetation reserve (KVR), where my study site was located, corresponds to the 'limestone' form of Parr-Smith and Calder (1979). It is considered a recognizable variant of the 'small bladder' form, which is the most common and widespread, but was not clearly separated from the latter in a canonical variates analysis. There are also populations with bladders intermediate between the 'limestone' and 'large bladder' forms, both of which occur on Koonamore station, but in most cases these two were easily separated.

There is no experimental evidence of physiological differences between the various forms of *A. vesicaria* but the possibility of such differences should be kept in mind when evidence on the functioning of plants in one area is used to help interpret results for those in another. For example, much of the work outlined in this short review was done on the Riverine Plain of New South Wales which supports a form of saltbush restricted to that area. It is apparent from the work of Parr Smith and Calder, described below, that the saltbush adjacent to KVR belong to a form which is widely distributed throughout the arid and semi-arid zones. Together with the 'large bladder' form with which the 'limestone' form intergrades in some areas, these forms constitute the bulk of the species. Results from saltbush at Koonamore may thus have wider application. However, even within the 'small bladder' form there is considerable variation. According to Parr-Smith and Calder, as well as being the most common, it is the most complex and least understood form of the species.

1.2 The use of chenopod shrubland by the pastoral industry

A comparison of the maps published by Oxley (1979) and Costin and Mosley (1969) shows that large areas of chenopod shrubland (dominant or understorey) near the border between South Australia and Western Australia and elsewhere are classed as vacant crown land but most of this land is contained within pastoral leases carrying sheep and/or cattle. The saltbush (*Atriplex* spp.) and bluebush (*Maireana* spp.) pastures of South Australia, New South Wales and Western Australia are grazed mainly by sheep. Those north of the dog fence in South Australia, however, are considered unsuitable for sheep because of the presence of wild dogs (*Canis familiaris*, dingo) and in these areas they are replaced by cattle even though the vegetation is considered more suited to sheep (Newman and Condon, 1969).

Williams (1968) found that saltbush communities supported exceptionally high rates of wool growth, but it is unlikely that these rates can be attributed to the shrub itself as sheep show a marked preference for the herbaceous plants growing between the shrubs (Leigh and Mulham 1966). Saltbush has a lower *in vitro* digestibility than the herbaceous component of the vegetation and hence cannot be expected to support such high rates of growth as the latter even if the voluntary intake is high. Nevertheless seasonal values for digestibility and nutritive quality do not fluctuate as markedly as those for grasses, and saltbush can provide a maintenance diet, if good quality water is available, at times when the animals are forced to browse because of the lack of grasses and herbs.

In the early 1970s the cattle industry became relatively more profitable and there was an appreciable increase in the number of cattle on pastures dominated by *Atriplex vesicaria* (Graetz and Wilson, 1980). In the years between 1950 and 1968 cattle numbers, expressed as sheep equivalents, were about one tenth that of the sheep population but this figure had risen to about one fourth by 1972 (Wilson, 1976). According to Graetz and Wilson (1980) it is a widely held view among pastoralists that cattle

are unable to forage as selectively as sheep and therefore cannot maintain as high a quality diet when grazing saltbush pastures, especially in dry times. The assumed loss of production was a reason for excluding cattle from these areas in the past. However, Wilson and Graetz (1980) found that the mean weight gains (kg/ha) of equivalent numbers of sheep and cattle grazing saltbush pastures were similar. Economic factors were responsible for the increased number of cattle carried on chenopod shrublands and Wilson (1976) suggested that it will be these, rather than foraging ability, which influence the number of cattle carried on pastoral leases normally used for sheep.

Although there is no conclusive evidence available on the effect of cattle on the long-term population dynamics of the perennial shrubs in these patures (Fatchen and Lange, 1979) the following outline of what is known of sheep and cattle diets does allow some conclusions on the possible consequences of higher cattle numbers. Conclusions about vegetation change from dietary studies should be treated with caution as such information fails to account for the response of the ingested species to frequent defoliation (Graetz and Wilson, 1979). Both Barker and Lange (1970) and Fatchen and Lange (1979) observed vegetation changes contrary to an expectation based on supposed preference by stock for the species involved. However, in the case of *A. vesicaria* a more confident prediction can be made as the response of the species to heavy grazing has been well documented (see Sections 1.3, 2.2) although less is known of the response to partial defoliation.

The diet of sheep grazing pastures dominated by chenopodiaceous shrubs has been extensively studied in a series of experiments carried out on the Riverine plain in New South Wales (Leigh and Mulham, 1966, 1967; Wilson *et al.*, 1969). The data for an *A. vesicaria* community were summarized by

Leigh and Wilson (1970). In this case saltbush provided only 10 per cent of the diet during the wetter months when herbaceous plants growing between the bushes were abundant. In summer and autumn the intake of bush was higher, although the amount eaten depended on rainfall and stocking rate. Saltbush constituted 25 per cent of the diet if rainfall was high and stocking rate low, or as much as 90 per cent if either rainfall was low or stocking rate high. The sheep ate less saltbush when herbs were present following summer rains.

Pen feeding trials (Wilson, 1966) have shown that *A. vesicaria* can provide a maintenance diet for sheep with access to fresh water at all times. In the grazing experiment summarized below stocking rates were 0.4, 0.81 and 1.62 ha/sheep yet even at the lowest stocking rate sheep lost body weight during periods of low rainfall when *A. vesicaria* was the major component of their diet. These sheep also had unrestricted access to fresh water but were not confined to metabolism cages. The availability of water is important for sheep on a diet of saltbush as the intake of salt is high and more water is required for the urinary excretion of sodium (Wilson, 1966). The lowest stocking rate corresponds to the average district stocking rate for similar pastures, in an area with a mean annual rainfall of 335 mm (Wilson *et al.*, 1969). In drier areas sheep are probably forced to rely on shrubs for a high proportion of their diet earlier in the year and consequently weight losses over the whole season may be greater. The location and number of watering points within a paddock is also important in saltbush communities. Large flocks of sheep confined to sparsely vegetated paddocks in the drier areas graze 2-3 km or more from water in summer and autumn and the high sodium intake from a diet of saltbush often forces them to walk to water twice a day (Wilson, 1978) resulting in a larger energy requirement for maintenance. A high salt diet may also reduce the tolerance of sheep to saline water which, when the only source of water, can

cause loss of body weight and poor lambing performance even when the animals are grazing more mesic pastures (Peirce, 1968; Potter and McKintosh 1974).

Cattle grazing saltbush pastures do not select from as wide a range of plants as sheep, but basically have the same preference for herbaceous species (Graetz and Wilson, 1980). Since they are unable to graze as closely as sheep (Leigh, 1974) the herbage available to them is less and hence saltbush forms a greater proportion of their diet especially in dry periods or when they are grazing pastures in common with sheep. The effect of such a diet on production from cattle is not well understood. Wilson and Graetz (1980) found no evidence of poor performance in cattle on saltbush pastures but in that experiment neither food nor water were limiting.

When grazing large paddocks, traditionally used for sheep, cattle may be less productive as they have a higher water turnover (McFarlane and Howard, 1974) and consequently may have to spend more time walking to water. They also have a lower tolerance to saline water than sheep (Weeth and Haviland, 1961). The amount of water consumed by cattle in the grazing experiment reported by Wilson and Graetz (1980) was 50 per cent higher than that by an equivalent number of sheep. Part of the increased water intake can probably be attributed to the higher proportion of saltbush in the diet of these cattle. Graziers using such pastures for cattle may therefore be forced either to increase the water supply by at least 50 per cent or to carry less cattle than the pasture can support.

Indirect evidence suggests that cattle may have a bigger impact on the shrub population because of their higher intake, although according to Wilson (1976) cattle are not as adept at removing the last leaves from among the woody stems of *A. vesicaria* so that more may survive heavy grazing.

Atriplex vesicaria appears to be neither highly preferred by domestic stock nor particularly nutritious. *In vitro* measurements return low values

(ca. 50%) for digestibility but these may be lower than *in vivo* values. The shrub can provide a maintenance diet in some circumstances but its main value to the pastoral industry, at least in the short term, is that its presence enables stock to be carried, albeit with some weight loss, over short periods of drought. Williams (1960) concluded that the reduced fluctuation in stocking rates on saltbush pastures at Hay in New South Wales compared with that for the wetter grasslands at nearby Deniliquin was due in part to the more stable forage production from the saltbush pastures. However, in a prolonged drought increasing use is made of the shrubs and ultimately the population may begin to decline if stock are not removed.

1.3 The impact of stock on saltbush pastures

1.31 Vegetation

Since the introduction of stock to the arid and semi-arid zones over 100 years ago, the general pattern has been one of high but variable stock numbers, followed by a decline to a number much less than that carried in earlier years (Newman and Condon, 1969). According to these authors it was a common occurrence in South Australia, where water was scarce in the early years, for 10 000 sheep to water at one watering point during a drought. It is current practice on some stations in the north-west pastoral district of South Australia, with well managed shrub populations, to set-stock each watering point with less than 300 sheep. The high numbers carried during drought in earlier years had a big impact on the perennial shrub population which was seriously depleted by the mid-thirties (Ratcliffe, 1936).

Osborn *et al.* (1931) describe the conditions which led to the removal of all but a few badly overgrazed bushes of *A. vesicaria* from part of a paddock on Koonamore station in the north-east pastoral district of South Australia. This episode occurred in 1925 when an estimated 1 425 sheep (Crisp, 1975) were watering at a bore about 10 km away. The severe deterioration of the vegetation at that distance from water indicated a high paddock stocking rate but was partly due to the fact that sheep congregated in the area because of the shade provided by trees and because they could smell water in the adjacent paddock to the south. The estimate of sheep numbers was based on a district stocking rate (15 sheep/km^2) considered conservative at that time. No records were available for 1925 but in the following year an isolated entry in the Koonamore files records 1 400 sheep grazing the paddock in question. The Koonamore Vegetation Reserve, an area of 400 ha in the 'worst eaten out' corner of the paddock, was fenced off in 1926 and subsequent studies of regeneration have shown that recolonization of the area by perennial shrubs was initially very slow,

especially on eroded land (Hall *et al.* 1964; Wood, 1936).

Osborn *et al.* (1932) described less extreme examples of the effects of sheep grazing saltbush pastures. They studied populations in four concentric zones around a watering point, and expected the vigour of the plants, as measured by degree of foliation, to improve as grazing pressure decreased with increasing distance from water. In order of increasing distance from water the four zones were as follows:

A	0 - 400 m	:	heavily grazed and trampled
B	400 - 1 600 m	:	moderate to heavy grazing
C	1 600 - 3 200 m	:	lightly grazed
D	over 6 500 m	:	unstocked

The A zone was generally bare to a distance depending on the number of stock using the watering point; the bush density then rapidly inceased until it merged with the B zone where the density was not significantly different from that of the unstocked country. The C zone had a higher bush density but a high proportion were wilting in contrast to those in the B zone where vigour was greatest. Osborn *et al.* attributed the poor vigour of the bushes in the lightly stocked zone to competition between the bushes, presumably for water and nutrients. They considered the grazing pressure insufficient to remove moribund plants yet enough to contribute to a significant increase in bush density through planting of seed by trampling hooves. On the other hand, the high grazing pressure in the B zone resulted in the mechanical removal of moribund plants and the production of more compact, vigorous bushes due to the stimulation of lateral growth by frequent removal of the terminal apices. It is difficult to know the value of a visual assessment of vigour. A compact bush, presenting a dense area of foliage, may simply look healthier than its more open neighbour. However, it is probably true, as Leigh and Mulham (1971) have pointed out, that a compact bush is less prone to complete defoliation and more likely to survive heavy grazing than is an open straggly bush where all the foliage

is accessible to sheep.

The concept of a radial gradient in vegetation was later used by Lange and his graduate students who refined the technique and applied it to the analysis of vegetation around watering points in much more conservatively stocked country. Lange (1969) called the area affected by animals grazing around such a watering point, the pioshpere. From among the piospheres that she studied, Barker (1979) found that only *A. vesicaria* was disappearing close to water points as a direct result of sheep stocking. The significance of such changes, which often include the invasion of the trough area by less preferred species, is that they are taking place under conservative stocking policies. The observed changes in *A. vesicaria* populations around one watering point occurred over a short radial distance, numbers increasing to a higher value beyond about 200m from water (Barker and Lange, 1970). In this case the watering point, a trough fed from a pipeline, was independent of topography (cf. dam or earth tank) and therefore of pre-existing pattern on the scale detected. They also concluded that the presence of small populations of seedlings, young and mature plants in the vicinity of the trough was an indication of recolonization of the area which had previously been denuded of *A. vesicaria*; and that the present stocking rate (250 - 300 sheep/water) allowed some recovery without spelling.

However, change is not always restricted to the immediate vicinity of the watering point. On some stations surveyed by Lay (1972), the shrub cover had been completely eaten out for up to 1 km from the water point. Nor, it seems, does a conservative stocking policy guarantee survival of the shrubs as he observed appreciable bush loss on apparently well managed stations. It is possible, of course, that these deaths were due to some influence other than grazing pressure, such as drought. The mean annual rainfall for the area surveyed is only 150-160mm, a value close to the lower

limit for areas occupied by chenopod shrublands. These changes, recorded by Lay (1972) had occurred in the 20 years since a survey of the same area by Jessup (1951).

1.32 Soils

The most serious consequence of mismanagement of chenopod shrublands is erosion leading to truncation of the soil profile and loss of nutrients. It is still commonly held that arid zone soils are potentially very fertile and that plant growth is limited only by the lack of water; the main evidence for this belief is the dramatic response of plant growth in arid areas following unusually heavy rains. However, arid zone soils, and in particular those of the Australian arid zone, are poor in nitrogen, phosphorus and organic matter (Charley and Cowling, 1968). The high plant production following heavy rains often receives comment but according to Trumble and Woodroffe (1954) it is not so widely known that when two good seasons occur in succession plant growth in the second is slight. The poor response in the second season they ascribe to the fact that the small circulating pool of readily available nutrients is largely depleted by the previous season's growth.

Of the total nitrogen and phosphorus in a perennial saltbush community only a small fraction was found in the above ground portion of the plant (Charley and Cowling, 1968). Thus, even the removal of all the shrub cover by overgrazing in that community, would not constitute a serious loss of nutrients provided that the soil surface remained intact. However, arid zone soils are particularly susceptible to erosion by both wind and water (Marshall, 1972; Condon, 1972) and since most of the nutrients are concentrated near the surface (Charley and Cowling, 1968) the potential loss is serious. Wind erosion is possible in less extreme cases than the hypothetical example cited above. Jessup (1951) considered that a bush density

of about 3 000/ha was the minimum required to prevent wind erosion. The bushes serve to decrease the wind speed over the soil surface (Marshall, 1972). Water erosion is also promoted by the action of many hooves which break up the lichen crust, pulverise the soil surface and compact the subsurface soil (Barker, 1979). As a result infiltration is poor and runoff carries away the powdery surface soil. The dispersal of the surface layers may eventually result in a poorer nutrient status in the underlying soils as nitrogen fixing organisms have been shown to occur in the lichen crust (Rogers *et al.* 1966).

Charley and Cowling (1968) observed that the loss of 5 cm of the surface soil is a common result of erosion in arid regions and that a 10 cm loss is not unusual. A 10 cm truncation of the soil profile in the community studied would mean a loss of 27 per cent nitrogen, 21 per cent phosphorus and 38 per cent organic matter from the total in a rooting zone 45 cm deep.

Erosion of the texture contrast soils supporting many perennial saltbush communities, as well as resulting in a loss of nutrients, may expose a sealing clay B horizon (Condon, 1972) which promotes runoff and hence poor water storage. Such conditions are unfavourable for germination and establishment and subsequent recolonization by perennial vegetation is slow (Hall *et al.* 1964; Charley and Cowling, 1968).

The use of these shrublands by the pastoral industry has resulted in degeneration of much of the perennial vegetation. In a review of condition and trend in arid communities Newman and Condon (1969) classed 25 per cent of shrublands as severely degenerated, while only 10 per cent were considered in excellent condition. Most of the damage occurred in the early years of settlement but erosion begun then is often aggravated during drought. Jones (1966) has found evidence of natural reclamation of scalds in the Hay area of New South Wales, and in this and other areas where stock numbers have been reduced (Newman and Condon, 1969; Barker and Lange, 1970) the

trend seems to be towards improvement. In other areas the trend is still towards deterioration (Lay, 1972; Dawson and Boyland, 1974). The vegetation is most subject to abuse during droughts following a run of good seasons when stock numbers have been allowed to increase.

The results of overstocking are not confined to removal of shrubs and erosion but may be found in the replacement of palatable shrubs by less palatable or inedible vegetation (Newman and Condon, 1969; Barker, 1979). Though less damaging to the country, in that loss of soil and nutrients is avoided, such changes must eventually place more pressure on the remaining edible shrubs. Perhaps the most subtle consequence of stocking, in some areas, is the lack of recruitment of seedlings to long lived tree (*Acacia papyrocarpa*) and shrub (*Maireana sedifolia*) populations (Lange and Purdie, 1976; Crisp, 1975). In the case of *M. sedifolia* the problem is aggravated by infrequent seeding. Edible shrubs, such as *Rhagodia* and *Enchylaena* spp., associated with *A. papyrocarpa* may also be lost if the tree population eventually disappears, thus placing more pressure on the remaining edible shrubs. The loss of shade may also result in reduced animal production due to heat stress when radiation loads and air temperatures are high (Brown and Hutchinson, 1973; Lynch and Alexander, 1973). Another example of the less obvious effects of stock on vegetation is the preferential grazing of female plants of *A. vesicaria* (Williams, 1972; Graetz, 1978) reducing the female/male ratio and possibly the reproductive capacity of the population. However, Graetz (1978) considered that intra specific diet selection was of little consequence for the shrub population as a whole, or to management.

Managers are primarily concerned with maintaining good animal condition but, as Perry (1974a) has pointed out, greater emphasis should be placed on the maintenance or improvement of vegetation and soil condition since in the long run animal production will depend on the stability of these resources.

Because the changes occurring at present are slow, and often not

immediately obvious, one approach to the development of management principles to counter these changes is to set up standards for assessing the health (condition) of plant communities and to monitor the condition of those communities at regular intervals. According to Perry (1967,1974a) the development of useful condition and trend standards is being held up because of our poor understanding of all the interactions between plants, grazing animals and their environment. Only a few of these have been outlined in this and previous sections.

In contrast to the approach taken in the arid regions of North America, one area of research which has received little attention in this country is the biology of major plant species and in particular their physiological response to the environment and to defoliation. Although physiological condition is not likely to be used as a direct measure of community condition an understanding of the physiological responses of major plant species to environment and defoliation may influence the choice of management strategies designed to arrest any trends towards poor condition which are identified. Perry (1967) considered such studies basic to the precise definition of condition and trend standards, and to our general understanding of the effects of various management practices. Some progress has been made since these articles were written but it is still true that very little is known of the biology of key species in the Australian arid zone.

A.vesicaria, for example, is not persistent under heavy grazing because of its brittle easily damaged stems and its inability to recover from severe defoliation (Leigh and Mulham, 1971). *Atriplex nummularia* and *Maireana sedifolia*, on the other hand, are able to resprout from stem stumps after complete removal of the shoot (Hall *et al.*, 1964; Leigh and Wilson, 1970). The physiological reasons for these differences are not known. Leigh and Wilson (1970) and Hodgkinson and Baas-Becking (1977) have advanced a number of explanations for the response of *A. vesicaria* to complete and severe defoliation which will be discussed in the next chapter.

CHAPTER 2

2. Physiological responses to defoliation and water stress

2.1 Introduction

The two main factors affecting the growth and survival of individuals of *Atriplex vesicaria* are overgrazing and drought.

It became apparent in the early years of settlement that *A. vesicaria* is unable to withstand heavy grazing. On Koonamore Station in South Australia, for example, a large flock of 4 500 sheep reduced a dense stand of saltbush and other chenopod shrubs to dust, within a radius of 2 km from water, in about six weeks (see Crisp, 1975). On part of the southern Riverine Plain, a much wetter area in New South Wales, disclimax grasslands have been produced by overgrazing of chenopod communities dominated by *A. vesicaria*. The ease with which this may be accomplished has been shown by Wilson *et al.* (1969). All but 2 per cent of saltbush in experimental plots, stocked at twice the district rate for similar pastures, were dead within three years from the start of the experiment.

Although *A. vesicaria* is well adapted to frequent periods of little or no rainfall the effects of an extended drought may be almost as drastic. Osborn *et al.* (1932) estimated that 95 per cent of saltbush in a stand in unstocked country were leafless as a result of drought. Individuals of *A. vesicaria* can resprout from the base after complete defoliation but it is doubtful whether they can survive for long without leaves. Regrowth of droughted shrubs probably depends on a substantial rain soon after defoliation.

On the other hand, saltbush apparently cannot survive complete defoliation by grazing animals under any conditions. Leigh and Mulham (1971) followed the persistence of shrubs after complete defoliation in an

experiment where both irrigated and control plots of saltbush were defoliated in mid spring and late summer. All died irrespective of soil moisture status or season. They attributed the death of these shrubs to the removal of all potential growing points. They found no evidence of the regrowth, from near the base of stems, recorded for plants recovering from drought-induced defoliation (Osborn *et al.*, 1932) and suggested that all primordia, from which new growth could be initiated, are located on the young terminal stems removed with the leaves by grazing animals.

Prior to the publication of details of this work the main results were outlined in a short review by Leigh and Wilson (1970) who indicated a number of areas of research which might help explain the high mortality observed. Among these was the suggestion that the use of stored photosynthate may be important for regrowth following partial defoliation, the implication being that in completely defoliated plants, where reserves must be used, the failure to resprout was due either to a lack of reserves or an inability to mobilize those stores existing at the time of defoliation.

However, there are aspects of the effect of water deficit on plant tissues, and the water relations and growth of arid zone plants which lead to the conclusion that shallow-rooted species, such as *A. vesicaria*, are likely to maintain high concentrations of stored photosynthate in their tissues (Section 2.3).

Both grazing and seasonal (or aseasonal) water deficit, among other factors, are known to affect the carbon balance, carbohydrate content and growth of arid zone shrubs (Mooney, 1972; Moore, 1977; Trlica, 1977; Depuit, 1979). The influence of season and intensity of use on the amount and rate of regrowth and carbohydrate content of pasture species has been the subject of extensive research in the United States (see Trlica, 1977).

Trlica and Singh, 1979). Some of these studies have shown that the vigour of regrowth (as measured by some aspect of production) is correlated with the carbohydrate concentration in plant tissues at the time of defoliation. In general, regrowth is slower and the amount of regrowth is less for plants with lower levels of carbohydrate reserves. Conversely, defoliation often results in a lower level of carbohydrate reserves after regrowth. If plants are defoliated late in the growing season regrowth and subsequent carbon gain and storage may be limited. Possible consequences are poor survival if adverse conditions follow (e.g. temperature or water stress), reduced growth during the next growing period, or a further decline in vigour after the next defoliation. Frequent heavy grazing may lead to a severe depletion of reserves and eventual death of the plant, but defoliation need be neither heavy nor frequent to affect plant vigour. Cook and Child (1971) showed that desert shrubs defoliated to the extent that vigour was only moderately reduced had not fully recovered seven years after the last treatment.

The evidence for the role of reserve carbohydrates in growth following a period of dormancy or quiescence and in regrowth after partial defoliation is largely circumstantial, mainly based on correlation between growth and carbohydrate content. Some workers have questioned the role of reserve* carbohydrates in regrowth (e.g., May, 1960; Jameson, 1963) an issue which is still not resolved. Nevertheless, carbohydrate content has been strongly (though not universally) implicated as an important influence on the response of plants to both grazing and environment and later articles have provided some evidence for direct use of labelled reserve carbohydrates at the growing

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*May (1960) has also questioned the use of the term 'reserves' because of the false impressions it may give about the role of stored carbohydrate. It is retained here for convenience and is used to denote stores of non-structural carbohydrate accumulated as a result of relatively high rates of photosynthesis during periods when growth and maintenance respiration are low.

point (Pearce *et al.*, 1969; Smith and Marten, 1970).

Much of this work was done with a veiw to using the results as a basis for sound management of vegetation supporting livestock. Of the two main factors affecting the survival of *A. vesicaria* grazing pressure is the most damaging and also the only one that can be manipulated. A knowledge of the interactions between carbohydrate content, growth and grazing is therefore of prime interest but since carbohydrate content may vary widely in response to phenological events a knowledge of its seasonal variation is also important, if only as a guide in planning defoliation or clipping treatments to best advantage.

The seasonal pattern of accumulation and depletion of total non-structural carbohydrate (TNC) has been described for a number of species from the American arid areas as a prelude to studies on plant response to defoliation (Coyne and Cook, 1970). Most species showed marked short-term fluctuations, which were not explained, superimposed on definite seasonal trends. This report has since been supplemented by papers on the effects of defoliation on vigour and carbohydrate content. (Cook and Child, 1971; Trlica and Cook, 1971).

To my knowledge no work of this kind has been done on any shrub species of the Australian arid zone except for the defoliation treatments imposed by Leigh and Mulham (1971) and Hodgkinson and Baas-Becking (1977). A start has been made on research into the response of *A. vesicaria* to clipping treatments (Bluff, 1980) but this work is not yet far enough advanced for any firm conclusions to be drawn. The only report on carbohydrate concentration is that by Wood (1932) who analyzed the leaves of three arid zone species, on two occasions, in connection with an hypothesis on drought resistance. The work presented in this thesis, a study of the pattern of accumulation and depletion of TNC and some of the factors affecting it, was

done as a first step towards an understanding of the carbon balance of *A. vesicaria*.

The work that has been done on *A. vesicaria*, although not specifically designed to answer questions about carbohydrate content and plant growth, is of interest here because of information on the response of the species to defoliation which allows some predictions about the location and level of reserves. This work is discussed in the next section and is followed by a discussion of the water relations and growth of arid zone plants, in which the factors most likely to affect the carbon balance and storage of carbohydrate in *A. vesicaria* are outlined.

2.2 The response of *A. vesicaria* to defoliation

In the grazing experiment reported by Wilson *et al.* (1969) the shrub population declined over a period of three years (Section 2.1). The authors later speculated about physiological responses to grazing which might have led to the death of these shrubs (Leigh and Wilson, 1970). Because the plants were defoliated by sheep the removal of leaf material from individual shrubs took place over several seasons and it was not clear whether shrubs died because they could not withstand the loss of existing leaves or whether the repeated removal of regrowth ultimately led to a complete exhaustion of reserves.

To obtain more information on shrub death in response to grazing, particularly on the influence of soil moisture status and season at the time of defoliation, Leigh and Mulham (1971) designed an experiment in which irrigated and control plots of saltbush were defoliated by sheep over a much shorter period of 8-12 days. The experiment was done in late summer and repeated in spring. The defoliation treatments were: complete defoliation, severe defoliation (1-40 leaves remaining) and control (ungrazed). All completely defoliated bushes died and the survival rate of shrubs in the severe defoliation treatments depended on the number of leaves remaining after defoliation; the higher the number of leaves the higher the survival rate. Leigh and Mulham suggested that completely defoliated shrubs died because of the removal of all growing points and that the survival rate of severely defoliated plants was a direct function of the number of sites left from which regrowth could occur.

However, whether the location of buds is sufficient explanation of shrub response to such treatments is not clear. Leigh and Wilson (1970) suggested that anatomical and morphological studies might provide an answer but these have not yet been done. Hodgkinson and Baas-Becking (1977) showed that bud removal need not necessarily result in shrub death. They removed all leaves

and visible buds from young saltbush plants and all recovered from this treatment. The fact that new growth by these plants occurred on young stem is consistent with the hypothesis of Leigh and Mulham but it appears, nevertheless, that the cambium can differentiate new buds when the existing ones are removed. From the pattern of regrowth observed by Osborn *et al.*, (1932) on drought-defoliated shrubs and the general growth habit of well watered plants it can be inferred that some buds must be located near the base of the shrub on old woody stem. On the basis of these observations it is perhaps more likely that regrowth is limited because severe defoliation inhibits the development of epicormic buds on old stem in some way, rather than by a lack of growing points.

More information on the response of *A. vesicaria* to defoliation has been provided by Hodgkinson and Baas-Becking (1977). To assess the effect of defoliation on root growth they imposed a number of treatments on plants grown in large sand filled boxes fitted with perspex windows. These plants, well watered and adequately supplied with nutrients, all recovered from quite severe defoliation and clipping treatments. The treatments were complete defoliation, complete defoliation with removal of all visible buds and lastly, clipping to a height of 50 mm from the soil surface. They observed a long period of depressed root growth following defoliation and suggested that reduced root growth may be responsible for the death of shrubs severely defoliated by grazing animals. Leigh and Mulham had observed some regrowth on completely defoliated plants but none of these shoots survived more than a few weeks even when the soil was well watered. Hodgkinson and Baas-Becking suggested that the shoots may have died because of the inability of the root system, which they described as stationary and dying, to supply the shoot with enough water to replace that lost by transpiration. The loss of roots and depression of root elongation may contribute to the death of defoliated shrubs in some circumstances but they do not explain the death of

severely defoliated plants which had been well watered before and after defoliation. Complete defoliation resulted in only 10 per cent loss of roots, and those remaining, although their extension rate declined, did not stop growing and began to recover four days after defoliation, attaining the original growth rate (approx. 30mm d^{-1}) in about 20 days. Considering the small amount of material left on severely defoliated plants, the effect on the root system is relatively minor. Even in the treatment involving complete defoliation and bud removal 50 per cent of the roots remained alive and eventually recommenced growth. However, since the remaining live roots did stop growing it is probable that such a treatment would contribute to the death of unwatered plants in the field (e.g. those defoliated by Leigh and Mulham) but it is difficult to explain the death of shrubs in well watered field plots on the basis of water stress. Such a large proportion of living root material (50-90%) should be able to supply enough water to the much reduced shoot in both severe and complete defoliation treatments especially when soil moisture status is high; yet even in the irrigated plots (75 mm every 3-4 weeks) there was a total loss of shrubs in the complete defoliation treatment. The survival of small shoots on these shrubs for extended periods indicates that the root system was able to supply water to maintain turgor in these leaves for some time. The fact that they did not develop further remains unexplained. The lack of regrowth by plants in unwatered plots, on the other hand, is not surprising as turgor, even in healthy fully foliated shrubs is probably not sufficient for rapid leaf expansion.

Leigh and Wilson (1970) in their review of the work by Leigh and Mulham suggested that auxins synthesised in the leaf may play a role in regrowth. This idea could be extended to include any hormone, or other stimulus to growth, normally supplied by the shoot to growing points in the plant. Defoliation in this case would act to remove the source of some necessary

stimulus for growth. However, the relatively rapid resumption of shoot growth by completely defoliated plants in the experiment by Hodgkinson and Baas-Becking leads to the conclusion that specific stimuli synthesised by the leaf are not of primary importance in the initiation of regrowth. The regrowth of drought-defoliated *A. vesicaria* must also be independent of such stimuli as is the growth of *Atriplex nummularia* and *Maireana sedifolia* from stem stumps after removal of the entire shoot. On the other hand, since removal of buds as well as leaves results in a fivefold increase in root loss, a hormonal effect cannot be ruled out. It is difficult to see how bud removal could elicit such a response on any other basis.

None of the mechanisms discussed so far can be ruled out as a contributing factor in the overall response of saltbush to grazing but the evidence is strongly indicative that some other factor is involved.

The observed responses are most easily explained by recourse to the hypothesis that *A. vesicaria* is unable to withstand heavy grazing because of a lack, or low level, of stored carbohydrates or an inability to mobilize the stores existing at the time of defoliation. Completely defoliated saltbush must rely on stored photosynthate for regrowth except perhaps for a possible contribution from stem photosynthesis. Their failure to respond to any great extent, despite being well watered could result from a low level of accessible carbohydrate, a condition which would also explain the eventual death of the plant. As suggested by Hodgkinson and Baas-Becking, the plant might eventually die because of the death of the root system but this response, I suspect, is due to the eventual exhaustion of reserves rather than an initial inability to supply the shoot with sufficient water and nutrients.

The survival of severely defoliated plants (Leigh and Mulham, 1971) was considered to be a direct function of the number of sites remaining for regrowth, but could also be a direct function of the amount of carbohydrate

left in young stem and leaf tissue, or of the amount of photosynthetic tissue providing assimilates, to maintain the plant body and support new growth. In the latter case, the dependence of survival on photosynthetic capacity also implies a lack of stored carbohydrate.

If, however, availability of stored carbohydrate is proposed to account for the lack of regrowth of plants in the field the reason for the resumption of growth of plants in the root growth experiment remains to be explained. There are at least two possible explanations, both related to the method of defoliation. Firstly, in the latter experiment most treatments did not involve the removal of stem thus leaving a bigger potential source of carbohydrates. When stem was removed, in the clipping treatment, the initial decline in root growth was more rapid, a response which the authors attributed to the removal of carbohydrate reserves contained in the stem. Secondly, and perhaps more importantly, the plants were defoliated by hand whereas those in the field were defoliated by sheep over a relatively long period. Twelve days is probably enough time for any reserves present to be committed to regrowth. Repeated removal of new growth would tend to lower the non-structural carbohydrate content of the plant. A single rapid defoliation by hand would not reduce the amount of carbohydrate in the remaining plant parts.

A similar explanation could account for the higher survival rate of plants defoliated by drought. Wood (1932) measured high concentrations of non-structural carbohydrates in the leaves of *A. vesicaria*. Withdrawal of carbohydrates and other organic and inorganic compounds, before leaf abscission, and storage elsewhere in the plant would provide a relatively large source of assimilates to maintain the plant during drought and to support subsequent regrowth.

Some aspects of the response of root growth to shoot pruning can also be explained on the basis of low carbohydrate content. Root growth could

be inhibited because of an increased demand for assimilates by the shoot. The root/shoot ratio is considered a dynamic equilibrium (Wareing, 1972) and shoot pruning in many plants results in a redistribution of assimilates in favour of the shoot (Brouwer, 1966; Wareing, 1972; Fick *et al.*, 1975). The failure of saltbush shoots to develop, despite this priority, again indicates a lack of stored carbohydrate. The increasing proportion of root loss with increasing severity of defoliation, and especially the response to bud removal, is more difficult to explain. The death of some roots, rather than simply a general reduction in the rate of growth of all roots, is perhaps a consequence of competition for a limited supply of assimilates. Strong sinks are known to attract a disproportionate amount of available assimilates (Evans, 1975) in which case less active roots may have been unable to compete successfully. Nevertheless, much of the response of *A. vesicaria* to both drought and defoliation still remains unexplained. There are quite complex exchanges of metabolites and nutrients between root and shoot, during normal growth (Wareing, 1972). Defoliation may disrupt the traffic of vitamins, amino acids or hormonal precursors between root and shoot (Pate, 1966; Crozier and Reid 1971).

The proposal that the observed responses are due to low levels of non-structural carbohydrates is also not entirely satisfactory since, as discussed in the next section, there are aspects of the water relations and growth of *A. vesicaria* that would favour a high carbohydrate content. If this is true it is possible that the response of the species to defoliation reflects an inability to mobilize stores of carbohydrate rapidly, perhaps as a result of hormonal imbalance, rather than a low concentration of carbohydrate in the tissues. A second possibility is that the bulk of carbohydrate which could support regrowth is located in the tissues removed by grazing animals.

2.3 The carbon balance of arid zone plants.

Arid lands have been defined (Noy-Meir, 1973) as "water controlled ecosystems with infrequent, discrete and largely unpredictable water inputs." Because of the low water input to these ecosystems a good deal of attention has been focused on the drought resistance of arid zone perennials which survive the often long intervals between rains by avoidance or tolerance of water stress. (e.g. Levitt, 1972). These forms of resistance are not mutually exclusive, however, as some species may tolerate an internal water stress which has developed despite adaptations which minimise water loss. Some species do not restrict water loss but avoid stress because they have access to large reserves of soil water, stored at high potential, from which transpiration losses can be rapidly replaced. Others avoid stress by early stomatal closure or a reduction of transpiring surface area (e.g. leaf rolling, leaf shedding) during the dry season (Parker, 1968; Orshan, 1972). A few ultimately survive because of an ability to tolerate dessication (e.g. Oppenheimer, 1960; Gaff 1971) but such a high degree of tolerance is more common among the lower plants.

The morphology of many of the water conserving drought avoiders also results in a high resistance to water vapour loss but since the pathways of water loss and CO_2 uptake are essentially the same, morphological and physiological adaptations which minimise transpiration also tend to reduce CO_2 uptake. Hence an important aspect of the survival of plants which do restrict water loss is the maintenance of a positive carbon balance over long periods (Anderson *et al.*, 1972; Levitt, 1972). This applies particularly to those plants in warm arid areas where the conditions favourable for high rates of growth are usually infrequent and of short duration.

During long intervals without rain these drought-persistent plants (terminology of Noy-Meir, 1973) are in danger of secondary drought injury (starvation) due to a negative carbon balance. The likelihood of such an

occurrence is offset to some extent by biochemical and physiological adaptations which allow CO_2 uptake under conditions of environmental or internal water stress. The high water use efficiency of succulents, for example, allows some carbon gain during prolonged droughts, due to their ability to close stomata during the day and fix CO_2 at night by the Crassulacean acid metabolism (CAM) pathway, when evaporative demand is relatively low. When environmental stress becomes severe they can apparently reduce carbon loss by recycling respiratory CO_2 internally (Szarek *et al.* 1973; Szarek and Ting, 1974). Woody shrubs which restrict transpiration less effectively are often tolerant of water stress and CO_2 uptake continues at a low rate when tissue water potential is low. Examples of an increase in water use efficiency at low leaf water potential have been reported for leaves of both C3 (Mooney *et al.*, 1977) and C4 (Pearcy *et al.*, 1974) species, a response which would prolong positive net CO_2 uptake. Acclimation of photosynthesis and respiration to prevailing seasonal temperatures in some species (Strain, 1969; Caldwell, 1972) also indirectly increases the capacity for carbon gain during hot dry weather.

Nevertheless despite adaptations which allow continued CO_2 uptake when internal water stress is high, water potentials may often fall below the hydration compensation point (water potential at zero net CO_2 uptake) in arid environments (Strain, 1969; Kozlowski, 1972). The activity of *Artemesia herba-alba* under severe stress illustrates this point. The leaves of *A. herba-alba* are capable of activity at low water potential as shown by Kappen *et al.*(1972) who recorded positive net photosynthesis by shoots of this dwarf shrub for two hours on a day when pre-dawn xylem water potential was -10 MPa. During the remainder of the 24-hour period the carbon balance was negative. Xylem water potential fell to -12.3 MPa on that day. A week later, when the minimum water potential had fallen to -16.3 MPa, carbon balance was probably negative for the whole day. Since root respiration was

not accounted for, the whole plant carbon balance was probably negative for some time before these measurements were taken.

The xylem water potential of *Atriplex vesicaria* falls to comparable values (-13 MPa) for considerable periods (Anderson *et al.*, 1972). Unless this species can match the photosynthetic capability of *A. herba-alba* carbon balance will be negative at such lower water potentials. According to Moore (1977) studies of *Eurotia lonata* and *Atriplex confertifolia* (both chenopods) have indicated photosynthetic capability comparable to that of *A. herba-alba* (White *et al.* unpubl.). There is also some evidence that *A. vesicaria* is capable of positive net CO_2 uptake at low water potential. Wood (1932) measured positive net CO_2 uptake for shoots of this species in summer at the end of a sequence of years of below average rainfall. He did not measure internal water stress but, judging from the pattern of rainfall in the months prior to this measurement (see Hall *et al.*, 1964) and the conditions prevailing at the time, it is likely to have been high.

Although photosynthate may be produced by some species throughout the dry season (Oechel *et al.*, 1972), most net production of structural biomass occurs during the short favourable periods when tissue water potential is high (Noy-Meir, 1973). The rates of cell division and elongation are often the most sensitive to water stress (Hsiao, 1973; Boyer, 1977). As water deficits develop these processes slow down and thereafter most production is channelled to storage except perhaps for a small proportion translocated to support continued root growth in localized pockets of high soil water potential (Newman, 1966). As a result, species which are able to assimilate CO_2 at low water potential are likely to have amassed considerable reserves before net CO_2 uptake is reduced to zero by severe stress. The subsequent rate of depletion is probably low (see Kappen *et al.*, 1972) as water deficit will have reduced the rate of respiration as well as that of photosynthesis (Brix, 1962; Crafts, 1968). The expected

increase in carbohydrate reserves, during periods of water stress, has been observed for a number of species (Brown and Blaser, 1965; Sosebee and Wiebe, 1971; Dina and Klikoff, 1973). In general therefore, it seems likely that apart from a slow drain during times of severe stress, the level of carbohydrate reserves in plants such as *A. vesicaria* will be high during the dry season.

However, if falls of rain heavy enough to result in high turgor and growth occur during the dry season reserves may be depleted for short periods as *A. vesicaria* is capable of very rapid growth when water potential is high (Jones and Hodgkinson, 1970; Williams, 1972). Wood (1932) stated that "maximal growth" of this species occurred following summer rains. This statement is consistent with the fact that *A. vesicaria* is a C4 plant, with the high temperature optimum for net photosynthesis associated with such species, but whether rapid summer growth could occur largely at the expense of current photosynthate, as it apparently does in the evergreen *Larrea divaricata* (Oechel *et al.*, 1972) is uncertain. Wood (1936) was of the opinion that *A. vesicaria* did not live on its reserves at any time but there is reason to believe that reserves may be used at least to initiate new growth following periods of severe water stress. For a shallow rooted species such as *A. vesicaria* leaf production and stem elongation in summer must be rapid if they are to occur before direct evaporation and transpiration have reduced soil water potential to a level below that necessary to allow the plant to maintain a critical turgor (Hsiao, 1973) at the growing points.

In many crop species the development of water deficits in the tissues results in a slow recovery on rehydration (Slatyer, 1967; Hsiao, 1973). Depending on the species and the severity of the water deficit, such plants can take one to several days to regain their full photosynthetic capacity. The slow recovery of these plants when rewatered may be due wholly or partly

to impaired stomatal function (Fischer *et al.*, 1970) or in some cases to persistent tissue water deficit (Boyer, 1971a; Slatyer, 1967). In one instance (Brix, 1962) photosynthetic capacity was not fully regained on rehydration. Although many arid zone species are more tolerant of water stress, their relative success in a drier environment does not guarantee perfect adaptation to severe water stress. Since arid zone plants develop water potentials very much lower than those required to produce an after-effect in crop species it is possible that some impairment of photosynthetic capacity results. In an environment where quite substantial falls of rain (12.5 mm) may evaporate within a single day (Cowling, 1969) a slow recovery would severely limit the growth of plants relying on current photosynthate as a source of energy and dry matter. Use of reserves would allow rapid production until the demand for photosynthate at growing points could be met by new leaf.

Low leaf water potential does not necessarily imply temporary or lasting damage to gas exchange capacity of shrubs since some dessication tolerant plants recover rapidly on rehydration. Ziegler and Vieweg (1970) described a rapid recovery of net CO_2 uptake in *Myrothamnus flabellifolia*, although Hoffman (1968) found that oxygen evoluation in this species took much longer to respond (7-24 hours) than respiration, which began immediately on rehydration. Studies on drought-persistent species of the warm arid regions of North America have shown that the recovery of photosynthetic capacity is rapid in some but not in others (Odening *et al.*, 1974).

Several days elapsed before the phyllodes of *Acacia aneura*, a dessication-resistant species of arid Australia, reached maximum relative water content (RWC) after a rain of 13 mm. Slatyer (1961) considered this period to be a measure of the rate of resumption of normal metabolism and growth. In contrast *Panicum maximum*, a tropical grass with a hydration compensation point of about -1.2 MPa recovered full photosynthetic capacity (adjusted

for leaf age) in two days (Ludlow, 1975). These plants were rewatered after leaf water potential had fallen to -9.0 MPa whereas the water potential of *A. aneura* phyllodes which took 3-4 days to recover was only about -8.0 MPa (RWC = 45%) when rain fell. The slow recovery of high RWC in *A. aneura* may have been partly a result of slow infiltration to a root zone of high density although this was not considered as a possible cause in the original article. The apparently anomalous difference between the latter two species may also be due, in part, to differences in the rate of dessication or in the length of the stress period.

Apart from the possibility of after-effects induced by drought when stress is severe and prolonged, there may be a further limitation to rapid structural growth for *A. vesicaria* and other drought-deciduous species in that when precipitation does occur these plants must photosynthesise with a reduced amount of leaf material. There may be an immediate demand for photosynthate in these species to replace the fine drought-deciduous roots in the nutrient-rich surface soils (Cowling, 1969). Although some new shoot growth may be supported by nutrients withdrawn from abscissing leaves root growth may have to precede rapid shoot growth and presumably must draw on reserves to do so. However, if net CO_2 uptake continues at low water potential the general level of reserves will remain high as any used during periods of rapid summer growth will be quickly replaced because high rates of structural growth are unlikely to be maintained for long in summer conditions.

The advantage of such prompt growth is not the replacement of, or increase in, reserve levels, which no doubt would have increased in the absence of structural growth but, in the short term a possible increase in drought resistance due to the productions of leaves with a higher water use efficiency (Cunningham and Strain, 1969; Orshan, 1972) and in the long term, the replacement of ageing leaves of reduced photosynthetic capacity, with young leaves better able to contribute favourably to the carbon balance of the plant.

In addition to the work on phenology and carbohydrate content outlined earlier, the following chapters include details of work on some issues discussed in this section. Studies of CO_2 exchange and growth during drying cycles were done to further define the periods of demand for and accumulation of non structural carbohydrates.

CHAPTER 3

3. Seasonal variation in total non-structural carbohydrate content

3.1 Introduction

It has been suggested in the previous chapter that the amount and availability of total non-structural carbohydrates are likely to be important factors controlling the extent and vigour of growth by individuals of *A. vesicaria* following partial defoliation or a period of water stress. Stored carbohydrates may serve as a source of energy for respiratory activity during growth and associated processes or as a source of structural components incorporated into new growth. As in previous studies of this kind the importance of stored carbohydrates, if present, has been assumed pending further evidence for their direct use at the growing point. In the long run the practical information required is the relationship between grazing, carbohydrate content and plant growth but since carbohydrate concentrations in plant tissues are known to vary seasonally, or at different stages of regular growth cycles, studies of such relationships may be more effectively planned and carried out if seasonal trends in carbohydrate concentration and content are known. In *A. vesicaria*, which has an opportunistic growth pattern, the seasonal variation in carbohydrate content may be more complex than that for species in temperate zones where the seasons and the annual growth cycle are well defined.

The results of a series of observations on the concentration of total

non-structural carbohydrate (TNC)* in the tissues of *A. vesicaria* are reported in this chapter. This work was done to establish firstly the existence of carbohydrate stores, secondly whether there are seasonal or aseasonal trends in concentration of carbohydrates and thirdly the location of such stores within the plant. The observed changes in TNC concentration are compared with the phenology of shrubs at the time of sampling and the prevailing weather conditions, particularly rainfall, in the interval before sampling.

The field site on Koonamore Station and the climate of the general area are also described to allow this work, as well as that in subsequent chapters, to be placed in perspective by others working on similar species elsewhere.

Because there has been a suggestion that regrowth following partial or complete defoliation of *A. vesicaria* is limited by the number of available growing points rather than carbohydrate concentration, as suggested here, the results of a series of observations on regrowth after complete defoliation are included in this chapter.

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* Total available carbohydrate (TAC) is a phrase often used by botanists to describe the carbohydrate energy readily available to plants. As defined by Coyne and Cook (1970), TAC may consist of one or all of starch, dextrins, fructosans, sucrose and reducing sugars but not structural carbohydrates such as pentosans, hemicellulose or cellulose. This definition will apply to TNC as used in this thesis. Smith (1969) has pointed out that total available carbohydrate is defined differently in studies of ruminant physiology and that interchange of information between plant and animal physiologists interested in grazing systems would be less confused if the more descriptive phrase TNC was adopted by botanists.

3.2 Study area

Field work was done at a site on Koonamore Station in the north-east pastoral district of South Australia about 400 km north of Adelaide (figure 3.1). Koonamore Homestead (32^{o} 04'S, 139^{o} 23'E) is more or less centrally located on a pastoral lease of 1200 km^2, near the southern edge of the winter rainfall arid zone (see Perry, 1967). The mean annual rainfall at the homestead calculated from 83 observations since 1888, is 214 mm. The annual rainfall of warm arid areas is typically highly variable and Koonamore is no exception with annual totals as low as 35 mm during a drought year and as high as 850 mm in the extremely wet year of 1974. Such extremes result in sustained alterations to the long-term running mean. The median annual rainfall, a statistic which is less affected by rare extreme values, is 184 mm. Rainfall and other climatic conditions at or near Koonamore are treated in more detail in Osborn *et al.* (1935), Hall *et al.* (1964) and Carrodus *et al.* (1965). Some of this information, as well as more recent figures for mean monthly rainfall, temperatures and relative humidity are summarized in figure 3.2. As rainfall figures only are regularly collected at Koonamore the remaining data are for Yunta, 65 km to the south.

Since Koonamore is located in the winter rainfall zone it receives rain from southern depression systems in the winter. However, there is also a large summer component, derived from northern monsoon systems, in the annual total. Hall *et al.* (1964) claim that there has been a marked increase in the incidence of summer rainfall at Koonamore since early this century. The high mean rainfall relative to the median in the summer months implies that much of this rain falls in light showers which probably do not result in plant growth. The relatively low mean number of rainy days, on the other hand, implies that summer rainfall is increased by a small number of very

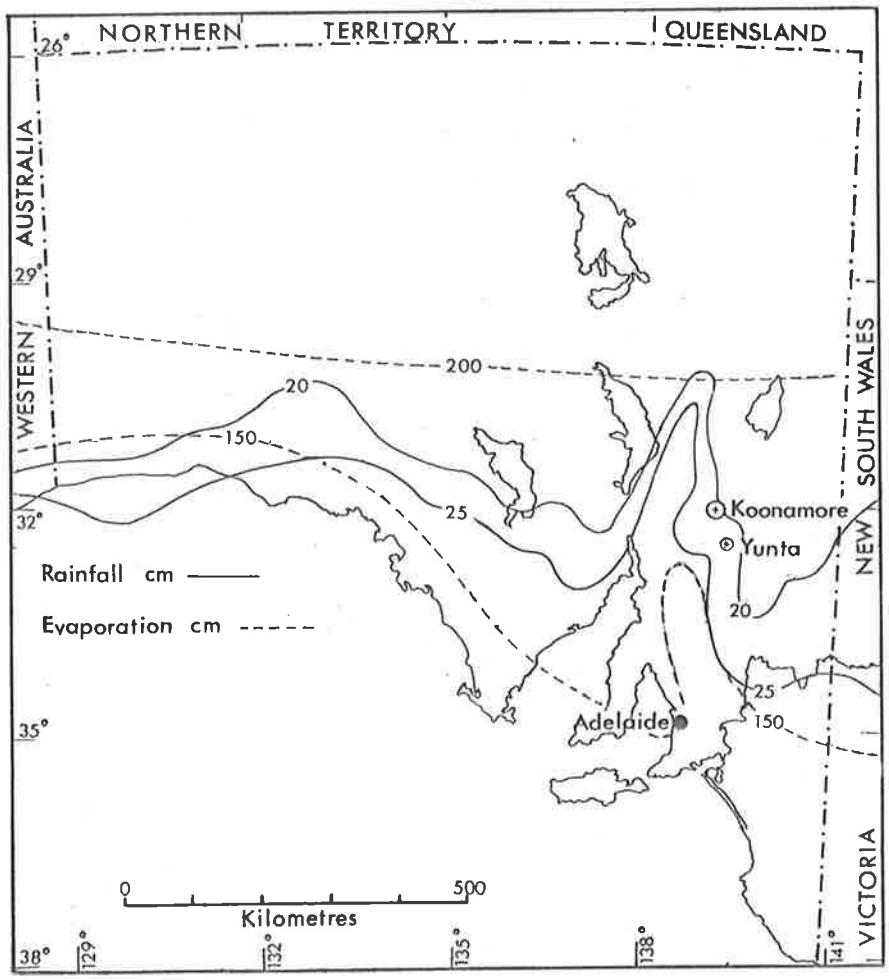


Figure 3.1 Map of South Australia showing the location of Koonamore and relevant isolines for annual pan evaporation and rainfall.

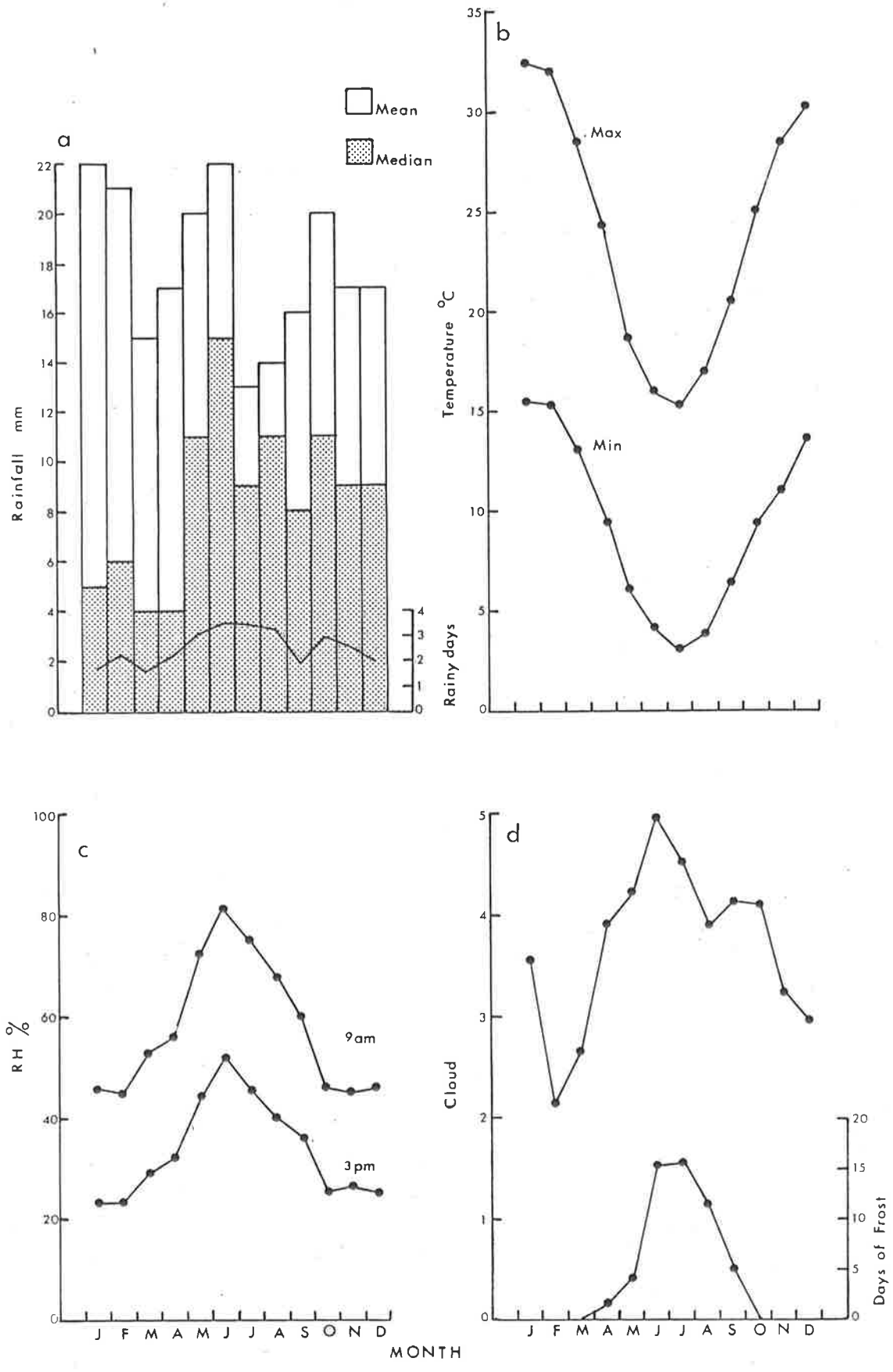
Figure 3.2 Mean monthly climatic conditions at Koonamore and Yunta

(a) Rainfall statistics (Koonamore)

(b) Maximum and minimum temperature (Yunta)

(c) Relative humidity (Yunta)

(d) Cloud cover on a scale of 0-10 (Yunta). Mean of 9am and 3pm.
Days of frost (Koonamore)


a
Mean
Median
Rainfall mm
Rainy days
b
Max
Min
Temperature °C
c
9am
3pm
RH %
d
Cloud
Days of Frost
J F M A M J J A S O N D
MONTH

heavy showers. Figures in Hall *et al.* (1964) show that although the probability of a dry month is greatest in summer and autumn, the heaviest recorded falls of rain have occurred at these times. Most falls of rain greater than about 40 mm (over 125 mm in one instance) have been recorded between November and March inclusive.

Carrodus *et al.* (1965) also described the landscape, vegetation and soils of Koonamore. Six vegetation sub-forms, ranging from ephemeral herb and grassland to arid scrub were recognised and subdivided into 15 associations. *Atriplex vesicaria* dominates some areas of low shrubland and is codominant with *Maireana sedifolia* and *M. astrotricha* in others. It is also prominent in the understorey of low open woodlands dominated by *Myoporum platycarpum*, *Casuarina cristata* and *Heterodendrum oleifolium* Annuals or biennials, mainly *Stipa* (spear grass) and *Sclerolaena* (bindy-i) species, occupy the area between the shrubs.

Soil profiles were sampled by Carrodus *et al.* at frequent intervals along traverses across the property, usually wherever changes in vegetation occurred. The 15 vegetation associations described for the station were found on six intergrading groups of soils. The groups are a local classification and often include more than one of the main classes of soils described, for example, in Northcote (1960). The heavy textured soils found in low-lying saline areas ('Lakes' soils) are replaced by lighter textured soils in hilly areas ('Hills' soils). *A. vesicaria* grows in extensive stands on the shallow uniform calcareous loams and duplex soils of the 'Oopina' soil group and in mixed stands with *Maireana astrotricha* and/or *M. sedifolia* on the transition zone between the Oopina and Hills soils.

A wide belt of country across the centre of Koonamore is characterized by sand plains and sand dunes supporting communities dominated by

Acacia aneura (mulga) *Casuarina cristata* (black oak) and to a lesser extent by *Eucalyptus socialis* (mallee). Distributed among the dunes and between dune systems are large areas of solonized brown loam (Gc 1.12 of Northcote, 1960) in which the texture gradually becomes finer with depth due to the flocculation and leaching of clay colloids in the presence of sodium and calcium ions in the soil solution. Limestone nodules are present at varying depth in the profile of all the soils in this group (Bindy-i soils). In the upper profile of the loams there are often many nodules which may be cemented into a prominent kunkar layer close to the soil surface. These soils support low shrublands of *A. vesicaria, M. sedifolia* or a mixture of the two. Large areas of low open woodland on these soils have a similar shrub stratum. *Myoporum platycarpum* provides a sparse canopy in these areas.

A field site adjacent to the Koonamore Vegetation Reserve was set up by fencing an area of about one hectare, to exclude domestic stock. The paddock in which the enclosure was located is normally used only when sheep have been mustered for shearing and is lightly grazed, if at all, at other times of the year. Grazing is intermittent but heavy. No extra measures were taken to exclude rabbits and kangaroos from the experimental site. The vegetation in the immediate area is a low open woodland as described above, with *A. vesicaria* and *M. sedifolia* dominating the shrub stratum. Other species of *Maireana* are scattered over the area with *Lycium, Chenopodium* and *Nitraria* species occupying slight depressions. The annual component of the vegetation consisted mainly of *Stipa nitida, Sclerolaena patenticuspis* and *Dissocarpus paradoxus*. Soils are as described above with much nodular limestone in the upper layers of the profile. A solid crust of limestone near the surface was evident in only a few places. Representative profiles and mechanical analyses can be found in Osborn *et al.* (1931) Carrodus (1962) Carrodus and Specht (1965) and Carrodus *et al.* (1965).

Individual profiles, give some indication of the variation of soils in the field. Mechanical analysis of the surface layers of 'saltbush soils' by Osborn *et al.* (1931) for example, revealed a high proportion of coarse sand. The surface soil in another profile of this soil type was composed mainly of silt and fine sand. A survey by Crisp (1975) also showed that the surface soils of the nearby reserve graded continuously from sand through loam to silty loam.

3.3 Bud growth after defoliation

At various times during 1978-1979 and on one occasion in 1980 (autumn) several shrubs were defoliated completely and the buds which put out new leaves were counted for the following few months. The plants were defoliated by hand. The majority of leaves on a young stem were removed in a single action by stripping the stem between thumb and fingers, a process which also removed small laterals and non woody apices. Any leaves remaining were then picked off individually. Young growth in less accessible parts of the plant, such as that among the old woody branches at the base of the shrub, was removed completely. On most occasions during 1978 half the number of defoliated shrubs were irrigated with 25 mm of rainwater immediately after defoliation. In 1979, a relatively wet year none of the defoliated plants were watered. Details of the irrigation procedure can be found in Chapter 7. The results of bud counts made 1-2 months after defoliation are shown in the following table.

Table 3.1 Mean number of buds per plant

	Season	Month	n	Mean $\pm$ s.e.	Range
1978	Summer	Dec-Feb	8	13 $\pm$ 3.8	3-31
	Autumn	Mar-May	4	12.8 $\pm$ 5.5	1-23
	Winter	June-Aug	6	4.3 $\pm$ 2.6	0-16
	Spring	Sept-Nov	6	7.5 $\pm$ 5.3	0-32
1979	Winter*	July	10	16.5 $\pm$ 3.0	3-38
	Spring*	September	6	0	-
1980	Autumn	March	8	18.6 $\pm$ 5.8	4-47

*Not irrigated. n = number of plants.

In 1978 most buds did not produce more than two small expanded leaves subtending the apex, which was enclosed by 2-3 very young folded leaves.

Rare instances of shoots with 5-10 leaves, including a few fully expanded leaves, were observed; these developed from epicormic buds on old stem near the base of the shrub. All died within five months from the date of defoliation except those defoliated in March, 1980. The smaller shoots described above had usually withered within two months of defoliation of the shrub. Bud expansion was apparently initiated soon after defoliation. In midsummer 1978, for example, expansion and leaf production were evident within two days. During that year unwatered plants showed no sign of regrowth except for a few buds produced in midwinter.

One of the main features of the data presented in table 3.1 is the variability in the number of buds produced per plant, as shown by the relatively large standard errors. In the first year part of the variation may be attributed to the fact that not all plants in a given season were defoliated in the same month. However, in 1979 and 1980 the figures given are for plants defoliated on the same day. The range of values is similar to that for plants in 1978.

These results are in sharp contrast to those of Leigh and Mulham (1971) who monitored plants defoliated by sheep in spring and midsummer (Chapter 2). Even with a much larger sample size (3 plots of 20 plants in each season) they recorded regrowth from only one point on each of several plants in the summer group. At Koonamore, a complete lack of regrowth, by any of the shrubs, was observed on only two occasions, both in spring. At other times of the year while there were some plants which produced very few, if any, shoots others resprouted from a large number of sites. This was true even of the shrubs defoliated in March 1980. when two individuals produced enough regrowth to survive the treatment. Regrowth was prolific when compared with that produced by shrubs on previous occasions. Although many of the buds did not develop into large shoots and a proportion had withered by the time regrowth was first recorded in June, about 2 months after defoliation, a few plants produced up to 20 large shoots with 10 or more fully expanded

leaves. Some of these shoots with about 30 leaves had begun to produce laterals. On this occasion the unirrigated controls also resprouted from 4-12 sites but most had died by June. The 75 mm of rain which fell between late March and early June no doubt contributed to the uncommon response from both control and irrigated plants. Two of the shrubs which produced a large amount of regrowth were still alive 10 months later although the degree of foliation was less at that time. Figure 3.3, Plate 2 shows the extent of regrowth by the most successful of these two shrubs. The photograph was taken seven months after defoliation.

Another aspect of the overall response to defoliation was the distribution of buds between old and young stem. In 1978 over 65 per cent of expanded buds were located on old woody stem. In 1979 the number of buds located on old stem was only 39 per cent of the total. This difference is significant ($P < 0.01$) despite considerable variation between plants. The difference between the two years may be a function of the age of the plants at the time of defoliation. In 1978 the size of the plants and hence presumably their age was much less than that of shrubs defoliated in the following year. The dry weight of leaf and young stem stripped from plants in 1978 and 1979, for example, was 8.7 ± 1.2g and 34.8 ± 3.0g respectively. Adventitious buds forming on the vascular cambium or the periphery of the vascular cylinder of woody plants may become buried in the bark as the stem increases in diameter (Berg and Plumb, 1972). In *A. vesicaria* where secondary growth arises from a series of cambia successively further out from the primary vascular cylinder early formed buds may also become more and more isolated from the outside, although this process should not reduce the number of epicormic shoots unless later formed cambia produce fewer adventitious buds. This explanation relies on the assumption that buried or isolated buds do not sprout readily.

Regardless of the reason for these differences it is evident that

Figure 3.3 Regrowth of *A. vesicaria* after complete defoliation.

Plate 1 shows the predominantly basal pattern of regrowth two months after defoliation.

Plate 2 shows the extent of regrowth after seven months.

1

2

regrowth by *A. vesicaria*, at least at Koonamore, is not limited by a lack of growing points. In particular, the potential for regrowth from adventitious buds on old woody stem appears to be quite high. Figure 3.3 Plate 1 shows the predominantly basal pattern of regrowth by one of the shrubs defoliated in March 1980. Other shrubs produced shoots on younger stem in addition to those arising from old woody stem.

This result is at variance with the suggestion by Leigh and Mulham (1971) that all growing points are located on youngstems. It is possible that the observed difference in the response to defoliation between plants at Koonamore and on the Riverine Plain (Emmet Vale) is related to the method of defoliation. Shrubs at Emmet Vale were defoliated by sheep over a period of 12 days. If any regrowth did occur during this time sheep may have reduced the number of available buds by nipping off young emerging shoots. As mentioned earlier on one occasion at Koonamore buds on defoliated shrubs began to expand within 2 days.

If on the other hand their speculation on the location of buds is correct then there are, apparently, differences between saltbush on the Riverine Plain and those at Koonamore. This may well be an example of anatomical and/or physiological differences between two forms of the species defined by Parr-Smith and Calder (1979). A discussion of these forms was included in section 1.12 where it was noted that the Riverine Plains form is restricted to that area. However, although shrubs at Koonamore which belong to a much more widespread form, usually produced many small shoots after defoliation at various times of the year, in most cases the potential for regrowth was not realized. Shrubs survived defoliation on only one occasion (March, 1980). Apart from that instance the longest surviving shoots of appreciable size (10 leaves) also grew on shrubs defoliated at the same time of the year (March, 1978). A possible explanation for this coincidence is suggested in the discussion of the results for TNC analyses which are presented in section 3.5.

3.4 Materials and methods

3.41 Sample selection

All plant material for TNC analysis in the laboratory was collected from the field site described in the previous section. During 1977-78 3-5 female and an equal number of male plants, were collected at approximately monthly intervals from a population of young plants protected from grazing. In 1979 five female plants were selected at each sampling date. Plants were chosen at random from a tagged population chosen for uniformity of size and appearance. Old, very open plants with a high proportion of dead branches and twigs, for example, were avoided. In general the plants sampled were young, mature plants between 30-40 cm high and 20-30 cm in diameter. At different sampling times throughout the year the amount of leaf on these plants varied but when initially selected they were all well foliated. Collections were made at the same time of day, shortly after sunrise, on each occasion to avoid the possibility of fluctuations in the measured value for TNC due to diurnal changes in carbohydrate content. The entire shoot and a subsample of the root system of each plant was collected and later processed in the laboratory. It was found impractical to attempt a thorough excavation of the root system because of the soil structure and the high concentration of limestone nodules in the soil profile. The brittle saltbush roots were readily broken on removal of the rubble. The root system was subsampled by loosening the soil around the plant to a depth of 20-30 cm and easing the plant from the soil to retain as much of the unbroken root system as possible. This procedure resulted in a large sample of both young and woody roots and had the advantage of reducing the time between disturbance of the plant and processing of the sampled material. Most of the root system to a radius of about 30 cm was retained on the plant. Only those roots still attached were used for TNC analysis.

In the first few months individual plants were collected on successive days and separated into fractions within two hours. The various plant parts

were then killed in boiling 80% ethanol and air dried. The plant material together with the residue of solids extracted by the ethanol was later milled, oven dried and stored in sealed glass vials. Although it is desirable to kill the plant material as quickly as possible, to minimise respiratory losses after collection, the procedure adopted for the first sampling dates was found to interfere with other work during field trips and subsequently all plant material was stored under dry ice until returned to the laboratory where it was stored at $-20^{o}C$ until processed. Dry ice was manufactured in the field from a cylinder of compressed CO_2. A mixture of gaseous and liquid CO_2 forced up an eductor tube reaching to the bottom of the cylinder was sprayed over the plants with a device of the type used to freeze water in pipes during maintenance work in large buildings. This consisted of a hand held section of high pressure tubing fitted with a fine nozzle and attached directly to the cylinder outlet. During rapid expansion of the CO_2 a thick layer of dry ice forms over the plants. Because the CO_2 is forced out under full cylinder pressure the plants were sealed in plastic bags to avoid loss of leaf material. The device is simple and robust and it can be left attached to a small cylinder of CO_2 enabling easy replenishment of the dry ice during transport to the laboratory in hot weather.

3.42 Sample preparation

The plants were separated into six fractions in the laboratory. Those were leaf, young stem, young woody stem, old stem, young root and woody root. The young stem and root fractions were separated from older tissue on the basis of a subjective assessment of their woodiness. The young stem, for example, could be bent before it broke whereas young woody stem although of similar diameter was brittle and snapped easily. Old and young woody stem were separated, arbitrarily, at the point where splits in the bark became noticeable. The leaf fraction in 1977-1978 contained a small

amount of non-woody young stem. During that period the shoot was subsampled by selecting entire branches until enough of the smaller fractions had been sorted. Root samples were washed in cold water to remove residual soil and any obviously young tissue removed with the soil was separated from the bulk of the root system by flotation and sieving. Only non-woody root was classified as young; the remainder was bulked with older material irrespective of its diameter. The fractions were killed as described earlier and the ethanol evaporated under lamps in a fume hood. The air-dried material was then ground to pass a 1 mm screen in a small hammer mill, oven dried at 70°C and cooled in a dessicator before being sealed into glass vials.

Priestley (1962) emphasised the importance of calculating the weight of TNC in various plant parts in order to identify the major storage sites. Since this calculation depends on the size of the storage site as well as the TNC concentration, in 1979 the whole shoot was separated into fractions and the dry weight of each measured. The crown, defined by Coyne and Cook (1970) as the woody tissue between the first stem branch and the first concentration of roots was included as a seventh fraction. The calculation of TNC content for the various fractions is described later.

In the previous year it was found difficult to separate leaf and stem completely when the shoots were fresh and since soluble carbohydrates are extracted when tissues are killed in boiling 80 per cent ethanol no further attempt was made to separate the young stem contained in the leaf fraction after the solvent had evaporated. For these reasons in 1979 the leaf and young stem tissues were killed by drying in an oven for up to an hour at 100°C. The remaining plant parts were also treated in this way. Because carbohydrate losses can occur if tissues are dried completely at 100°C (Smith,1969) drying was completed at 70°C. The high initial temperature was used to rapidly denature respiratory enzymes and to decrease the overall drying time. The dried tissue was then easily separated into

fractions composed entirely of leaf or young stem. The samples were then milled, redried and stored in glass vials with tightly fitting caps.

3.43 TNC extraction and analysis

Non-structural carbohydrates were removed from dried plant material by extraction in hot dilute sulphuric acid. Arguments for and against the dilute acid extraction method have been presented by Priestly (1962) and Smith (1969). The merits of those arguments as they apply to this study are examined in the discussion of results reported in this chapter.

Accurately weighed samples of about 250 mg were refluxed in 25 ml of 0.2 N sulphuric acid for one hour. The hot solution was then filtered to remove woody material and cooled to room temperature. Proteins were not removed.

The concentration of hexose sugars in the extract was assayed by the anthrone method described in Yemm and Willis (1954). The intensity of the blue-green colour produced when carbohydrate solutions are heated with anthrone in concentrated H_2SO_4 is linearly related to carbohydrate concentrations in the range 0-100 µg/ml.

The anthrone reagent (9,10 dihydro-9-oxoanthracene) was prepared by dissolving 0.2 g of the compound in 100 ml of sulphuric acid made by adding 500 ml of concentrated acid (AR) to 200 ml of water. The solution was allowed to stand for at least an hour, with occasional shaking, until perfectly clear. The reagent was freshly prepared each day and used within 6-8 hours. The acid solvent concentration used, viz. that chosen by Trevelyan and Harrison (1952) to minimise the heat of mixing on addition of the aqueous sample, is only just high enough to prevent precipitation of anthrone from the final solution. For this reason the solvent was also prepared regularly rather than in bulk to prevent gradual dilution by water vapour from the atmosphere. As found by Yemm and Willis (1954), some commercial samples of anthrone were not sufficiently pure and did not dissolve completely in the

acid solvent. These batches were recrystallized from benzene and light petroleum (p. 741 Vogel, 1957) before use.

The anthrone reagent (7.5 ml) was pipetted into thick-walled Pyrex tubes and chilled in ice water. Appropriate dilutions of the carbohydrate extracts were made and 1.5 ml aliquots were layered on the anthrone solution. The tubes were chilled for a further 5 minutes before the contents were rapidly and thoroughly mixed and rechilled. The tubes, capped with glass marbles, were then lowered into a vigorously boiling water bath, in a fume hood, and heated for 10 minutes. The solutions were cooled to room temperature and the absorbance at 625 nm read immediately against a reagent blank in either a Beckman DB or Coleman 295E spectrophotometer. The reagent blank consisted of 1.5 ml of glass distilled water treated in the same way as the samples. Each run included duplicate samples of several standard glucose solutions. The TNC concentration of plant extracts was estimated from the mean absorbance of triplicate aliquots and a calibration curve based on the absorbance readings of the standard solutions.

The colour reaction with anthrone is specific for carbohydrates although other compounds may interfere with the reaction. One of the main interfering substances is the chloride ion. Chloride concentrations as low as 0.02 M resulted in a 10 per cent higher colour production in test solutions (Scott and Melvin, 1953). Use of the anthrone method for saltbush may result in overestimates of TNC concentration, particularly in the leaf fraction, to a different extent at different times of the year unless the interfering ion is removed. In this study chloride was removed by ion exchange. The sample dilutions for TNC analysis were deionized by shaking for 10 minutes with a mixed bed resin composed of equal parts of a weakly basic anion exchange resin (Amberlite IR45(OH)) and a strongly acid cation exchange resin (Amberlite IR120 H). Sugars are not taken up by ion exchange resins of the type and form used in this study (Kressman, 1956; Morries and Stuckey, 1956).

The mixed bed resin was regenerated when necessary by separating the components and treating with the appropriate acid or alkali. Separation of the two resins was achieved by pouring the mixture into a column and back-flushing the column slowly with distilled water. The chloride form of the anion exchanger is less dense than the sodium form of the cation exchange resin and a sharp boundary is quickly formed between the two. The upper layer can then be siphoned off with very little if any contamination from the cation exchanger. The chloride concentration of the sample dilutions was checked, before and after ion exchange, by titration of duplicate sub-samples with silver nitrate (Mohr titration p.260, Vogel, 1961).

3.5 Results: Seasonal variation of TNC

3.51 TNC concentration

The results of TNC analyses for shrubs collected over a period of 28 months are shown in figures 3.4 to 3.6. The data points in these diagrams represent the TNC concentration, expressed as glucose equivalents in mg/g dry weight, for various plant fractions. The TNC concentrations were calculated from the mean of three absorbance readings for each dilute acid hydrolysate. Extracted samples were composed of equal subsamples of the dried material from 3-5 plants collected on a given sampling date. The phenological stage of the plants at the time of collection is shown by the discontinuous horizontal bars above the set of TNC curves. A solid line to the left of a particular date indicates that the majority of plants included in the TNC sample on that day were, for example, flowering or showed evidence of recent root growth. Broken lines indicate that some evidence of vegetative or reproductive growth was noted but in less than half of the sampled plants. The extension of the bar back to the previous date signifies that growth occurred at some time in the interval between the two samplings.

The accompanying vertical bar diagrams depict two sets of rainfall data. The upper diagram shows the rainfall accumulated at the field site between sampling dates, while the lower shows daily rainfall figures for Koonamore Homestead, about 7 km to the north. Because of the possibility of local thunderstorms, particularly in summer and autumn, the incidence of rainy days at the two recording stations cannot be expected to correspond exactly at all times (e.g. May 1978). Nevertheless the latter are included to indicate the probable distribution of rainfall at the field site during the intervals between readings.

Figure 3.4 shows seasonal trends in TNC concentration for the various fractions from samples of female plants collected between October 1977 and December 1978. Until February 1978 young woody stem was included as old stem. Similarly young root was combined with woody root before that date. The TNC concentration of the older material is unlikely to have been altered significantly as the dry weight of the younger tissue was only a small proportion of the total. As shown by the horizontal bar for root growth young root did not appear in appreciable amounts until May. Leaf samples during 1977-78 contained a small amount of young stem, which is also considered negligible.

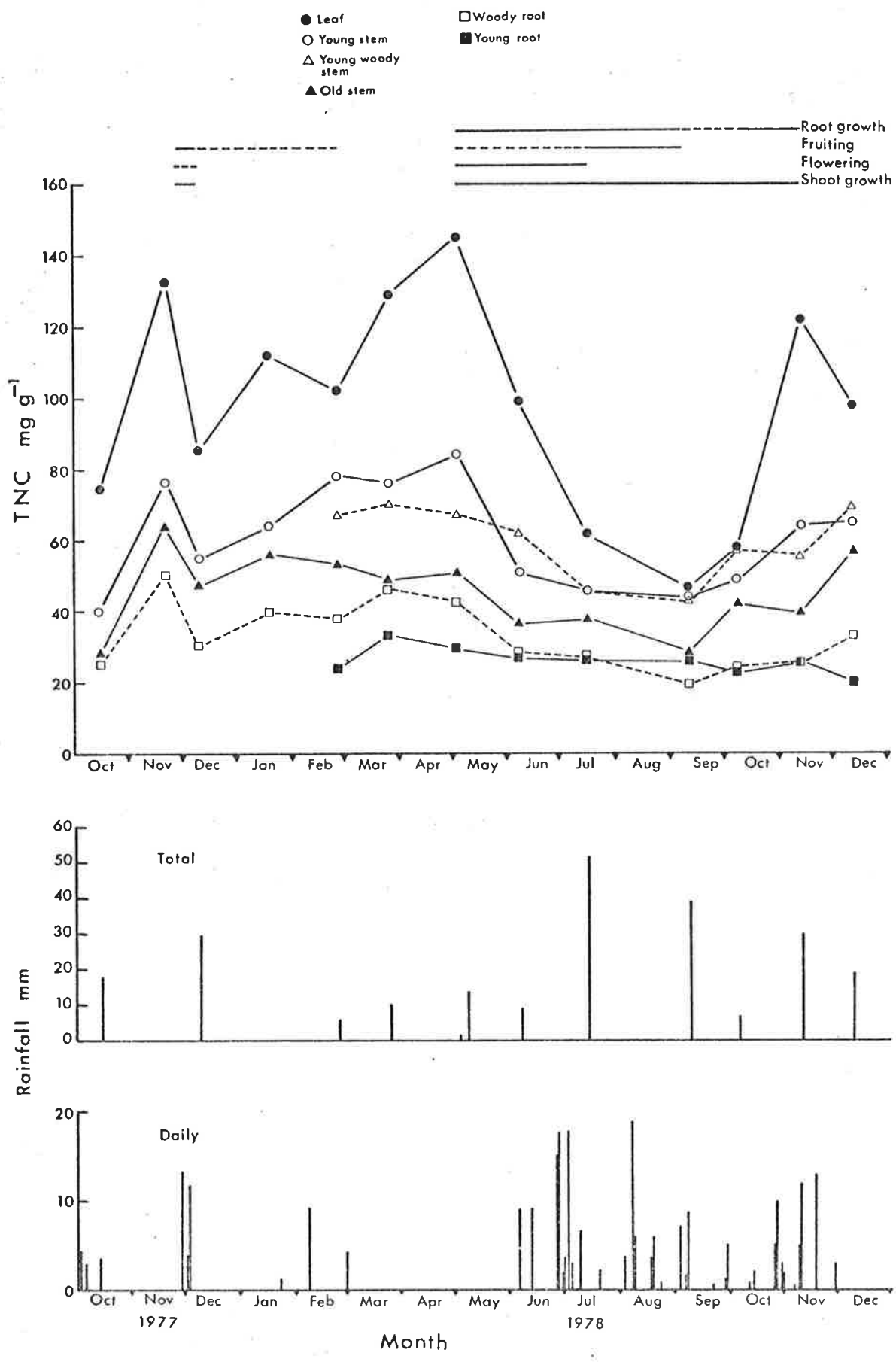
A marked seasonal trend in carbohydrates is evident from figure 3.4. TNC concentrations were highest in summer and autumn and lowest in winter and early spring. This pattern applied to all the fractions measured but the range of values was greater for the above-ground organs. The concentration of TNC was higher in leaves than in young stem and while there appeared to be little difference between the two young stem fractions these had concentrations higher than that of old stem. TNC concentration in root samples was in turn lower than that in old stem.

The fluctuations in TNC concentration were largely consistent with the pattern of growth and rainfall. A substantial rain in early December 1977 resulted in a short flush of growth and a large depression of TNC concentration in all the fractions relative to those recorded for the November samples. In the following low rainfall period, when little if any vegetative or reproductive growth was apparent, there was an increase in carbohydrate concentration in most fractions. TNC concentrations began to fall before the major winter rains in July. The onset of growth prior to the sampling date in June illustrates the effectiveness of small amounts of rain at this time of the year. According to the daily rainfall figures from Koonamore

Figure 3.4 Total non-structural carbohydrate concentration (TNC mg g^{-1}) for combined subsamples of the dried tissue from samples of 3-5 female plants collected from the field at monthly intervals between October, 1977 and December, 1978.

The horizontal bars show the pattern of vegetative and reproductive growth assessed from notes on the phenology of sampled shrubs. The continuous line indicates that half or more of the shrubs were active and the broken line, less than half.

The accompanying diagrams show total rainfall accumulated at the experimental site between samplings, and daily rainfall for Koonamore Homestead, 7 km to the north.


Leaf
Young stem
Young woody stem
Old stem
Woody root
Young root
Root growth
Fruiting
Flowering
Shoot growth
TNC mg g⁻¹
160
140
120
100
80
60
40
20
0
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Rainfall mm
Total
60
50
40
30
20
10
0
Daily
20
10
0
1977
1978
Month

the 10 mm of rain collected from the field site gauge in June, fell only a few days before sampling. The second and larger of the two falls in May occurred a few days after the sampling date in that month and hence total rainfall in the interval between samplings was 24 mm. Thus both the timing and amount of rainfall may have contributed to growth and the fall of TNC concentration between May and June. There are, nonetheless, some fluctuations in TNC concentration which cannot be explained on the basis of rainfall or phenology notes, notably those for leaf material in February and December 1978. This may simply be due to the inadequacies of using phenology notes to assess the stage of growth. This point will be referred to in the next chapter where results of shoot and root growth measurements for other plants are reported. Other possible reasons for the fall in TNC concentration on those occasions include a redistribution of carbohydrates from leaf to stem or their use as a source of energy for osmotic adjustment during periods of high evaporative demand and relatively low rainfall.

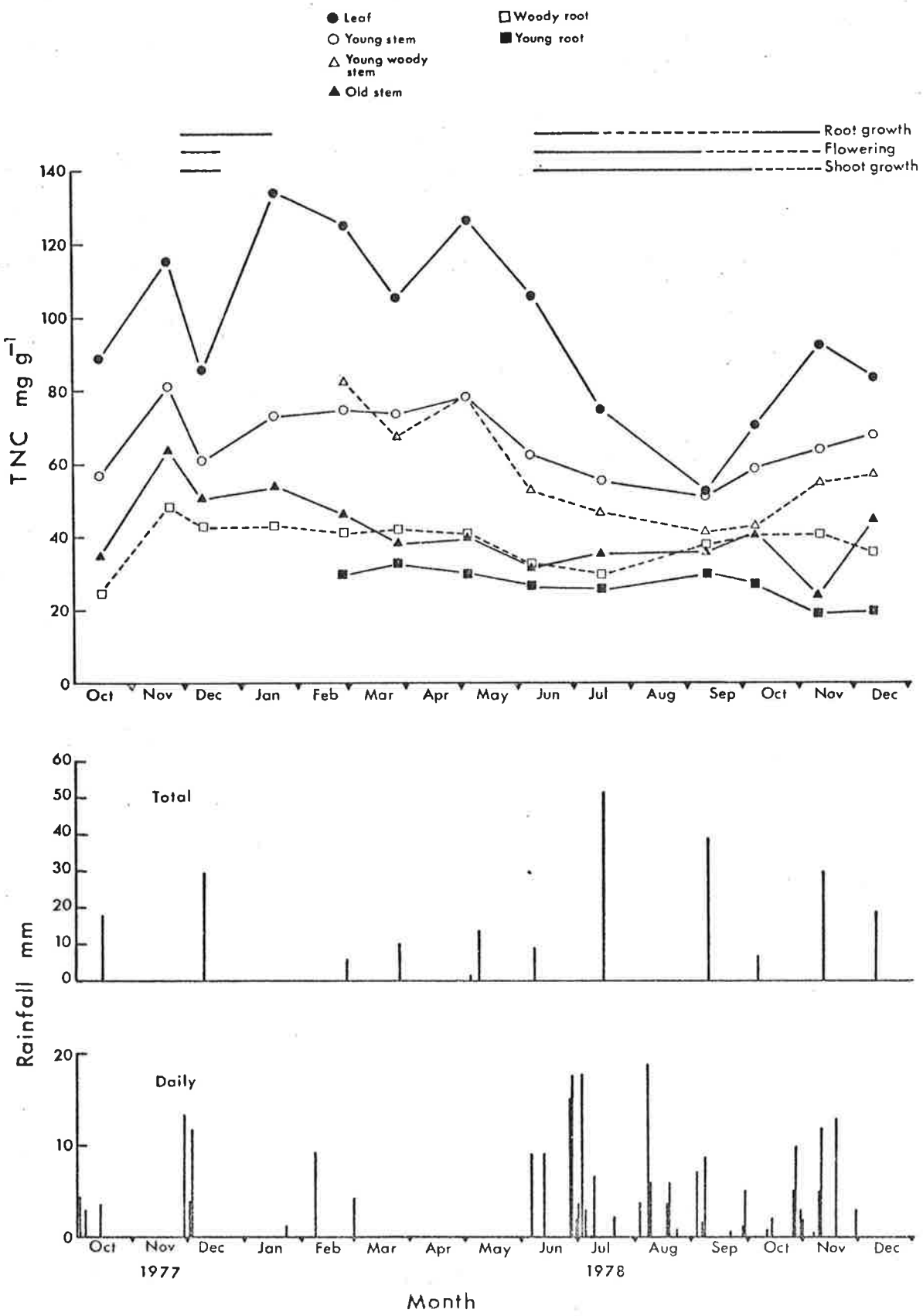
Figure 3.5 shows the seasonal pattern of TNC concentration in the various fractions from male plants collected on the same dates as the female plants. The trends are similar to those for female plants and hence the comments in the preceding pages also apply here.

The large fall in carbohydrate concentration for the old stem fraction in November 1978 coincided with a period of prolific root production. Many young root tips were obvious on male plants on that occasion. The depression of TNC concentration in the same fraction of the female plants was less pronounced. The amount of root growth was more variable for female plants. For example, one of the females had produced long sections of large succulent young roots 2-3 mm in diameter but others showed little or no evidence of recent root growth.

The replenishment of TNC stores in old stem between November and December may partly account for the previously unexplained fall in TNC

Figure 3.5 Total non-structural carbohydrate concentration (TNC mg g^{-1}) for combined subsamples of the dried tissue from samples of 3-5 male plants collected from the field at monthly intervals between October, 1977 and December, 1978. The horizontal bars show the pattern of vegetative and reproductive growth assessed from notes on the phenology of sampled shrubs. The continuous line indicates that half or more of the shrubs were active and the broken line, less than half.

The accompanying diagrams show total rainfall accumulated at the experimental site between samplings, and daily rainfall for Koonamore Homestead, 7 km to the north.


Leaf
Young stem
Young woody stem
Old stem
Woody root
Young root
Root growth
Flowering
Shoot growth
TNC mg g⁻¹
140
120
100
80
60
40
20
0
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Total
Daily
Rainfall mm
60
50
40
30
20
10
0
1977
1978
Month

concentration in the leaf fraction during that interval.

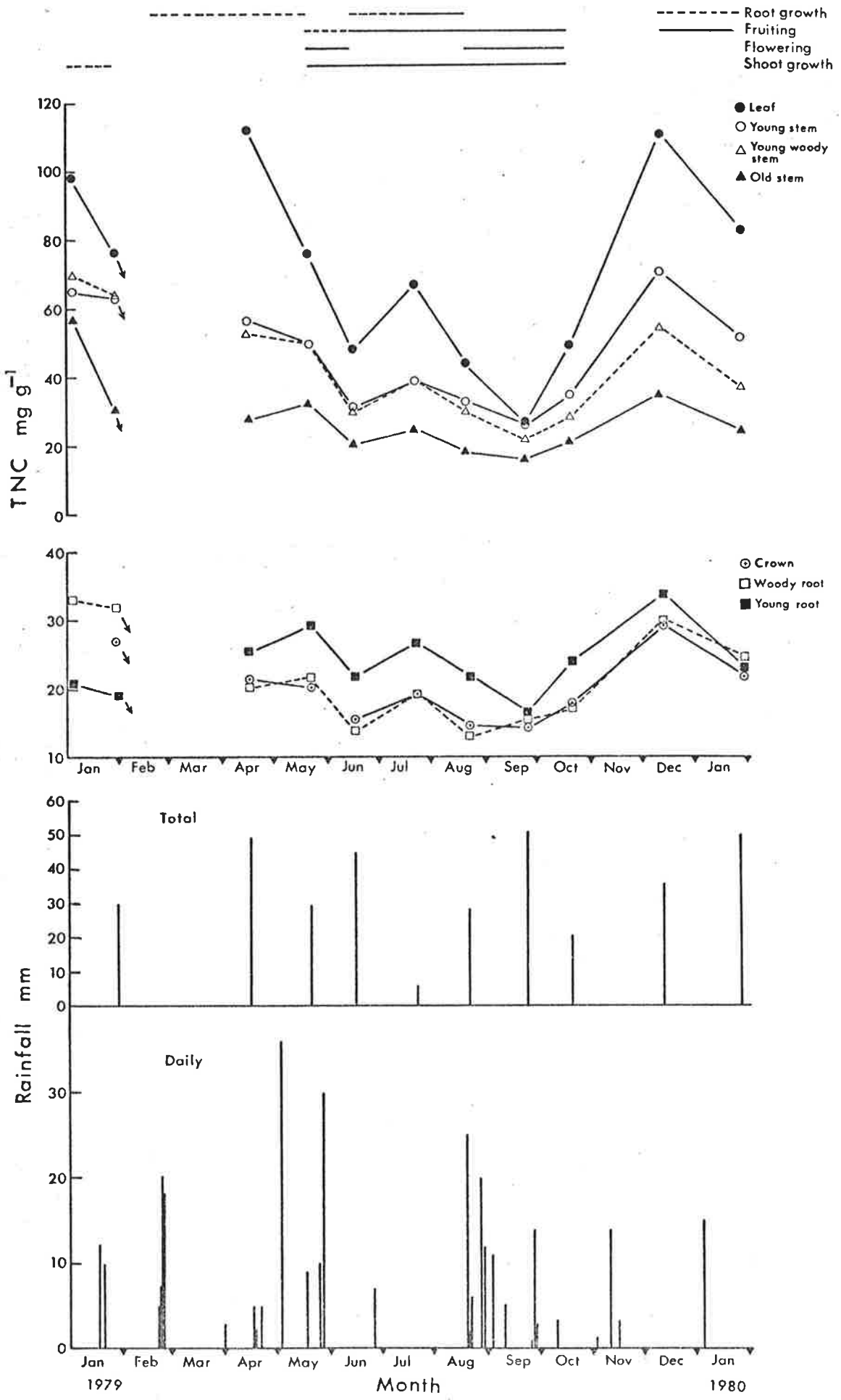
The last set of curves in this series, figure 3.6, shows the seasonal variation in TNC concentration for samples of 5 female plants during 1979. An additional fraction, the crown, defined in section 3.3, was included in the analyses for 1979. These results confirm those observed in the previous year. No samples were collected in February and March. The arrows attached to the data points in January indicate the probable direction of change in TNC concentrations following the substantial rains at the field site during the interval. According to daily rainfall figures these probably fell at the end of February. The first set of data points are those for female samples from December 1978. They are included to provide a reference for continuity between years. The fall in TNC concentrations, which occurs earlier in 1979 than in the previous year, coincides with the heavy rains in May. One further point of interest is the rise in TNC concentration during a period of low rainfall in July. Concentrations, at least in the leaves, were significantly larger ($P < 0.05$) than in June or August. This response presumably also coincides with a reduction in the rates of shoot elongation and leaf production as well as the observed cessation of flowering.

3.52 TNC content

The next series of graphs (figures 3.7 - 3.9) depicts the TNC content of the various plant fractions at different times of the year. In 1979 the above-ground portion of each shrub was divided into five fractions. The dry weight of the fractions was measured and root dry weight estimated from the combined weights and published values for the root/shoot ratio of *A. vesicaria*. The total dry weight of TNC in shoot fractions was calculated from the dry weight of each fraction (g) and the measured values for TNC

Figure 3.6 Total non-structural carbohydrate concentration (TNC mg g^{-1}) for combined subsamples of the dried tissue from samples of 5 female plants collected from the field at monthly intervals between January, 1979 and January, 1980. The horizontal bars show the pattern of vegetative and reproductive growth assessed from notes on the phenology of sampled shrubs. The continuous line indicates that half or more of the shrubs were active and the broken line, less than half.

The accompanying diagrams show total rainfall accumulated at the experimental site between samplings, and daily rainfall for Koonamore Homestead, 7 km to the north.


Root growth
Fruiting
Flowering
Shoot growth
Leaf
Young stem
Young woody stem
Old stem
TNC mg g⁻¹
Crown
Woody root
Young root
Total
Daily
Rainfall mm
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
1979
1980
Month

concentration (mg/g). Similarly the TNC content of the root system was estimated from the appropriate figures. The combined values give an estimate of the TNC content per plant.

Figure 3.7 shows the TNC content of shrubs during 1979. The height of each bar represents the mean TNC content (g) per plant. The width of the divisions within each bar shows the proportion of TNC in the designated fractions. Figures given are for plants with a dry weight of 100 g.

The pattern of variation in TNC content per plant is much the same as that for TNC concentration shown in figure 3.6. The only deviation from that pattern is the relatively high value for January, 1979 which, due to loss of part of each of the sampled plants, was calculated from the average proportion of dry weight per fraction between April, 1979 and January, 1980. There is no reason, however, to expect exactly the same pattern for TNC concentration and content as the proportion of leaf, the fraction with the highest concentration of carbohydrates, varies from month to month. The extent of this variation can be seen in figure 3.8a which shows the mean proportion of dry weight in each fraction at various times of the year. The accompanying figure (3.8b) shows the proportion of TNC per fraction over the same period. The information in figure 3.8b is the same as that given by the width of the divisions in figure 3.7 but gives a clearer picture of the variation over the year. Inspection of these figures reveals that while the dry weight of old stem is the largest fraction of the total, the highest proportion of the TNC content is located in the leaves. Except in August the top three fractions, leaf, young stem and young woody stem contained at least 50 per cent of the TNC content of the plant. These figures are based on estimates of bush dry weight using a root/shoot ratio (below ground/above ground) of 0.32, measured for 1-2 year old shrubs (Jones and Hodgkinson, 1970). Shrubs older than 8 years in that study had a lower root/shoot ratio (0.21). The use of the latter figure to estimate shrub dry weight would

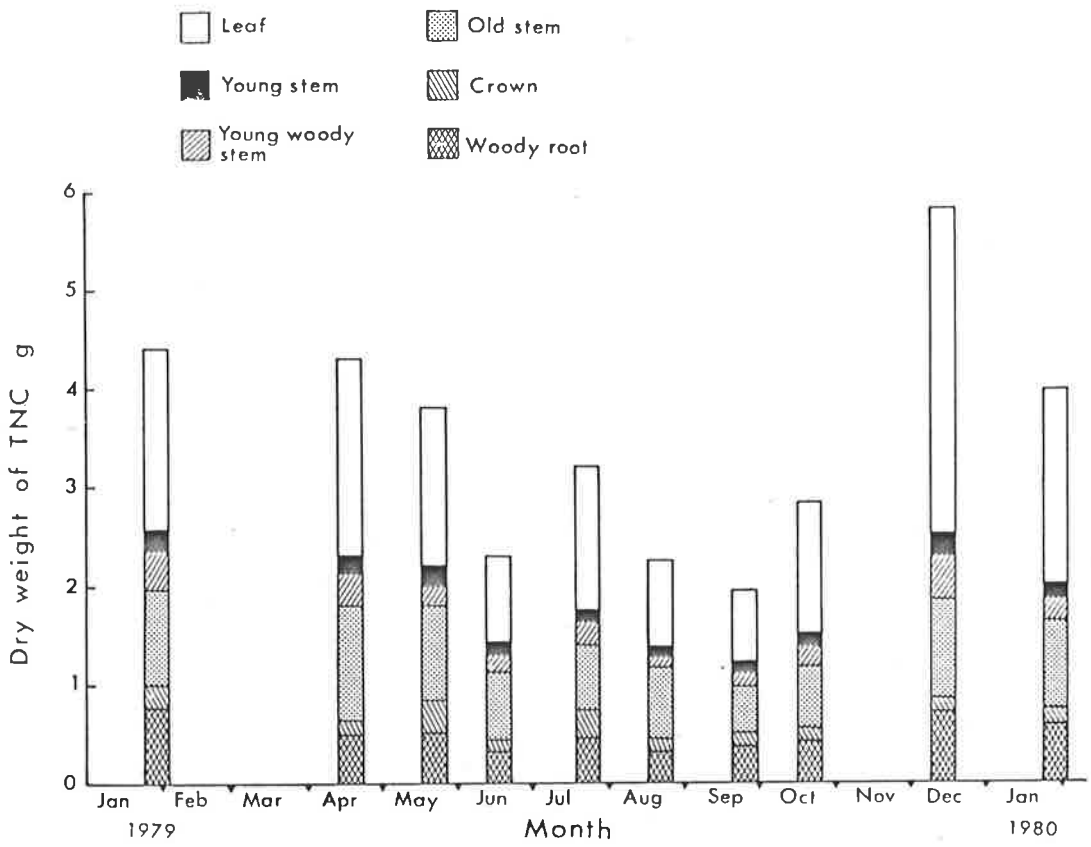
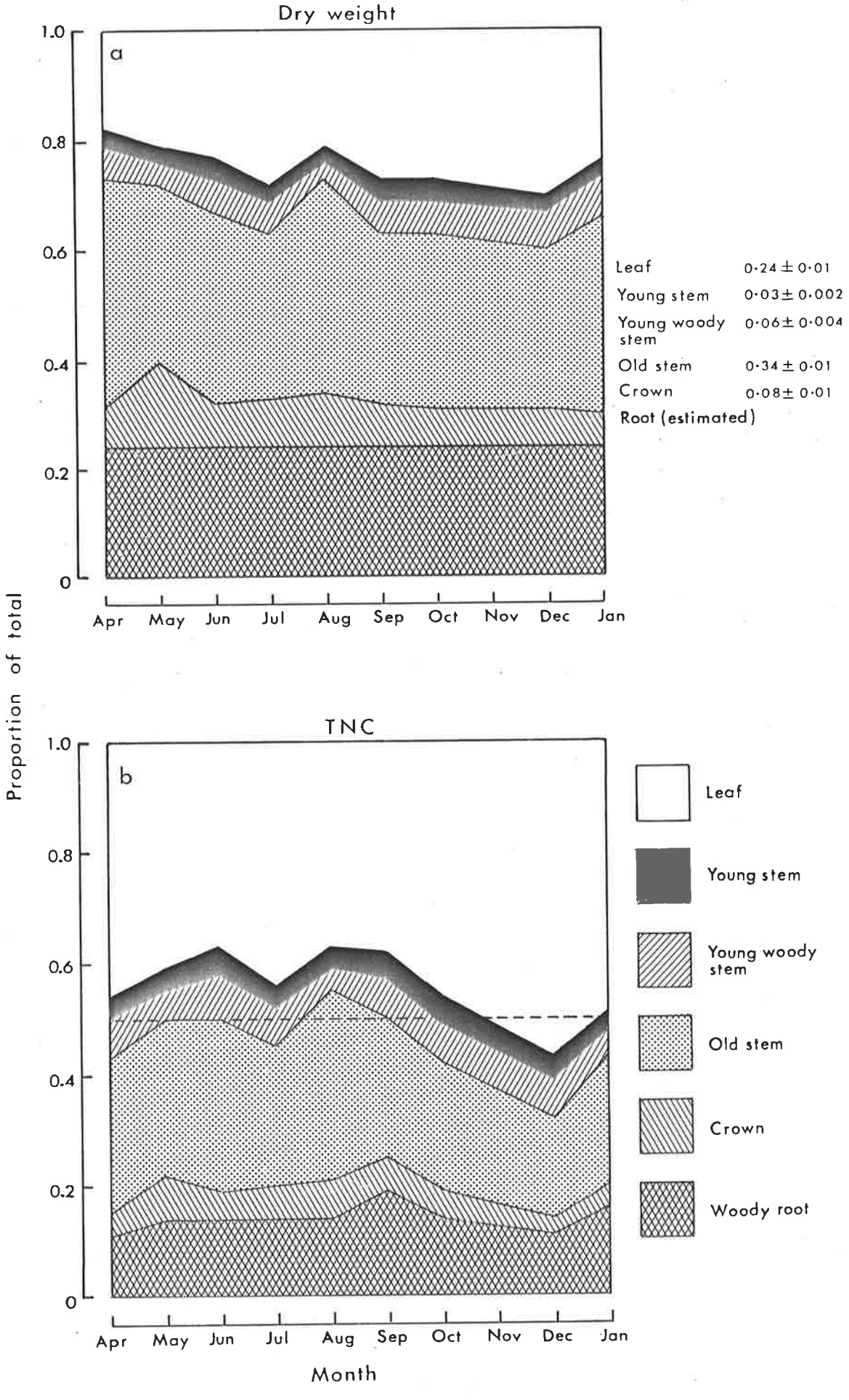


Figure 3.7 Dry weight of total non-structural carbohydrate (g) per 100 g dry weight of plant, calculated from TNC concentrations shown in Figure 3.6 and the dry weight in each fraction. The width of the divisions in each bar represent the dry weight of TNC in the designated fraction.

Figure 3.8 The mean proportion of dry weight (a) and TNC (b) in various fractions of samples of 5 female plants collected between April, 1979 and January, 1980. The values associated with Figure 3.8a are the overall means and standard errors for dry weight during that period.

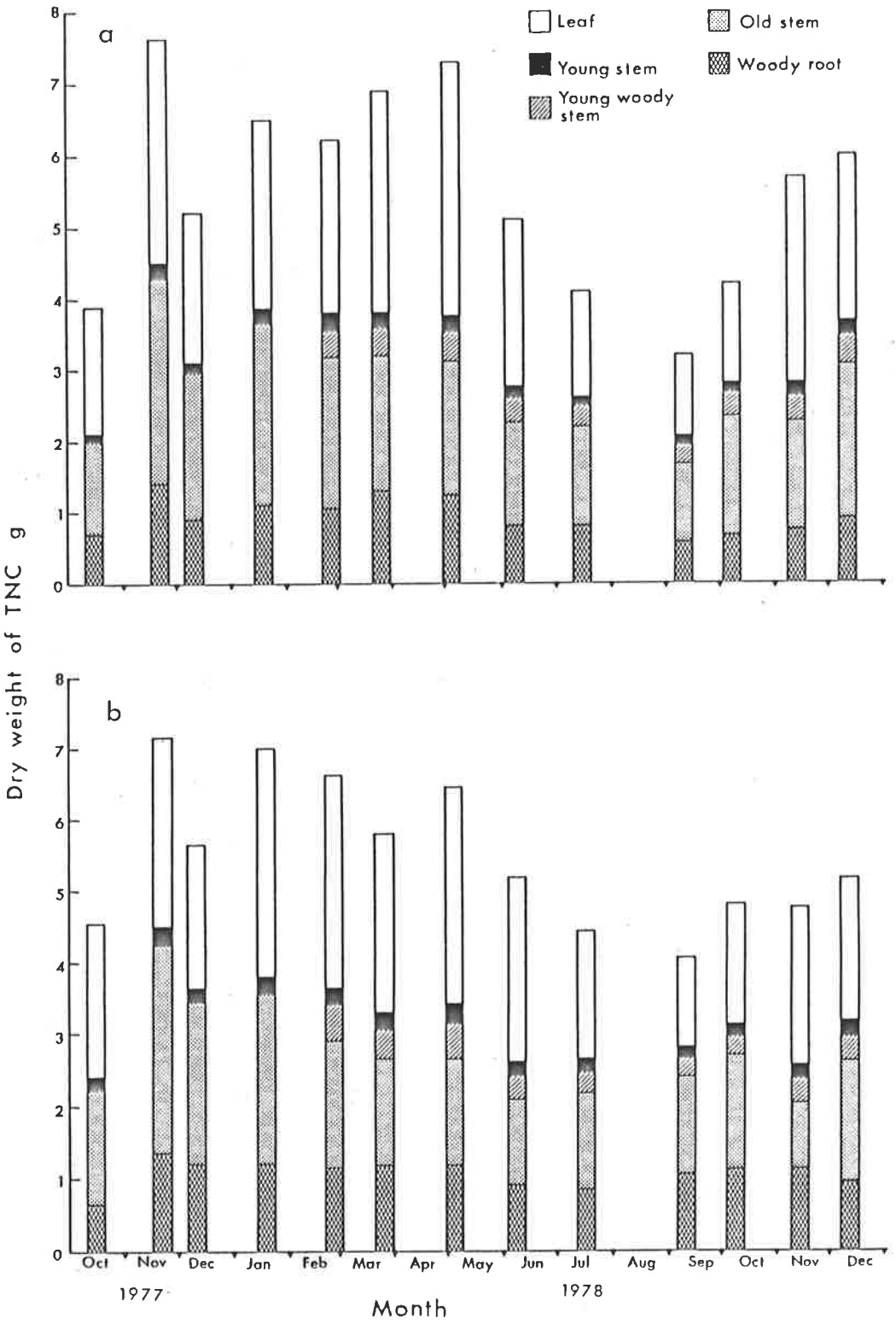

Dry weight
a
1.0
0.8
0.6
0.4
0.2
0
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Leaf 0·24 ± 0·01
Young stem 0·03 ± 0·002
Young woody stem 0·06 ± 0·004
Old stem 0·34 ± 0·01
Crown 0·08 ± 0·01
Root (estimated)
Proportion of total
TNC
b
Leaf
Young stem
Young woody stem
Old stem
Crown
Woody root
Month

increase the calculated proportion of total dry weight in the shoot fractions and since TNC concentrations in the younger shoot tissue are often much higher than those in other fractions the proportion of TNC in leaf and young stem may be increased to over 50 per cent of the total. Nevertheless, despite a lower carbohydrate concentration, because of its bulk old stem contains the second largest store of TNC in the plant.

Figure 3.9 shows the seasonal variation in TNC content over the period October 1977 to December 1978 for male and female plants. Because the collected plants were subsampled for analysis of TNC concentration no direct information on the proportion of dry weight in each fraction is available. The TNC content of the various plant parts, and hence of the whole plant, are estimates based on the overall mean of monthly figures for the proportion of dry weight observed in 1979. The mean and standard error for the proportions used are shown in figure 3.8a. Although there was some variation in measured dry weights between months, analyses of variance showed that the proportion of the total, for a given fraction, was not significantly different over the whole year (April 1979 - January 1980). The data were not transformed for the analyses as there seemed no reason to expect any deviation from normality or dependence of the variance on the mean as found, for example, in proportions derived from sampling binomial populations.

The TNC content of old stem and woody root are slightly inflated because the dry weight calculated for crown tissue was divided equally between these fractions. In the first four months young woody stem was also classed as old stem thus further increasing the estimated proportion of TNC in the latter.

Figure 3.9 Dry weight of total non-structural carbohydrate per 100g dry weight of plant for samples of 3-5 shrubs collected between October, 1977 and December, 1978. (a) Female plants (b) Male plants. Values for dry weight of TNC were calculated from the TNC concentrations shown in Figures 3.4 and 3.5 and the overall mean proportion of dry weight measured for shrubs in 1979 (see fig. 3.8a).


a
b
Leaf
Old stem
Young stem
Woody root
Young woody stem
Dry weight of TNC g
0
1
2
3
4
5
6
7
8
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
1977
1978
Month

3.6 Discussion

Before the main section of results from monthly samples for TNC concentration are discussed two points raised earlier will be dealt with. The first of these relates to the survival of shrubs after complete defoliation, the second to criticisms of the dilute acid method of carbohydrate extraction in the literature.

3.61 Defoliation

The results and discussion of work on survival of defoliated shrubs were presented together because essentially it was intended as a preliminary demonstration that, at least for the form of the species at Koonamore, shrub regrowth is independent of either the number or location of buds. The alternative suggestion, outlined in chapter 2, that TNC concentration influences regrowth is supported by the observation that buds are present yet do not usually develop into large shoots. It is perhaps significant that appreciable regrowth was observed on only two occasions (March, 1978, 1980) in the season, if not the exact month, when TNC concentrations were highest.

However, this is by no means conclusive evidence of the overriding importance of TNC concentration. Regrowth in the field is no doubt influenced by interaction between a number of factors. Grazing by sheep, for example, may alter the number of growing points on old stem by the time defoliation is complete, in which case the tentative conclusion reached by Leigh and Mulham about the location of buds on plants defoliated in that particular experiment may have been correct. Any regrowth from growing points on old stem may have been removed by sheep during the 12 days they were given access to the shrubs. All viable buds on old stem could have sprouted before sheep were removed from the experimental plots.

3.62 TNC extraction and measurement

According to Smith (1969) the two methods most commonly used to extract TNC from plant tissues are incubation of the tissue with diastatic enzyme preparations or hydrolysis with dilute acids. Extraction in hot water has also been used but although amylose is largely soluble in water, amylopectin, a highly branched molecule of high molecular weight, is not. The amylopectin content of starch ranges from 70-90% and therefore hot water extraction is not recommended for tissues rich in starch. It is, however, a useful method where TNC is composed largely of water soluble fructosans.

Smith *et al.* (1964) compared these methods by analyzing TNC concentrations of the various extracts from tissues of two species, one rich in fructosans and the other in starch. For both species the enzyme and dilute acid hydrolysates gave almost identical results, while hot water gave similar results only when little or no starch was present. They recommended the dilute acid method because the time involved in preparation for analysis was much less (8 vs. 56 hours). Later Smith (1969), in a review of methods, recommended incubation with takadiastase because of reports that 0.2N H_2SO_4 destroyed some fructose, hydrolyzed some structural carbohydrate, as indicated by the presence of xylose, and failed to hydrolyze over 60 per cent of the starch contained in some tissues (Grotelueschen and Smith, 1967). Priestley (1962) on the other hand cited references in which it was claimed that the dilute acid method gives more reproducible results. However, in view of the above there is little doubt that the use of takadiastase is the safest and probably the most accurate method especially when the composition of TNC is not known. Disadvantages are the long incubation during which the samples require attention and an increased number of steps in the analysis (e.g. removal of protein before analysis), a possible reason for

the poorer reproducibility claimed in Priestly (1962). Since diastatic enzymes do not hydrolyze fructosans, extracts from tissues containing fructose polymers still require acid hydrolysis after incubation with the enzyme, a procedure which may involve some loss of fructose.

The dilute acid method was chosen in this study for convenience because in practice there appears to be little difference between the two methods (see Smith *et al.*, 1964).

There are aspects of the use of anthrone for analysis of TNC concentration which may negate the criticisms of the dilute acid method of extraction. Smith (1969) used Fehlings solutions to analyse for carbohydrate concentration. Since this method depends on the reducing power of sugar monomers any fructose destruction, hydrolysis of structural carbohydrate or presence of unhydrolyzed nonstructural carbohydrate will lead to incorrect results for TNC concentration. However, the presence of unhydrolyzed sugar polymers in the extract, potentially the biggest source of error, is not important when the anthrone method is used as the reaction is carried out in the presence of 75 per cent sulphuric acid. Grotelueschen and Smith (1967) detected xylose in dilute acid extracts. They attributed the presence of xylose, which amounted to less than 2 per cent of the total nonstructural carbohydrate, to the hydrolysis of hemicellulose during extraction. The xylose would not be detected by the anthrone method as colour production by pentose sugars is much less than that by hexoses and under the reaction conditions used the rate of colour destruction during incubation is such that after 10 minutes little of the initial colour due to pentose remains. However, if the hemicellulose involved is a xyloglucan there is a possibility of contamination of the extract with small amounts of glucose. The fructose destruction reported for dilute acid hydrolysates is probably due to the production of 5-hydroxymethyl-furfural during extraction (pp. 441-443, Finar, 1963). Although furfurals possess an aldehyde group Fehling's

solution, a weak oxidizing agent, is probably not strong enough to attack the ethylenic bond in the ring structure. They are chemically very similar to benzaldehyde which does not reduce Fehling's solution. However, since colour production by sugars in the presence of anthrone in sulphuric acid is thought to be due to reaction of furfural derivatives with anthrone (Yemm and Willis, 1954) the formation of such compounds during extraction with dilute acids would not alter the outcome of the analysis as it may do when TNC concentration is measured by analysing for reducing power. Thus when used in combination with anthrone, the method of extraction with dilute acid, for which a higher precision has been claimed, may also yield results as accurate as those from enzymatic hydrolysis followed by analysis for reducing power.

The only known biological compound which interferes with the anthrone reaction is tryptophan. Colour production by glucose in the presence of an approximately equal weight of tryptophan was about 5 per cent less than that produced by glucose alone. D-galacturonic acid yields about 10 per cent of the colour produced by an equal weight of glucose. Tryptophan may also interfere with this reaction. As these compounds are unlikely to occur in such high concentrations their effect on the measurement of TNC is believed to be minimal (Yemm and Willis, 1954).

The chloride ion is known to affect the accuracy of the anthrone method by enhancing colour production. However, the chloride concentrations in the final dilution on the sample extracts were less than 0.005 M, a concentration which according to Scott and Melvin (1953) increased colour production by less than 1 per cent. Despite the low concentration of these ions it was considered safer to remove them. Since amino acids and uronic acids are taken up by the types of ion exchange resin used in this study some of the other interfering substances may also have been removed during this step.

Since the carbohydrate concentration is low in the final dilution, less

than 100 µg/ml, the main source of analytical error is contamination by extraneous carbohydrate. For this reason the glassware must be kept scrupulously clean and reaction solutions protected from contamination with cellulose fibre from the atmosphere.

In practice the method gave reproducible results. The standard error for the mean absorbance by individual concentrations of the standard glucose solutions over all runs was less than 1.5 per cent of the mean. For the 50 µg/ml standard the standard error was less than 0.6 per cent of the mean. These errors include possible contamination, dilution errors and differences in incubation time.

The largest source of error overall is likely to be that introduced by sampling for plant material. The rather frugal practice of combining subsamples for a given fraction, from all plants in the original sample, was not initially intended but was due to lack of time. However, standard errors calculated for the mean on 15 occasions when one or more of the fractions from each plant were analyzed individually were less than 10 per cent of the mean. On average the percentage standard error for those samples was 6.6 per cent. The average standard error quoted by McConnell and Garrison (1966) for two root fractions and 3 top fractions of *Purshia tridentata* over a whole season were 9.9 and 5.5 per cent of the mean respectively. Figures for arid zone shrubs referred to later in this thesis are not available as the authors either adopted the same practice of combining samples or did not quote standard errors.

The errors represented by the figures for *A. vesicaria*, quoted above, include variation between plants and errors introduced during the extraction procedures as well as those quoted earlier for the precision of the anthrone analysis. The variation between plants appears sufficiently small to allow an experimental approach to work on the details of TNC accumulation and depletion, in response to grazing or other manipulations of the environment,

without increasing the sample size beyond reasonable limits imposed by time and expense. For fractions such as old stem, however, in which TNC concentration does not vary widely during the year, the number of plants included in the sample would need to be relatively higher. On the basis of results presented here sample size might justifiably be increased by sub-sampling a larger number of plants rather than increasing the number of entire shrubs in the sample. The simultaneous variation in TNC concentration of all fractions particularly during 1979, as well as the apparently stable relative concentration in these fractions indicates that little information would be lost by selecting a single branch from each of an appropriate number of shrubs and ignoring roots and crowns. The four shoot fractions obtained by sampling in this way would account for most of the TNC in the plant. Given that roots are identifiable they could be included in the analysis if necessary by sampling with a soil auger. TNC contents could be calculated by adapting a procedure such as the point quadrat analysis used to estimate leaf area by Warren Wilson (1965) to estimate the dry weight in various shoot fractions. This procedure would also have the advantage of being much less destructive.

There are no figures available for comparing either the precision or accuracy of the methods used here for *A. vesicaria* with enzymatic hydrolysis and analysis for reducing power. However, the maximum values recorded for leaves of *A. vesicaria* in this study (100-145 mg/g) are much the same as those measured by Wood (1932) who analyzed for the reducing power of dilute acid hydrolysates of saltbush leaves. Hexose polysaccharides and soluble sugars together accounted for about 15.5 per cent (155 mg/g) of the dry weight of leaves collected from Koonamore in early summer. At the end of the preceding winter (August 1931) the concentration of these compounds (76.5 mg/g), although higher than that recorded at the same time of year in this study (ca. 30-50 mg/g), was approximately half the December value, a pattern similar to that illustrated in figures 3.4-3.6. The higher

concentrations are predictable as 1931 marked the end of a sequence of low rainfall years during which storage carbohydrates might have been expected to accumulate (see Ch. 2). The main reason for the work done by Wood was to establish the existence of high concentrations of non-structural pentosans, which he believed contributed to the drought resistance of arid zone species with tomentose succulent leaves. Irrespective of the effect of their presence, pentosans were found in significant quantities in the leaves of saltbush. In August and December the concentration of pentosans was 17 and 70 mg/g respectively. Some may have been derived from cell wall constituents during the relatively long 3-hour extraction in dilute acid but their presence in the protoplasm was checked in fresh sections by a staining technique.

The concentration of accessible carbohydrates in leaves, and possibly young stem, may therefore by substantially higher than estimated by the anthrone method used here.

3.63 Seasonal variation of TNC

TNC concentration

The pattern of TNC accumulation and depletion by saltbush during 1978 and 1979 is in general consistent with that expected from the amount and distribution of rainfall and hence the growth pattern. TNC was depleted at times of rapid growth and accumulated during periods of low rainfall or reduced growth as judged from observations on the phenology of the shrubs sampled for TNC analysis. While the results presented in this chapter provide no information on the role of storage carbohydrates during growth they do allow some conclusions on growth and photosynthetic activity of saltbush at various times of the year.

One of the suggestions made in section 2.3 was that the TNC concentration of arid zone plants, and other species, will increase during periods of low rainfall because structural growth is restricted earlier than net

photosynthesis as leaf water potential decreases. According to the references cited, with further decreases in water potential growth ceases well before net photosynthesis is reduced to zero. The relatively rapid rise in TNC concentration of most plant parts during spring and early summer (September-December) observed during this study is coincident with decreasing xylem water potential and presumably the sequence of events described above. Seasonal changes in xylem water potential are illustrated in Chapter 7. There were substantial rains during the spring in both years which, as seen from the October peak in mean monthly rainfall figures (fig. 3.2a) are quite common. Spring rains were followed by some growth which might have been expected to result in a depression of TNC concentration, as observed, for example, after summer rains. The most obvious explanation is that any fluctuations were missed due to the relatively long interval between sampling dates but the rise in TNC concentration is not inconsistent with expected patterns of photosynthetic activity and growth during spring. The apparently steady increase may be a consequence of rising temperatures and decreasing relative humidity and their effect on photosynthesis, growth and soil water storage. While the potential maximum rate of net photosynthesis by individual leaves of *A. vesicaria*, a C4 species, is likely to occur during a wet summer when temperatures are high (see Wood, 1932) the maximum rate of net CO_2 uptake in years with drier summers probably coincides with the increasing air temperature and relatively high soil water potential during spring. Since shrubs are then relatively well foliated with young active leaves the highest rates of carbon gain per shrub may normally occur in mild spring conditions. Fluctuations in TNC concentration are likely to be small or short lived either because current photosynthate is able to meet all of the demand or because the depleted stores of TNC can be rapidly replenished. Structural growth increments will also be small if, as expected, rainfall becomes increasingly less effective in maintaining

turgor for long periods as the season progresses. The net result will be an increase in TNC concentration since most photosynthate under those conditions would be diverted to storage. There is some evidence for high rates of carbon gain during spring by individual leaves of *A. vesicaria* in the field. The peak rates of fixation of labelled CO_2 measured by Chapman and Jacobs (1979) approximate the highest rates of net CO_2 uptake measured under favourable conditions in the laboratory during this study (see Ch. 5).

Another factor contributing to the apparently steady increase in TNC concentration during spring may be the availability of nutrients. Trumble and Woodroffe (1954) and Charley and Cowling (1968) suggested that poor growth by arid zone communities in the second of two good years was probably due to depletion of small pools of readily available nutrients by the previous year's growth. The same sequence of events may also occur over a shorter period; depletion of soil nutrients during rapid spring growth may shorten the growing period after spring rains. Much of the available nitrogen and phosphorous is cycled through litter by soil microflora. In the normal course of events the pool of soil nutrients is increased in dry weather by small falls of rain which, although ineffective in producing plant growth, are able to initiate microbial activity and hence mineralization of organic nitrogen and phosphorous in litter. These rains called 'mineralization rains' by Charley (1972) may also activate nitrogen fixing organisms in the lichen crust and soil. Such rains are most effective in summer (pp. 245-247 in Osmond *et al.*, 1980). If the nutrient pool cannot be adequately replenished in the interval between rainy seasons in successive years, the rate of litter fall and reprocessing of organic nutrients may not be high enough to support rapid spring growth for long periods. Although growth rates in spring are high (Ch. 4) a combination of high photosynthetic rate and a short growing period may obscure any fluctuations in TNC concentration which do occur.

In contrast to the apparent lack of response to spring rains the short

growth flush observed in December 1977, initiated by rainfalls totalling 30 mm, resulted in a large depression of TNC concentration (figs 3.4-3.5). This response is relevant to a second prediction made in Chapter 2 where it was suggested that carbon gain may be restricted following summer rains due to the effects of prior water stress on the photosynthetic capacity of individual leaves or on the total amount of leaf material. Assuming that TNC is used to support new growth, the observed result implies that for either or both of these reasons the supply of current photosynthate was inadequate to meet the demand. The rainfall in 1977 was relatively low (86.5 mm) and midday xylem water potentials had fallen to between -9.1 and -10.7 MPa by November and possibly lower by the time rain fell towards the end of that month. Although not conclusive evidence of impaired photosynthetic capacity during rehydration the apparent use of stored carbohydrate does give some support for this possibility. The effects of low water potential on the subsequent rate of net CO_2 uptake by shoots of *A. vesicaria* during and after rehydration are examined further in chapter 5.

The fluctuations in TNC concentration during the low rainfall period after December 1977 may have been due to cambial growth or to the use of carbohydrate as an energy source during osmotic adjustment. The overall rise in TNC concentration of the younger shoot tissue of female plants during this period, when dawn water potential of some plants reached a minimum of less than -11.0 MPa, indicates that *A. vesicaria* is capable of positive net CO_2 uptake at low leaf water potential. However, while concentration in the leaves of male plants (fig. 3.5) was higher in April after the low in December the trend is less convincing, with an apparent fall in TNC concentration from a peak in January. The difference in the trend of TNC concentrations for males and females may be related to the difference in growth pattern. The determinate growth of shoots on male plants, many of which had produced terminal flower spikes in and before December may mean that the leaf population

on male shrubs was composed of leaves of a greater average age, and hence lower photosynthetic capacity, than those on female shrubs. There is also some evidence of a small decline in the TNC concentration of old stem of male plants between mid summer and autumn, and this decline coincided with that observed in their leaves, and possibly with one in young woody stem. The trend was less pronounced, in old stem of female plants (fig. 3.4), a response which could also be explained by a greater photosynthetic capacity of the leaf population as suggested above. In females current photosynthate may meet some of the demand for assimilates from old stem. However, as outlined earlier, the observed changes in TNC concentration of old stem may not be significant, although the fact that similar trends occurred in various other fractions and in the two independent samples (male and female) lends some support for the possibility that the measured decline was a real response to prevailing conditions. It is notable that a small change in TNC concentration of old stem involves a much larger loss or gain of TNC than a similar concentration change in young or young woody stem due to the smaller bulk of the latter.

If there were losses of TNC from old stem occurring during this dry interval the rapid rise in TNC concentration in the leaves of female plants relative to that in other plant parts may indicate some restriction on the movement of carbohydrate from leaf tissue. Although low water potential is not thought to affect translocation markedly there is some evidence that the loading of assimilates into the phloem is restricted (Wardlaw, 1968). The high TNC concentrations in leaf material during dry periods may be partly a result of reduced movement of assimilates although it is unlikely that traffic of carbohydrates and other compounds is stopped completely by low leaf water potential. Charley (1978), for example, observed withdrawal of some minerals and nutrients prior to leaf shedding.

The interpretation given to the fall in TNC after summer rains in 1977

can probably also be applied to the decline in TNC concentration after late autumn or winter rains, observed in both the following years. Jameson (1963) and Davidson and Milthorpe (1965) believed that, in general, storage carbohydrates contribute significantly to growth only in the first few days after defoliation. Jameson concluded that once the carbohydrate needs for new leaf have been met there is little reason to believe that additional stored carbohydrates will result in additional growth. If carbohydrate use by saltbush after a period of water stress in summer and autumn follows the same pattern, TNC concentrations might be expected to increase or at least remain steady after production of the first flush of new leaves. However, the continuing decline in TNC concentration of most fractions throughout the winter and early spring, except for a brief rise in midwinter (July) 1979, implies that stored carbohydrate contributes to growth during the whole period of rapid growth. On this basis it would appear that structural growth of saltbush depends primarily on the activity of the various sinks for assimilate rather than the rate of supply of current photosynthate. Hence, given different combinations of the environmental factors controlling the activity of meristems, TNC concentrations in winter may be reduced to even lower values than recorded here. A combination of relatively high air temperatures at night and cloud cover for part of the day, for example, may increase the use of stored carbohydrate. Although it is rarely suggested that the level of irradiance might limit photosynthesis by plants in arid areas it is possible that cloud cover in relatively wet winters could reduce the rate of net CO_2 uptake, particularly by C4 species, with the result that stored carbohydrates are drawn upon by actively growing tissues to compensate for the shortfall. Noy-Meir (1973) has suggested that the assumption of saturating irradiance should in general be treated with some caution. However, changes in TNC concentration are undoubtedly influenced

by a complex of interacting factors and their detailed interpretation requires a much more experimental approach than used here. It is not clear, for example, whether the small peak of TNC concentration in midwinter 1979 was due to the observed cessation of flowering, a reduced rate of vegetative growth or simply to an increase in the rate of net CO_2 uptake beyond the requirements of an unimpeded growth rate.

Nevertheless, the pattern of accumulation and depletion of TNC by saltbush does lend some support for the predictions made about the effects of environment on growth, net CO_2 uptake and the use of stored photosynthate at different times of the year. Similarly the figures for TNC content (figs 3.7, 3.9) allow some conclusions about the extent of the dependence of this species on stored carbohydrates.

TNC content

In comparison with other species which occupy areas with adverse climatic conditions the maximum TNC concentrations recorded here for *A. vesicaria* (100-145 mg/g leaf tissue) are low. Such diverse species as alpine herbs, tundra graminoids and drought-deciduous trees had TNC concentrations between 350 and 600 milligrams per gram dry weight in various storage organs (Mooney and Billings, 1960; Shaver and Billings, 1976; Mooney and Bartholomew, 1974). The bulbs and rhizomes of perennial ephemeroids in desert ecosystems also have large stores of carbohydrate and protein. In some species the dry weight of stored assimilates may be comparable to peak vegetative biomass (Noy-Meir, 1973). These species, however, are obliged to use stored carbohydrate to replace, or at least initiate replacement of, most or all of their photosynthetically active tissue after killing frosts in winter or drought-induced leaf fall in summer. The lower concentrations recorded for saltbush probably reflect a greater ability to maintain positive net photosynthesis for most or all of the year. Comparing concentrations of

the different fractions of a given species can be misleading as the relative amount of TNC available for growth or maintenance depends on the dry weight of the storage organ or fraction as well as the concentration (Priestly, 1962). This point is well illustrated in the literature. Sprague and Sullivan (1950), for example, found that although roots of orchard grass had a lower carbohydrate concentration than other storage organs, the total quantity stored in roots was higher because of the greater dry weight of the root system. In contrast, McConnel and Garrison (1966) found higher concentrations of TNC in the roots of *Purshia tridentata* (bitter brush) than in shoot fractions. In this case the root system also contained most of the TNC despite a smaller dry weight.

Calculation of the amount of TNC in the different fractions of *A. vesicaria* (figs 3.7 - 3.9) reveals that, due to their bulk, old stem and woody root which have relatively low concentrations of TNC are the second and third largest sites of storage respectively. Both fractions are relatively dry and brittle and at first sight appear unlikely storage sites for TNC. According to Trlica and Singh (1979) non-structural carbohydrate may be stored temporarily in all perennating plant parts but most storage occurs in living parenchyma cells. The anomalous secondary growth of chenopod stems has been likened to the pattern of growth in the roots of *Beta* spp. (Chenopodiaceae) where the vascular strands are separated by wide radial panels of storage parenchyma (p. 252 Esau, 1960). The stems and roots of *A. vesicaria*, which presumably have concentric uninterrupted rings of vascular tissue, may have some storage parenchyma although obviously not as much as the roots of sugar beet. Other possible storage sites for TNC in chenopods are the libriform fibres in woody tissue. Fahn and Leshem (1962) examined 60 species from the Negev desert and the hills around Jerusalem and found that the xylem fibres of the entire sapwood in 70 per cent of the species had living protoplasts. Their criteria for the vitality of the protoplasts were the integrity of the nucleus and the reduction of triphenyl tetrazolium

chloride (TTC) by dehydrogenases. Thirteen of the species were chenopods and of these twelve were found to have living protoplasts. There was no specific reference to the presence of starch in the fibres of these chenopods but they did suggest that since both living fibres and parenchyma cells can store starch the functional difference between the two cell types was diminished. The fibre lumens of *Calligonum comosum* (Polygonaceae) and *Tamarix* spp. (Tamaricaceae) were densely packed with starch grains and Fahn and Arnon (1962) found starch grains in the fibres of up to twelve of the most recent growth rings of *Tamarix aphylla*. Seasonal changes in the starch content of the outermost wood of that species had previously been described by Fahn (1958). As a result of their survey Fahn and Leshem (1962) speculated that the frequent occurrence of living fibres in subshrubs and shrubs may be associated with a diminishing support function and that such fibres represent transition forms leading towards the evolution of parenchyma cells, prevalent in the stem tissue of herbs. They also suggested that living fibres may appear more frequently in plants of arid habitats in which the woody plants are generally subshrubs or shrubs.

No attempt was made to check for the presence of living fibres in the woody tissue of *A. vesicaria*. However, considering the high proportion of chenopods found to have fibres with the potential for storage, such fibres may constitute a substantial proportion of carbohydrate-containing tissue in the apparently dry and brittle stems and roots of *A. vesicaria*. Irrespective of the exact site of storage, the carbohydrate contained within old stem tissues is apparently readily accessible to the plant during periods of high demand. Of the TNC used during the initial depletion between April and June 1979 23 per cent was derived from old stem, the second largest source of stored carbohydrate over that interval. During the growth flush at the beginning of 1980, on the other hand, only 9 per cent was supplied by old stem perhaps reflecting limited root growth in that

period. Sinks for assimilates are usually supplied by the nearest source (Evans, 1976).

Although stored carbohydrate is depleted during periods of rapid growth the total amount used appears relatively low. The TNC used between April and September 1979, for example, amounted to about 3 grams per bush of 100 g dry weight (fig. 3.7). Based on theoretical carbon costs for construction of leaf tissue (Mooney, 1972; Penning de Vries, 1975) only between 1.4 and 2.0 grams of leaf would result were the whole of that amount diverted to support new leaf growth. Such estimates are approximate and vary depending on leaf composition. If the value of 10 mg dry weight per cm^2 of leaf is used as a guide (Caldwell *et al.*, 1977) the TNC could have been used to produce a maximum of about 400 leaves, each with an area of 0.5 cm^2. For a bush with 100 primary and secondary shoots, a conservative figure, 2 g of TNC could support the growth of 4 leaves per shoot provided it was equally distributed among the shoots. However, since shoots produced an average of about 20 leaves of varying size during the interval (see Ch. 4) it appears that at best TNC would be able to support only about 20 per cent of the observed leaf growth. Since the figures quoted do not account for stem growth, the calculated number of leaves per shoot is an overestimate. It is also unreasonable to expect TNC from all parts of the shrub to be diverted to the growth of new leaves, over long or short periods, while other vegetative and reproductive organs are growing; in this case the allocation of TNC to the shoot apices and hence its contribution to new leaf growth would be reduced.

The pattern of use of TNC by *A. vesicaria* during a growth flush probably parallels that believed to occur after defoliation in other species (see Jameson, 1963) where TNC contributes significantly to growth only in the first few days. The first few leaves may be largely supported by stored

photosynthate and thereafter growth is probably increasingly dependent on current photosynthate from new and existing leaves, although as noted earlier TNC may be drawn upon further if for any reason net CO_2 uptake is depressed.

Nonetheless, the peak TNC content of the shrubs sampled during this study is equivalent to over twice the average net productivity of non woody shoot biomass (1 g m^{-2} yr^{-1}) estimated by Noble (1977) for a small population of *A. vesicaria* on a quadrat in the adjacent Koonamore Vegetation Reserve. The estimates of productivity were made by analysis of photopoint records and for much of the period between 1926 and 1970 the density of the bushes on the quadrat was about 0.5 per square metre (Hall *et al.*, 1964). If the net productivity approximated the long term average (1 g m^{-2} yr^{-1}) during that time the net increment per bush must have been about 2 g yr^{-1}, slightly less than the weight of TNC stored in shrubs of 50-60 g dry weight sampled in this study. The amount of TNC used by shrubs of that size during the winter growth period in 1979, again corrected for construction losses, could account for half the average yearly growth of non woody shoot mass. Of course, the yearly increments, which are underestimates because they do not account for litter turnover (Noble, 1977) must necessarily be the result of newly fixed carbon and are in part due to the increased storage capacity for TNC. The significance of the relative figures quoted above is that the weight of TNC stored appears to be sufficient to replace a year's growth in the event of loss due, for example, to grazing or drought. Exactly how significant the size of the TNC store is to the long and short term production of *A. vesicaria* must await more detailed studies on the relationship between TNC content and vigour of the shrub and the effects of the environment, including grazing, on both. If, as suggested, only a small proportion of TNC is used to initiate growth the quantities involved may be adequate to account for a number of small losses even over a short period, but studies by Cook and Child (1971)

on similar species indicate that relatively low usage results in a depression of vigour which may not be recovered for up to seven years. According to the data in Coyne and Cook (1970) these plants, including two *Atriplex* species, have much higher TNC contents than *A. vesicaria*.

Perhaps the most significant feature of the results of TNC measurements for *A. vesicaria* is the high proportion stored in young shoot tissue. In the absence of grazing the observed distribution could be seen as an efficient adaptation as TNC is located close to sites where it might be used (cf. Evans, 1976). The possibility of loss during drought-induced defoliation or senescence is presumably avoided by translocation before leaf abscission. Even moderate grazing, however, poses a twofold threat to the vigour, and possibly survival, of *A. vesicaria* in that it results in the removal of both a substantial part of the TNC within the plant and reduces the capacity for its renewal.

CHAPTER 4

4 Seasonal patterns of shoot and root growth in the field

4.1 Introduction

The data on shoot and root growth in the field, summarized in the following pages, were gathered, initially, for comparison with the results of measurements of TNC concentration. They are presented separately here for several reasons, partly to avoid the inclusion of a number of points of discussion on growth, unrelated to changes in TNC concentration, in the preceding chapter. Growth of individual plants, in a given interval, was variable and it was considered more appropriate to compare TNC concentrations with growth activity assessed from notes on the shrubs sampled for analysis. However, as shown in chapter 3, on occasion the phenology notes did not adequately explain the fluctuations in carbohydrate concentration. Where relevant, the results of regular measurements of growth, reported in section 4.3, will be compared with those changes in TNC concentration.

Measurements of shoot elongation, leaf production and root elongation are also compared with patterns of growth exhibited by other arid zone species and discussed briefly in that context.

4.2 Methods

4.21 Shoot growth

Changes in shoot length between October, 1977 and January, 1980 were assessed from a photographic record of tagged shoots. This approach was taken, rather than a direct measurement, to avoid excessive handling of the brittle stems and hence to minimise leaf loss and stem breakage.

Shoots were chosen initially by passing a needle point through the shrub canopy at randomly selected positions. The sample consisted of fifty shoots in total, five on each of ten plants. Five of the shrubs were female and the remainder male. Shoots chosen in this way were sometimes relatively inaccessible and when replacements were necessary, due to shoot death or breakage, they were selected from the outer canopy. In most cases shoots were laterals near the apex of secondary branches.

Photographs of these shoots were taken, at about monthly intervals (see ch. 3), against a background of graph paper supported on a steel plate which was in turn supported at a preset distance from the lens by a light metal rod fixed to the base of the camera. The background also included a counter, identifying the plant and the shoot, and a small magnet which could be aligned next to the tagged shoot to help locate the latter easily when subsequent measurements were made. With the focus preset the camera, held in one hand, was used to manipulate the backing plate behind the shoot which was lightly held flat against the graph paper for the photograph.

All measurements of shoot length were made from projected images of black and white negatives (1977-78) or colour positives (1979) using a segment of small diameter plastic tubing to follow curves or bends in the shoot. The length of tubing, measured against the shoot, was then compared with the background scale provided by the image of the graph paper.

This technique was also used to monitor shoot growth of several other groups of plants including a group of four plants adjacent to the root growth observation chambers described in the next section. In addition pairs of plants were irrigated at various times in 1978 and shoot growth was recorded in the following one or two months. Another group of four plants was irrigated regularly in 1978 and shoot growth was recorded each month. The response of irrigated shrubs is reported in chapter 7.

4.22 Root growth

In February 1977 two root growth observation chambers, similar to those constructed by Fernandez and Caldwell (1975) in Curlew Valley, northern Utah, were installed at Koonamore. A third was dug in August 1977. They consisted of square pits with vertical sides lined with perspex sheet, through which root growth could be observed. Between observations the perspex faces were covered with sheets of plastic foam and the chamber was covered by a lid supported on a wooden frame resting on the soil surface. A second cover fixed to a separate frame was placed over the inner lid enclosing an insulating air space of about 5 cm between the two. The chambers were about 1 metre square and 70-80 cm deep.

The depth to which the soil was excavated was governed partly by the distribution of roots expected for the species (e.g. Jones and Hodgkinson, 1970) and partly by the increasing amount of hard limestone rubble at depth. Because of the limestone nodules higher in the profile a clean straight surface could not be cut and, as found by Fernandez and Caldwell, it was necessary to backfill the gap between perspex and soil (2-5 mm) with some of the excavated soil.

An attempt was made to compare soil water potential down the profile next to the observation panels with that in the profile about 1 metre from one of the chambers by installing thermocouple psychrometers but these were found to be unreliable under field conditions at Koonamore.

The chambers were fenced to exclude stock and the area within the fences was kept clear, as far as possible, of other small species in the understorey. The bases of shrubs adjacent to the chamber were 20-30 cm behind the observation panels.

Root growth was mapped on transparent plastic sheets placed over the windows at about monthly intervals. A permanent record of the pattern and amount of root growth was kept by transferring the data to tracing

paper. The same method as that used for shoots was used to measure the length of curved traces.

4.3 Results

4.31 Shoot growth

Figure 4.1 shows the pattern of shoot elongation between October, 1977 and December, 1978. The data from five male and five female plants have been combined in figure 4.1a as there were no significant differences between the two groups. The solid bars represent the mean change in total length and the hatched bars the mean change in length of that segment of the stem bearing leaves, for fifty shoots, in the interval between sampling dates. The horizontal bars show the activity of shoots assessed from notes made on plants sampled for TNC analysis. As before (ch. 3) some indication of the variation between plants is shown. In this case the solid section of the phenology bar signifies that more than half the number of sampled plants showed signs of recent shoot growth. The hatched section indicates that half or less were active.

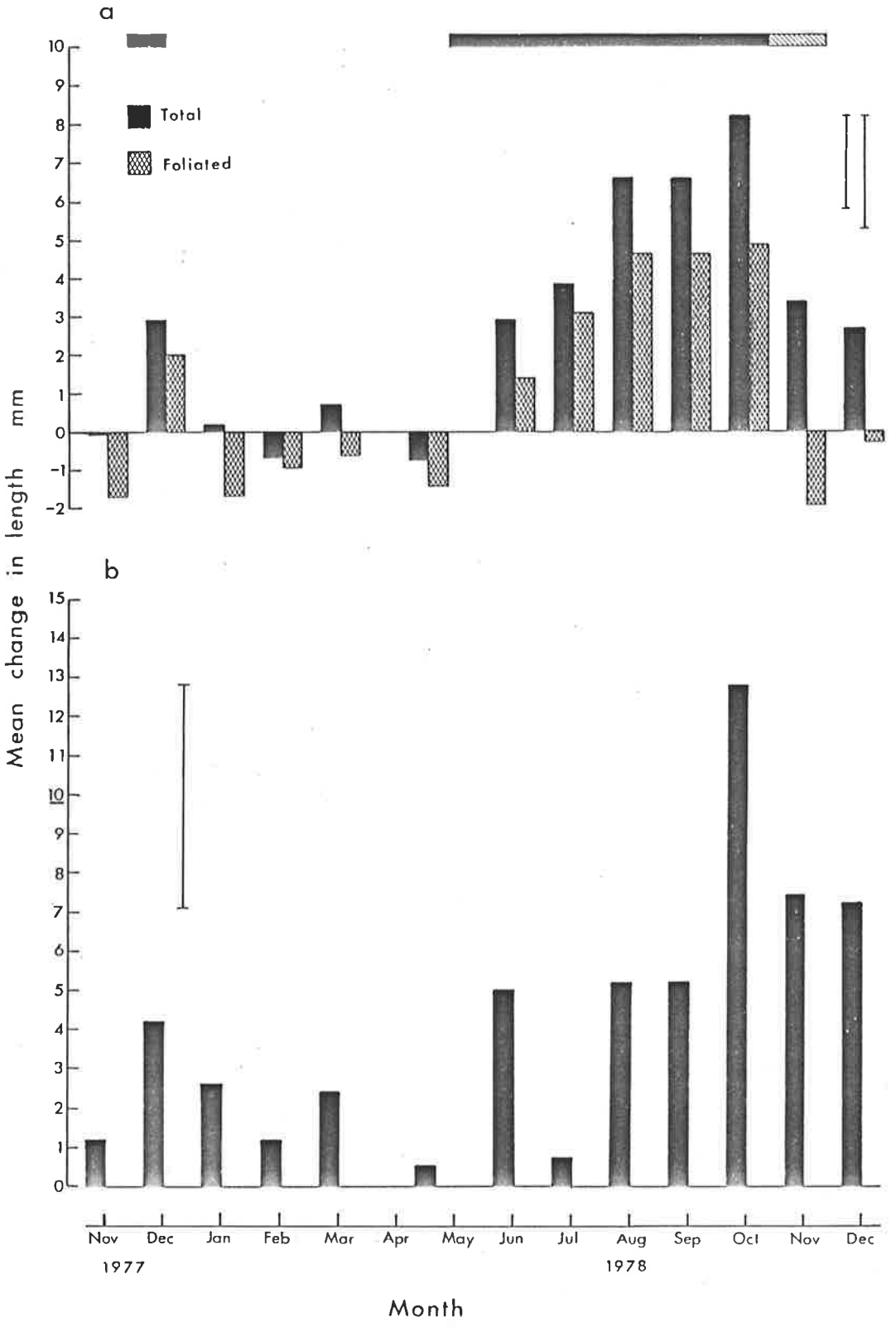
Figure 4.1b shows, for comparison, the mean increase in length of twenty shoots on four plants adjacent to two of the root growth observation chambers. Comments on this comparison are made at the end of this section. In both diagrams least significant differences (LSD, $P < 0.05$) for comparing means in different months are shown. From left to right in the upper diagram the LSDs refer to the mean increase in total and foliated shoot length, respectively.

Variation in growth between shoots on the same or different shrubs was such that only large differences between means are significant. In some instances some shoots on the one plant grew while others decreased in length. It is possible that, on occasion, small decreases recorded for shoots in a given interval were due to error. If shoots were not held flat against

Figure 4.1 Growth of tagged shoots in the field.

(a) Mean change in total length and foliated shoot length for 50 tagged shoots recorded between October, 1977 and December, 1978 (foliated length is that portion of the stem bearing leaves). LSDs for comparison of means over time ($P<0.05$) are, from left to right, for total and foliated length, respectively. The horizontal bar shows the activity of shrubs assessed from phenology of shrubs sampled for TNC analysis (Chapter 3). The solid section indicates that half or more of the shrubs showed signs of recent shoot growth; and the hatched section, that less than half were active.

(b) The mean change in total length for a sample of 20 shoots on plants adjacent to root growth observation chambers (LSD at $P<0.05$).


a
b
Total
Foliated
Mean change in length mm
10
9
8
7
6
5
4
3
2
1
0
-1
-2
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
1977
1978
Month

the background when photographs were taken the image would be foreshortened. On a few photographs this was obvious and such images were discarded from the sample. However, shrinkage and swelling of tissues, due to changes in water content, have been observed for many other plant species and hence the observed decreases are considered likely to be real. This phenomenon is discussed at length in Chapter 6, where measurements of growth of glasshouse grown seedlings are reported.

Figure 4.2a shows the mean change in total and foliated length for 25 shoots on 5 female plants in the interval between December, 1978 and January, 1980. The scale is smaller than that in figure 4.1a and the data from the last two readings in 1978 are reproduced in figure 4.2a for comparison with the mean monthly values in the wetter year of 1979. Figure 4.2b shows the growth of laterals on the tagged shoots in 1979. There was little lateral growth in the previous year, despite the presence of male shoots in the sample. The latter were expected to initiate lateral growth when flowering terminated vegetative growth at the apex.

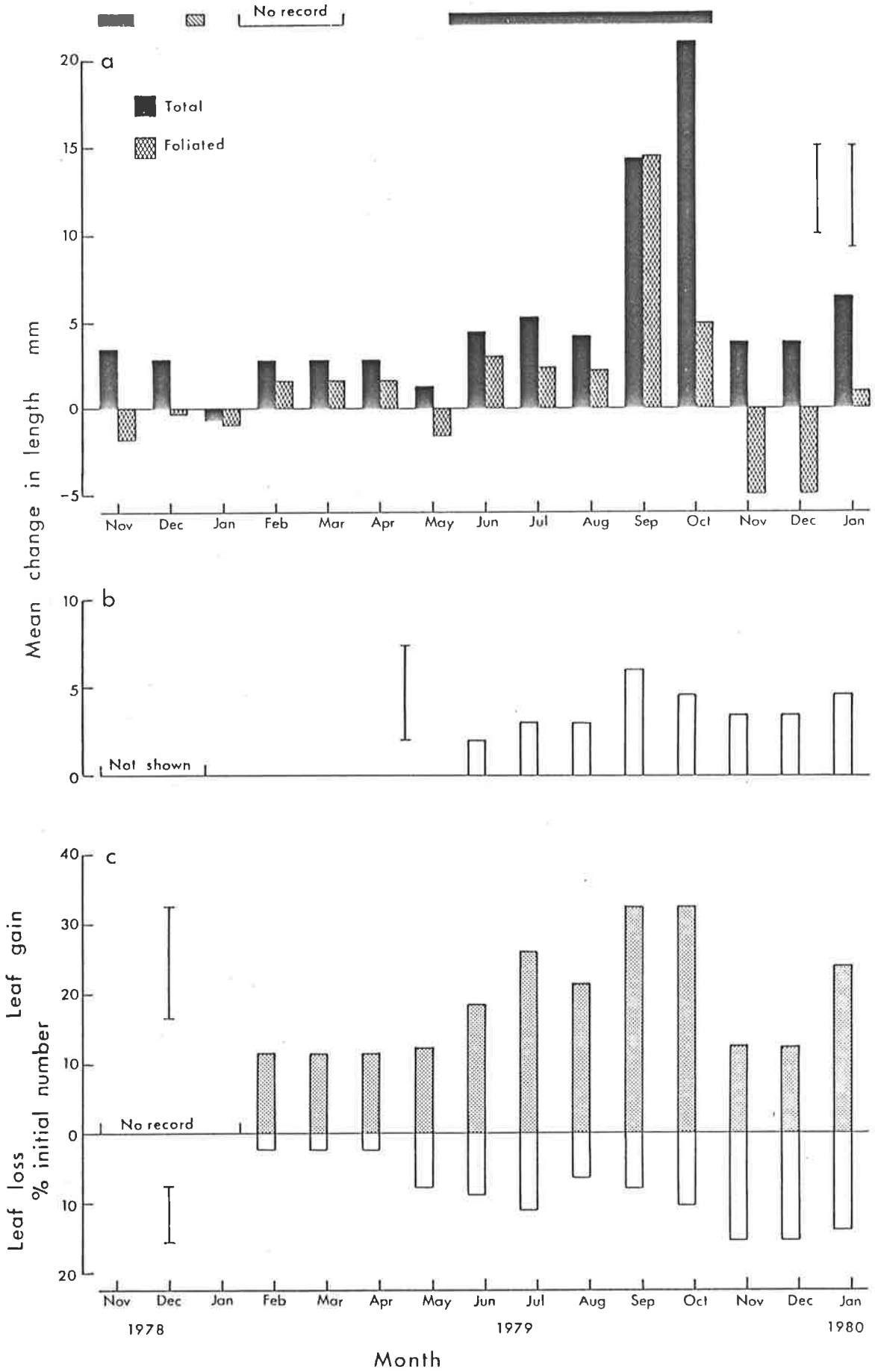
Figure 4.2c shows the gain and loss of leaves on tagged shoots, in 1979, expressed as a percentage of the number present at the last sampling date. Whereas the use of black and white film in 1977-78 made it difficult to assess the change in leaf numbers, especially at times of rapid growth when large numbers of axilliary leaves were produced, the use of colour positives in 1979 allowed accurate leaf counts.

The seasonal pattern of growth illustrated in figures 4.1a and 4.2a is similar in both years, with little growth in summer and autumn and a peak in late winter to mid-spring. However, the pattern of growth may be distorted in places in these two figures. On three occasions the intervals were longer than a month and the mean increase in length over the interval has been shown as two or three equal increments. No measure-

Figure 4.2 Growth and leaf production of tagged shoots in the field. (a) Mean change in total length and foliated shoot length for 25 tagged shoots recorded between November, 1978 and January, 1980 (foliated length is that portion of the stem bearing leaves). LSDs for comparison of means over time ($P<0.05$) are, from left to right, for total and foliated length, respectively. The horizontal bar shows the activity of shrubs assessed from phenology of shrubs sampled for TNC analysis (Chapter 3). The solid section indicated that half or more of the shrubs showed signs of recent shoot growth; and the hatched section, that less than half were active.

(b) Growth of laterals on tagged shoots (LSD, $P < 0.05$).

(c) Change in leaf number on tagged shoots, including leaves on small laterals, expressed as percentage of the number of leaves present at the beginning of each interval (LSDs, $P < 0.05$).


No record
a
Total
Foliated
20
15
10
5
0
-5
Mean change in length mm
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
b
10
5
0
Not shown
c
40
30
20
10
0
10
20
Leaf gain
Leaf loss
% initial number
No record
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
1978
1979
1980
Month

ments were taken between January and April, 1979 (fig. 4.2a) for example, and the three equal means are probably not an accurate representation of the growth pattern during that time. According to the daily rainfall figures from Koonamore Homestead (see figs 3.4-3.6) most of the rain during the interval fell over 3-4 days in February, probably resulting in a peak of growth as seen at other times when substantial rains fell in summer. Similarly the increase in length recorded in December, 1979 for the interval since October may have been largely produced in November as, apparently, no rain fell between mid-November and the sampling date early in December.

The change in foliated length shown in these two figures, gives some indication of the pattern of leaf gain and loss. As expected, on most occasions an increase in shoot length was associated with an increase in length of leaf-bearing stem and no doubt with production of leaves as well as an increase in internode length. On almost all sampling dates the change in foliated length differed from the change in total length. The difference between the two variables is a measure of leaf loss over a given interval. Given no leaf loss the changes should be equal and hence the shortfall implies a loss of lower leaves.

In one instance (September, 1979, fig. 4.2a) the recorded mean increase in foliated length exceeded the increase in overall length. Although such a result appears anomalous, and is in this case not significant, it is not inconsistent with the pattern of growth of individual shoots. If lower leaves, subtending viable leaf or stem buds, are lost before one measurement of foliated length then a period of growth before the next measurement may result in elongation at the apex and production of leaves at lower nodes thus leading to a change in foliated

length greater than the overall increase in shoot length.

The relative changes in overall and foliated length in September, 1979 also imply no loss of leaf at that time. This illustrates another limitation to the use of this approach to represent the pattern of leaf loss since such changes do not account for the loss of leaves on intermediate nodes. As shown in figure 4.2c some leaf loss, calculated from leaf counts, was evident on all sampling dates in 1979. Part of the difference in pattern of leaf production in figures 4.2a and 4.2c can be attributed to the inclusion of leaves on short laterals, and expanded axilliary leaves, in leaf counts. Nevertheless, these results show that substantial loss of leaf can be expected at times of rapid growth as well as during dry periods. Judging by the data in figure 4.1a part of the decrease in foliated length in summer and autumn (December-May) is due to both leaf abscission and contraction of the stem along its length.

Contractions of this nature have been observed for other species. Oechel *et al.* (1972), for example, measured a decrease in the length of primary and secondary stems of *Larrea divaricata* in summer, which they attributed to water loss and tissue shrinkage. There was no loss of tissue from the stems and apical buds remained undamaged. In most cases this was also true of *A.vesicaria* but the apex of some shoots was damaged when conditions were severe.

Dry conditions during the period of observations on shoot growth were not maintained for long enough to result in the massive loss of leaf sometimes observed for *A. vesicaria* (Osborn *et al.* 1932) but it is apparent that leaf loss is not restricted to summer. References cited in Syvertsen and Cunningham (1977) indicate that the rate of senescence of mature leaves of *L. tridenata* is closely associated with the rate of leaf production. Periods of greatest leaf fall and litter accumulation coincide with periods of maximum growth rate for that species. Leaf loss has also been shown to coincide with leaf production in the arid zone tree species,

Heterodendrum oleifolium (Maconochie and Lange, 1970).

In Chapter 3 it was shown that on several occasions fluctuations in TNC concentration did not correspond to the pattern predicted from notes on the growth stage or sampled shrubs. Because of the imprecision, due to natural variation between shoots of the estimates of growth activity reported here, these data are of little more use in predicting changes in TNC concentration. One problem associated with correlation of increases in shoot length over long intervals with changes in TNC concentration is that growth apparently occurs in short pulses. This subject is enlarged upon in Chapters 6 and 7. How well the increment in growth correlates with observed changes in TNC concentration over the same interval will depend on the timing of the growth pulse relative to the date of sampling for TNC concentration. A fall in TNC concentration resulting from a growth pulse early in the interval will probably not be detected due to subsequent accumulation of TNC before the next sampling date. The peak in TNC concentration in July, 1979 (fig. 3.6) was associated with low rainfall as expected but apparently not with a reduced growth rate (fig. 4.2). The rainfall for the interval, fell shortly after the sampling date in the previous month allowing a long period for accumulation of TNC.

There were also falls in TNC concentration in December, 1978 and January, 1980 which were not expected according to notes on phenology. Growth in summer is difficult to detect just by observation as young stem rapidly hardens and appears similar to older tissue. As far as can be judged from the data in figures 4.1 and 4.2, however, there was some growth in the intervals leading up to the above dates and this could account for the observed fall in TNC concentration.

The results of observations on root growth are described in the next section. Shoot growth of plants adjacent to the observation chambers over the period November, 1977 to December, 1978 is illustrated in figure 4.1b. The mean increase in length for shoots on these plants was greater than that for the undisturbed control plants shown in figure 4.1a. In particular, growth continued during summer whereas shoots on control plants apparently decreased in length.

These plants were growing in an area where the soil overlying the kunkar layer or high concentration of limestone nodules was much deeper than that for the control plants. Water potential readings at various times showed that while the difference between the two groups was small in winter (0.06-0.56 MPa) the water potential of the controls in summer was up to 3.3 MPa lower than that of plants adjacent to the observation chambers.

4.32 Root growth

The first roots appeared at the observation windows in June, 1977 and November, 1977 for chambers installed in February and August, 1977, respectively. For comparison, the first roots of *Atriplex confertifolia* (Fernandez and Caldwell, 1975) appeared at the windows of observation chambers installed in that community about 5-6 weeks after installation.

The photographs in figure 4.3 show the appearance of a few roots growing at the interface between soil and perspex. The hairs on these particular roots, first noted in September, 1978, were still visible in December, 1979. However, not all roots had obvious root hairs, nor did all roots remain visible for so long. It is not certain whether roots which disappeared from view died. In some cases root segments disappeared from view during a dry period and reappeared in the same place after rain.

Figure 4.4a shows the mean root production from 2-5 panels of the observation chambers between July, 1977 and December, 1979. A panel

Figure 4.3 Root observation panels.

Plates 1 and 2 show the appearance of roots observable at the face of perspex panels in the root growth observation chambers. On the left hand side of Plate 1 there is evidence of the condensation referred to in the text.

1

2

consisted of half of one of the observation chamber walls which were divided in two by a vertical strut supporting the perspex window. Root growth was mapped to a depth of 60 cm and hence panel area was approximately 3 000 cm^2.

The remarks made about the possibility of distortions in the pattern of shoot growth over intervals longer than a month due to their presentation as equal increments during the intervening months also apply here. Apart from this, however, the pattern of root growth is similar to that for shoot growth with increases in new root length at the observation windows showing a peak in late winter to mid-spring.

Fernandez and Caldwell (1975) found little difference between temperatures and soil water potentials at observation panels and in the undisturbed profile at a distance of 1 metre from their chambers in Curlew Valley but at Koonamore patches of condensation were sometimes observed at the soil-perspex interface, suggesting differences in temperature and soil water potential for different areas of the same panel. This patchiness was another reason, apart from the unreliability of thermocouple psychrometers, for abandoning the attempt to measure soil water potential. Figure 4.4b shows the mean number of new roots appearing at the observation panels during a given interval. Presumably for most of the time these roots would have been growing in undisturbed soil where conditions were probably similar to those in the profile some distance from the chamber. The pattern is essentially the same as that for root elongation at the interface suggesting that the latter may be representative of the pattern if not the amount of growth expected in undisturbed soil.

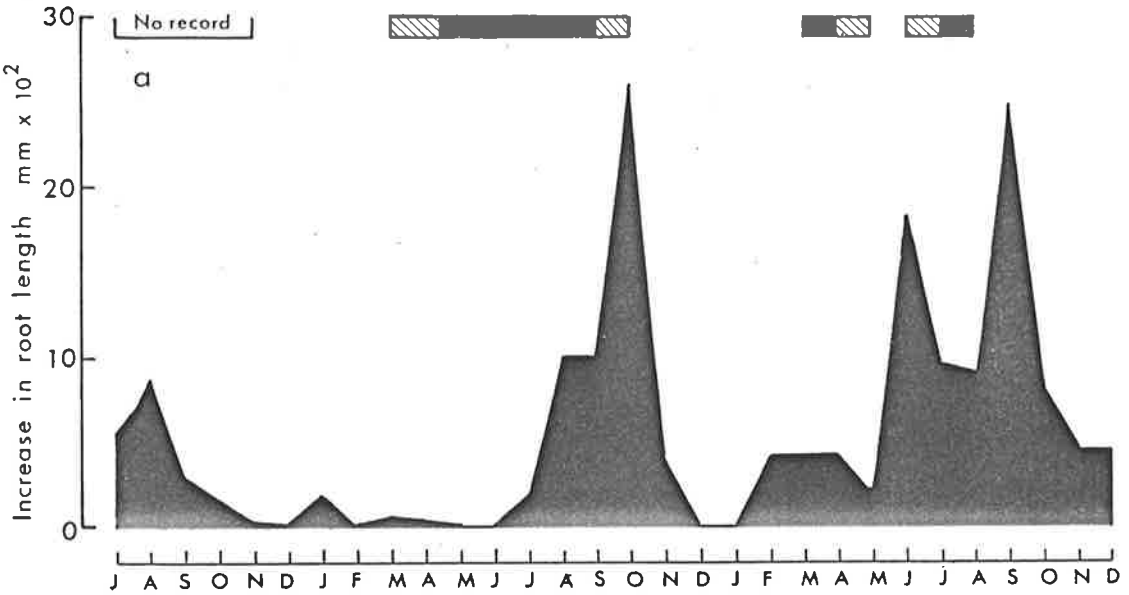
On several occasions during the period that root growth was monitored individual roots were active at the time measurements were taken. The rate of growth of some of these roots is shown in figure 4.4c.

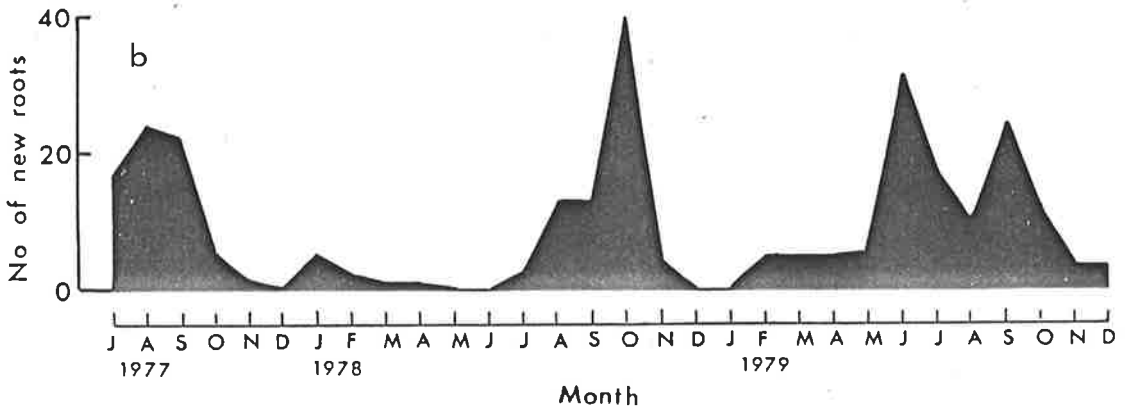
Figure 4.4 Root production.

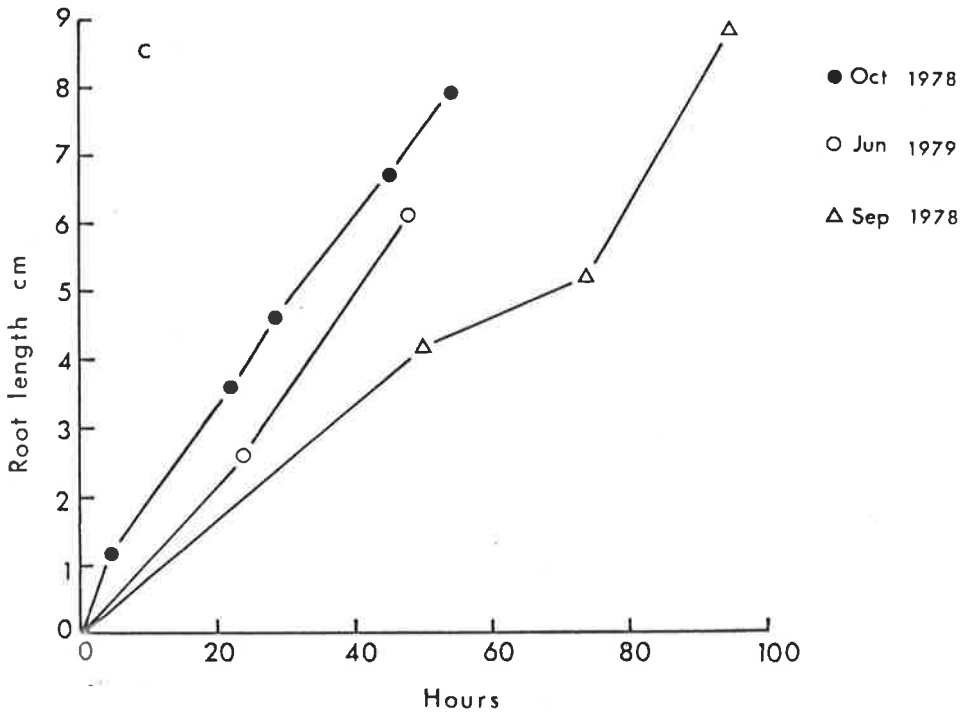
(a) Mean increase in root length observable at 2-5 observation windows in root growth observation chambers between July, 1977 and December, 1979. Each panel was 3,000 cm^2 in area. The horizontal bar shows the activity of roots assessed from notes on phenology of plants sampled for TNC analysis. The solid section indicates that half or more of those shrubs showed signs of recent root growth and the hatched section indicates that root growth was recorded for less than half of the sampled plants.

(b) Number of new roots appearing at the observation window during monthly intervals.

(c) The increase in length of individual roots up to 100 hours after they were first measured on the dates shown. The vertical axis shows the difference between initial and final length at various times.


No record
a
Increase in root length mm x 10²
30
20
10
0
J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D


b
No of new roots
40
20
0
J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D
1977
1978
1979
Month


c
Root length cm
9
8
7
6
5
4
3
2
1
0
0
20
40
60
80
100
Hours
Oct 1978
Jun 1979
Sep 1978

The maximum rate of root growth, from the slope of individual plots of elongation with time, was about 3.5 cm per day, a value similar to rates recorded elsewhere (e.g. Jones and Hodgkinson, 1970, Hodgkinson and Baas-Becking, 1977). However, because the gap between perpex and soil had to be back-filled with loose soil, root growth rates at the interface are probably higher than those in the undisturbed soil profile, due to the low mechanical resistance to root growth at the interface (see Drew, 1979).

A comparison of the root activity recorded in phenological notes on excavated plants, depicted by the horizontal bar in figure 4.4a, with the pattern of growth in the body of that figure shows some major discrepancies. In the interval leading up to the main peaks in root growth little or no young growth was recorded in phenological notes. However, most of the young growth recorded for excavated plants was concentrated near the root crown, or along primary roots, whereas that observed in observation chambers was probably the result of apical elongation of existing secondary or tertiary roots. Root production at or near the crown need not coincide with elongation of existing roots in which case the discrepancies are probably due to the loss of young apical root growth while root systems of plants sampled for TNC analysis were being removed from the soil.

Fernandez and Caldwell (1975) presented data on root growth at various depths in an *Atriplex confertifolia* community. Figure 4.5 shows similar data at 10 cm intervals down the profile in a stand of *A. vesicaria*. The mean increase in new root length, at depths down to 60 cm, is shown in figure 4.5a for the period between July, 1979 and December, 1979. Each subdivision in the panel encompasses an area of 500 cm^2.

Judging by the data in this figure there appears to be considerable root growth at depths as great as 50-60 cm. Part of the root presence at that and higher levels in the profile was, however, due to roots growing down the interface between window and soil, growth which may not have occurred in an undisturbed profile. Figure 4.5b also illustrates growth at depth for *A. vesicaria* but in this case increases in root length were recorded only while the apex remained in the zone of origin, as judged by its first appearance at the observation panel. Growth of roots after they had crossed the boundary of the subdivision was not recorded further. Even on this basis there was some root growth at depths below that expected for *A. vesicaria*. A diagramatic section of root distribution for this species in Jones and Hodgkinson (1970) shows little lateral spread of roots at depths below about 20 cm. On the other hand they did observe some root mass at depths as great as 90 cm. As noted earlier, the observation chambers at Koonamore were located in deep soil in a slight depression which apparently has allowed deeper penetration of the root system than in areas where a hardpan is found close to the soil surface (Cf. Osborn *et al.* 1932).

The data of Fernandez and Caldwell (1975) for *A. confertifolia* and two other species show a progression of root activity from the upper layers of the profile to the lower as the dry season advances. Little evidence of such a progression was observed for *A. vesicaria* at Koonamore, except for some semblance of a transfer of root activity to lower levels in the first year of observation between July, 1977 and June, 1978 (fig. 4.5b), most change occurring in the top 3-4 levels. This response may not be characteristic, however, as root growth patterns were possibly altered in the first year after installation of the observation chambers.

Figure 4.5 Root production at various depths.

(a) The increase in root length, at 10 cm intervals down the soil profile, recorded at observation windows between July, 1977 and December, 1979. Values for other than the top 10 cm include measurements on roots which grew down the interface between the perspex window and the soil from higher levels.

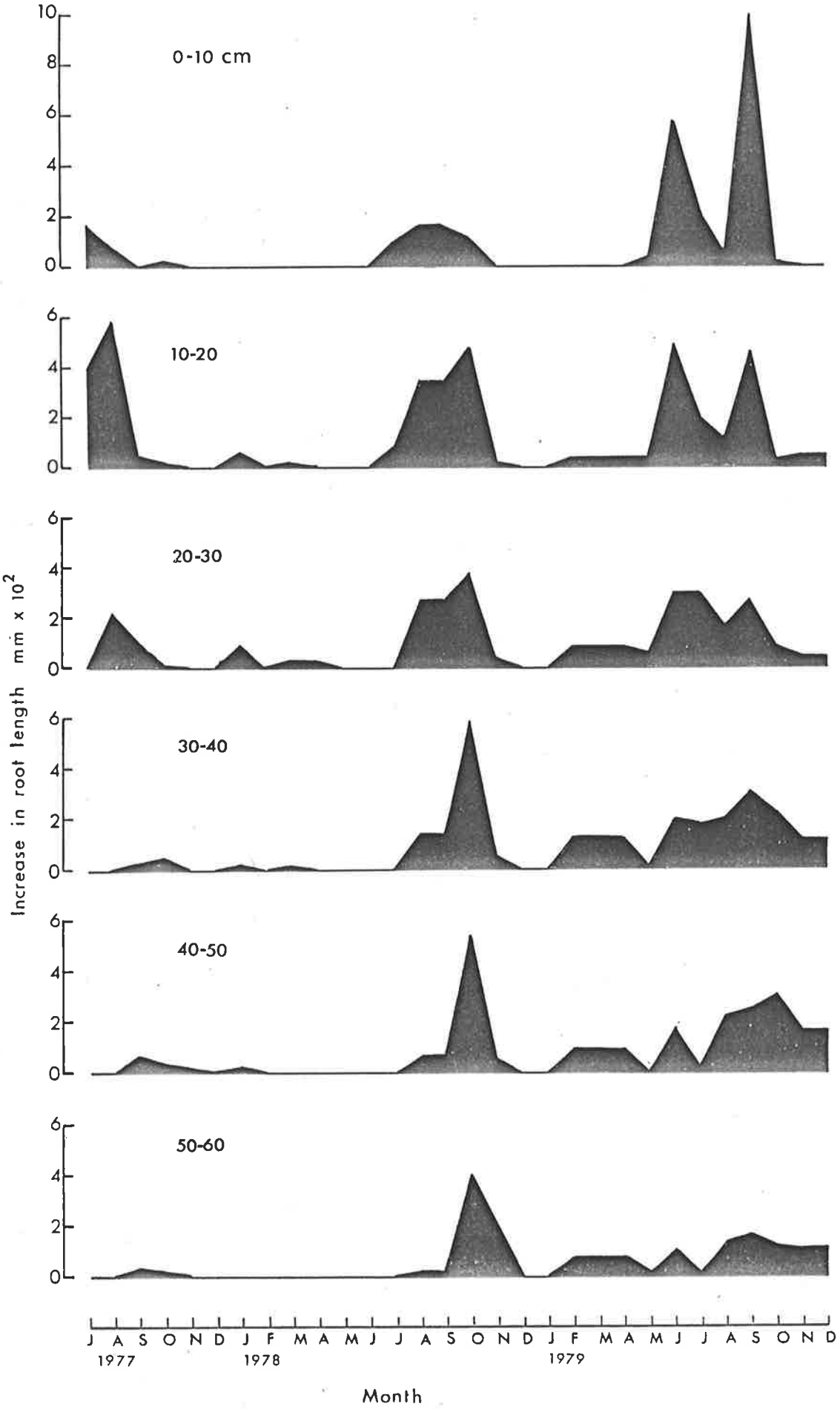
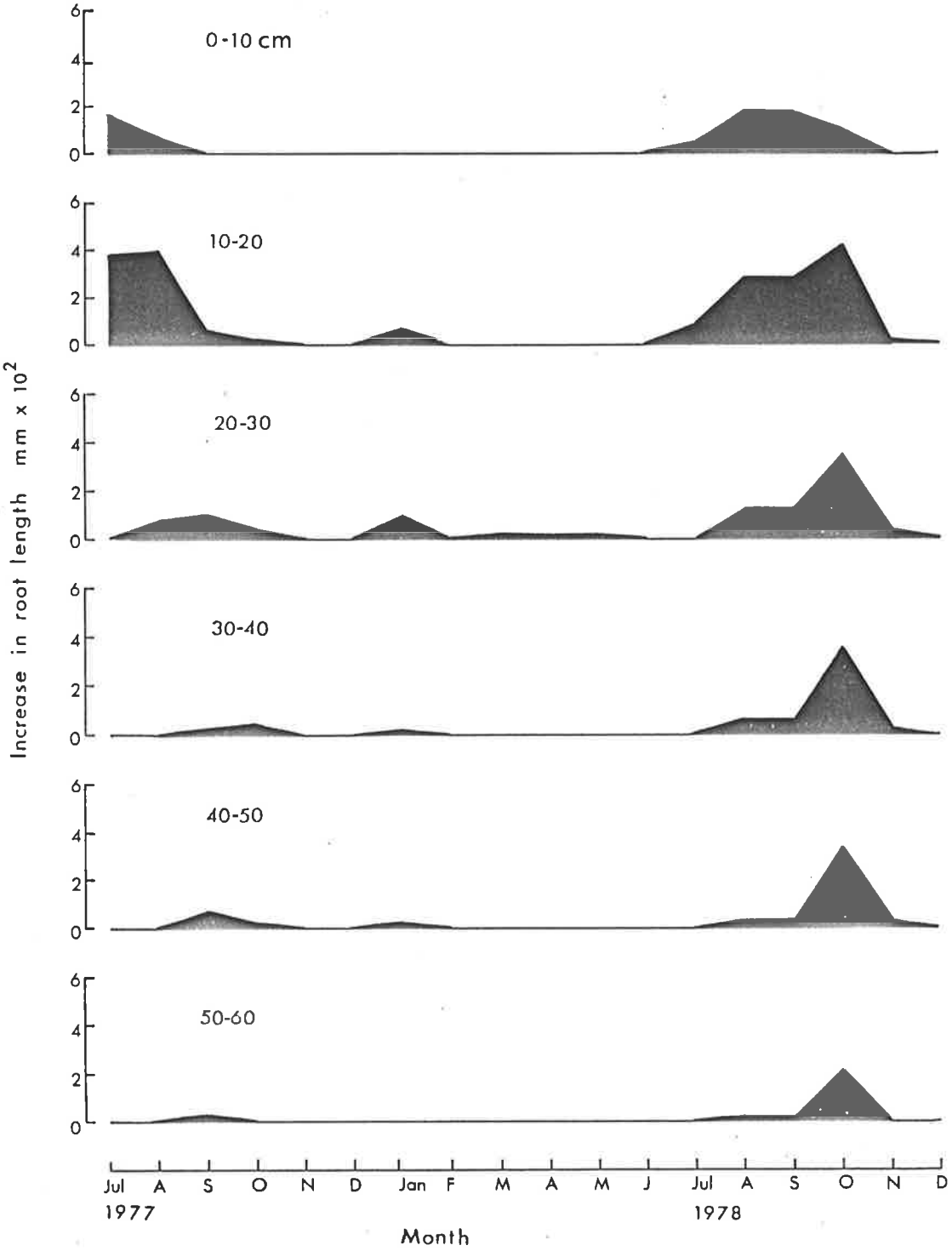

0-10 cm
10-20
20-30
30-40
40-50
50-60
Increase in root length mm x 10²
J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D
1977
1978
1979
Month

Figure 4.5 Root production at various depths.

(b) The increase in root length, at 10 cm intervals down the soil profile, recorded at observation windows between July, 1977 and December, 1978. Sections of root after they had grown out of their zone of origin were not included in summations of root length for lower levels (cf. fig. 4.5a).


0-10 cm
10-20
20-30
30-40
40-50
50-60
0
2
4
6
Increase in root length mm x 10^2
Jul A S O N D Jan F M A M J Jul A S O N D
1977
1978
Month

The following year was too wet to allow the development of a similar response. Nevertheless, a pattern of growth of this kind may occur in stands of *A. vesicaria* probably over a much shorter period than in the case of *A. confertifolia* in Curlew Valley, where the seasons are better defined. Regular observation at intervals much shorter than a month are probably required to detect such a progression of root production by individuals of *A. vesicaria*.

4.4 Discussion

An important feature of the data on shoot growth presented here is the high variability in the response of individual shoots at any given time. During the description of results the means were assumed to be accurate despite their statistical imprecision (see Sokal and Rohlf, 1969, p. 13) although strictly no significance can be attached to the details of small fluctuations in growth.

The overall pattern of growth suggests that plants will respond to heavy rain at any time of the year. The highest rates of growth in the two years of observation were recorded in spring and were presumably associated with the increasing air temperature during that time as well as high soil water potential.

The pattern of root growth was similar to that for shoots but it is not certain whether root growth in the undisturbed soil profile corresponds to that in observation chambers. The growth of roots down the observation panel makes it difficult to interpret these data. A project designed specifically for the study of root growth could yield useful results on root growth in the field using this method, provided that measurements are made to define the soil environment, but probably the best use of observation panels is that made by Hodgkinson and Baas-Becking (1977) or Billings *et al.* (1976) who used an experimental approach, involving

manipulation of the plant or its environment. For example, some useful results on root growth of *A. vesicaria* following defoliation of the shoot were described in Hodgkinson and Baas-Becking (1977).

The variability in the response of shoots suggests that more attention should be paid to the choice of shoots for detailed studies of shoot activity. The position of the shoot on the plant may be important and hence samples may need to be selected by a process of restricted randomization to ensure precise results. On the other hand, variable water absorption by roots in soil zones of differing moisture content and complicated patterns of xylem connections (see ch. 6) may be responsible for the variability between shoots which may still be evident in subclasses of shoots chosen in such a way.

As it stands the pattern of shoot growth broadly accounts for the observed changes in TNC, within the limitations discussed in the previous section. The notes on phenology also have limitations as a means of predicting changes in TNC concentration, for example, in summer when new growth is difficult to detect, but when such notes are combined with rainfall records they provide a good indication of likely changes.

5. Gas exchange

5.1 Introduction

The possibility of positive net photosynthesis at low water potential and its importance to the carbon balance of saltbush have been outlined earlier (Ch.2). The only published rates of photosynthesis for *A. vesicaria*, to my knowledge, are those reported by Wood (1932), Chapman and Jacobs (1979) and Caldwell *et al.* (1977). As the last named authors were interested in temperature acclimation of photosynthesis leaf water potential was maintained at a high level throughout their experiments. Wood (1932) and Chapman and Jacobs (1979) measured the rates of photosynthesis by shoots and individual leaves of field grown plants, respectively. Measurements were made at times when leaf water potential was likely to have been low but since water potentials were either not recorded or too low to measure with the equipment at hand no data on photosynthetic capacity at low water potential are available.

Another aspect of photosynthesis under conditions expected in the field, and outlined in introductory remarks, is the possibility of damage to metabolic and physiological systems, during dehydration to the very low water potentials observed in *A. vesicaria* (e.g. Anderson *et al.*, 1972; Sharma, 1976, 1978). The development of water deficits in plant tissues affects almost every aspect of plant function either directly or indirectly (e.g. Slatyer, 1967; Hsiao, 1973; Boyer, 1977; Osmond *et al.*, 1980). The progressive fall in the rate of net CO_2 assimilation, with increasing water deficit, can be attributed mainly to stomatal closure in many species but in others a non-stomatal component, increasing the resistance to CO_2 transfer through the mesophyll or to CO_2 fixation at the sites of carboxylation, may be equally or even largely responsible for the inhibition of net CO_2 uptake.

Associated with such changes are increased rates of protein and nucleic acid breakdown and changes in the level of hormones in the tissues. Severe levels of stress may induce migration of inorganic and organic nutrients from the leaves and structural damage to organelles. These few examples show the scope for damage to systems involved in the transport and fixation of CO_2 during photosynthesis. The result, as shown for many crop species, is often a slow recovery on rehydration. Part of the overall response in some species may stem from a reduction in the rate of rehydration due to increased suberization of the root system and death of root hairs, but in many species the rate of recovery depends on the ability of the stomata to function normally and an unknown degree of inhibition by non-stomatal factors.

In this chapter these two aspects of photosynthesis, activity at low water potential and recovery on rehydration, are examined and discussed in relation to some of the issues raised in the preceding paragraphs.

5.2 Materials and methods

5.21 Plant material

All gas exchange measurements detailed in this chapter were done on terminal shoots of 3-year-old field grown plants. The plants were raised as cuttings collected from a site adjacent to the Koonamore Vegetation Reserve (KVR). Once established, in small pots of sand from the Koonamore area, they were returned to this site and grown in 30 cm pots until ready for use. To ensure that the microclimate around the cuttings was similar to that around plants naturally established in the field the pots were buried up to their rims; the excavated silty loam soil (Osborn *et al.*, 1935; Carrodus *et al.*, 1965) was used to refill the pots, the deeper layers first, such that when the original surface layers were packed around the cuttings the surface was flush with that of the surrounding undisturbed soil. The cuttings were given supplementary water when transplanted and on two subsequent occasions, at intervals of 3-4 weeks, to aid their re-establishment in the field. After 2.5 years in the field these plants had grown to 0.2 - 0.4 m high and 0.2 - 0.3 m in diameter. In comparison Osborn *et al.* (1932) reported mean values of 0.32 m for height and 0.34 m for diameter of a sample of shrubs (n=5000) growing in the same general area.

5.22 Treatments

Individual plants were collected from the field for measurements of net photosynthesis and respiration in the laboratory. At this stage plants were maintained on a laboratory bench under Metalarc lamps (GTE Sylvania) providing a quantum flux of 150 nE $cm^{-2}s^{-1}$ (400-700 nm) at the top of the canopy for 16 hours per day. Air temperature and humidity were not controlled. At the time gas exchange measurements were taken (summer and autumn of 1979-80) day/night temperatures in the laboratory were as high as 37/25°C and as low as 30/21°C.

Net photosynthesis and respiration were measured during a single drying cycle which was imposed by withholding water from plants until the rate of net photosynthesis fell to zero. In most cases the plants were approaching their hydration compensation point at the time of collection from the field. The soil was then watered and maintained at field capacity until xylem water potential and the rate of net photosynthesis reached maximum values, when water was again withheld. Gas exchange and water potential were then monitored until net photosynthesis was reduced to zero.

5.23 Gas exchange

Rates of net CO_2 uptake in the light and CO_2 evolution in the dark were measured in an open gas exchange system (fig. 5.1) built during the course of this study. The central point of the system is a water-jacketed cuvette (fig. 5.2) designed to accommodate a single microphyllous shoot. Air supplied to the cuvette from a cylinder (CIG Medicalair) was pre-conditioned by passage through a humidifier immersed in a constant temperature water bath. A sample of air diverted from the main stream after this point served as reference for comparison with air leaving the gas exchange cuvette. The difference between CO_2 concentrations of reference and sample air streams was measured with an infra red gas analyzer (URAS 1, Hartmann and Braun) calibrated for a full scale deflection at 50 ppm CO_2 differential. Mixtures of CO_2 in nitrogen, generated by Wösthoff gas mixing pumps, were used to calibrate the gas analyzer, periodically, by using the appropriate mixture to set the end point relative to a zero established by passing air from a cylinder through reference and sample cells connected in series. The same air, which had a CO_2 concentration near ambient, was used as a reference for the gas mixtures of known CO_2 concentration used to construct the calibration curve. The CO_2 concentration of the standard reference gas was found by extrapolation of the calibration curve to zero. As the gas mixing pumps were not readily available at all times a standard calibration gas mixture of

appropriate CO_2 concentration was made by adding nitrogen to a partly used cylinder of compressed air. The standard reference and calibration gases were used to calibrate the instrument daily. To avoid changes in CO_2 concentration these gases were remixed for 1-2 hours before use by warming the bases of the storage cylinders (Sestak *et al.*, 1971).

The construction and dimensions of the gas exchange cuvette are shown in fig 5.2. A single attached shoot bearing several leaves was enclosed in the cuvette, before each run, by means of a split circular plate fitted around the stem and sealed with plasticine. Fine control of the air flow rate was effected by adjustment of a flow meter (Fischer and Porter, Triflat) inserted in the air line just before the cuvette. The CO_2 concentration of air within the cuvette, adjusted by alterations in the flow rate, was never allowed to differ from that of the reference air stream by more than 20 ppm during measurements of CO_2 uptake in the light. As the contents of compressed air cylinders used as an air supply for the system were chosen to have a CO_2 concentration between 310-330 ppm the CO_2 concentration of air within the cuvette did not fall below 290 ppm. Because of the design and size of the cuvette it was considered impractical to instal a fan; to avoid the formation of CO_2 and water vapour gradients, air entering the cuvette, at a rate of at least 30 litres per hour, was recirculated over the shoot by means of an external pump in closed circuit with the cuvette. Recirculation at a rate of 1 L min^{-1} was sufficient to minimise the boundary layer resistance to CO_2 diffusion for the leafiest shoot used, as judged by the response of CO_2 uptake rate to increases in the rate of recirculation.

Air and leaf temperatures were measured by shaded copper-constantan thermocouples inserted through access ports in the perspex rings supporting the copper-walled cuvette. Air temperature was controlled by adjusting the temperature of the water circulating through the water jacket. The fine wire thermocouple used for measuring leaf temperature was adpressed to the

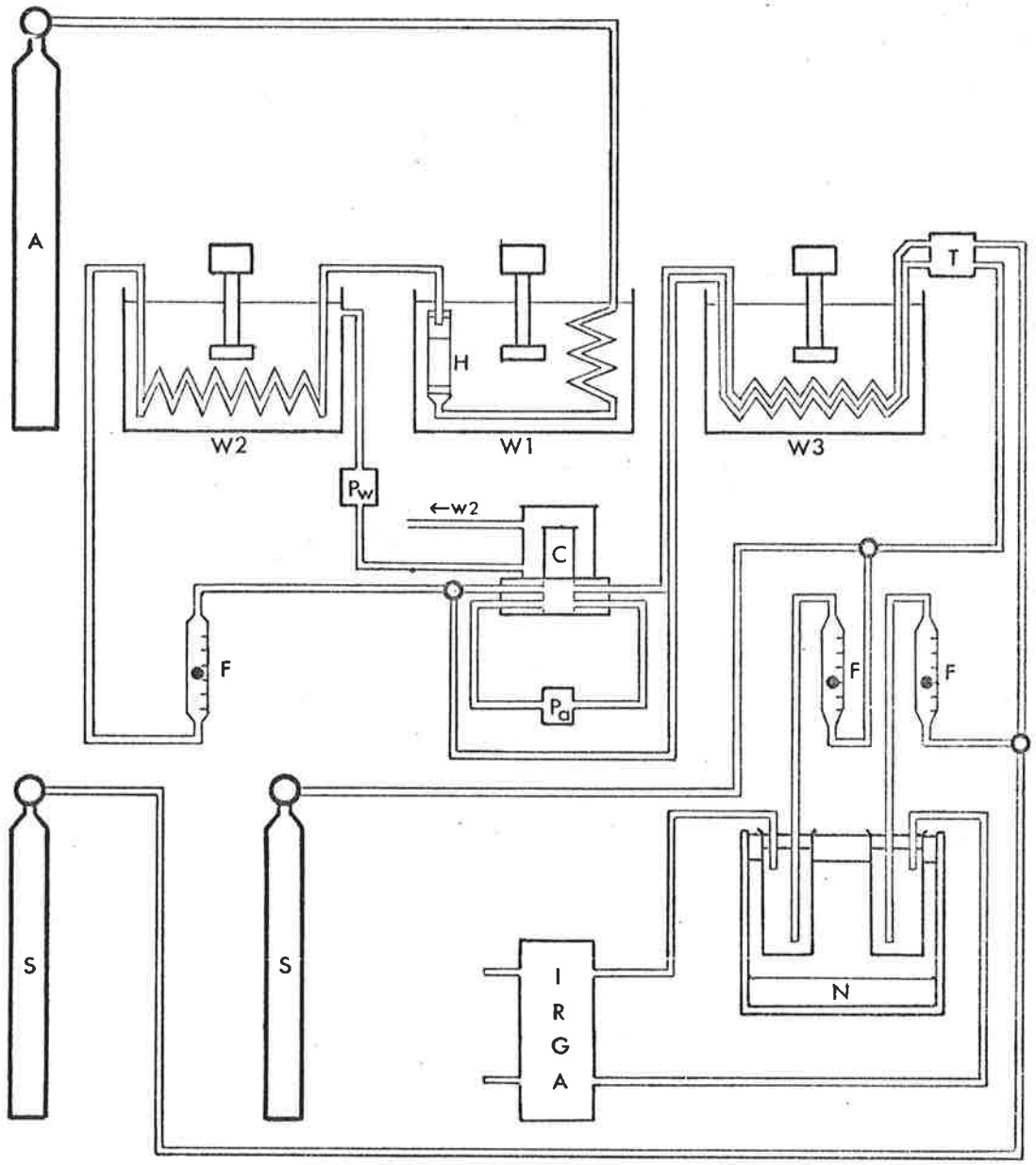


Figure 5.1 Diagram of CO_2 exchange system.

A - Air supply

C - Gas exchange cuvette

F - Flowmeters

H - Humidifier

N - Liquid nitrogen bath

P - Air or water pump

S - Standard calibration gases

T - Thermocouple psychrometer

Figure 5.2 (a) Section and (b) plan of gas exchange cuvette

A - Cuvette

B - Water jacket

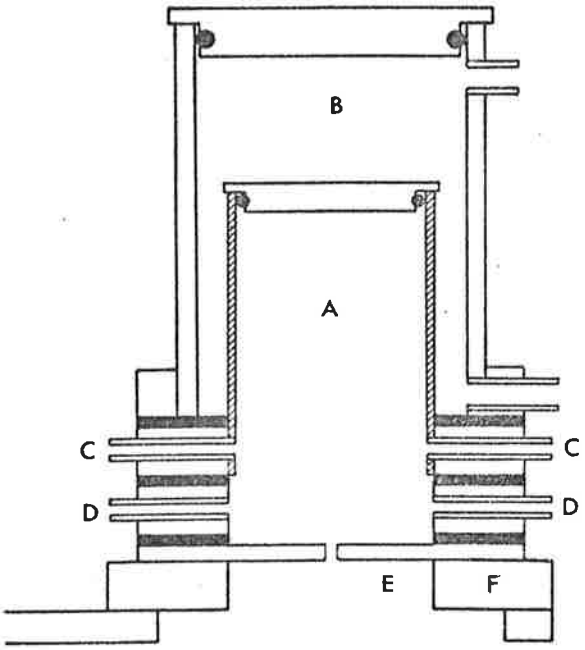
C - Ports for air entry and exit. Also represents ports for recirculation of air via an air pump in closed circuit with the cuvette (shown only in plan).

D - Thermocouple entry ports

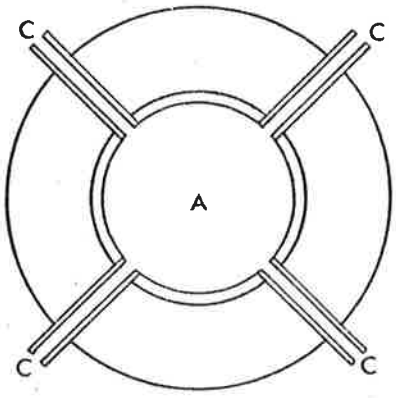
E - Split circular plate used to seal shoots in the cuvette

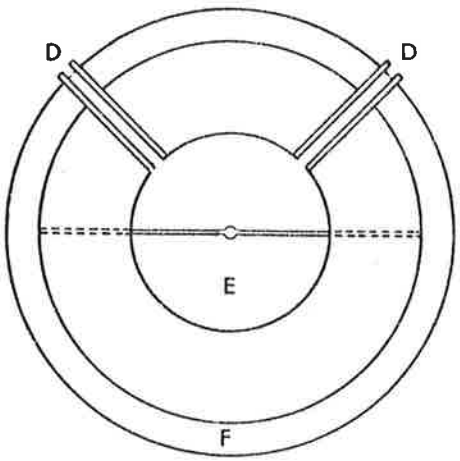
F - Base plate

a



b





ventral surface of a young leaf.

The relative humidity of the air entering the cuvette was adjusted to the required value by controlling the temperature at which air was saturated with water vapour, by passing it through a copper coil and humidifier immersed in a water bath, before it reached the cuvette. On reaching the cuvette air temperature was raised and in the process relative humidity was reduced to a value equal to the ratio of the saturation vapour pressure of air at the temperature of the cooler bath to the saturation vapour pressure at cuvette temperature (Bierhuizen and Slatyer, 1964). A differential thermocouple psychrometer was built according to specifications given in Slatyer and Bierhuizen (1964) and Bierhuizen and Slatyer (1964) to measure the increase in vapour pressure of the air leaving the cuvette due to transpiration. However, in order to obtain a realistic value for net photosynthesis by the microphyllous shoots of *A. vesicaria*, the number of leaves used for a measurement was limited by the necessity to reduce shading of lower leaves to a minimum. The small amount of leaf (2-5 cm^2) used was insufficient to significantly affect the vapour pressure of the air so that in practice the psychrometer was used simply to monitor the vapour pressure of the air entering the cuvette.

The reference and sample air streams passing to the gas analyzer were dried by freezing out the water vapout over a bath of liquid nitrogen. The efficiency of drying was checked periodically by venting the air streams leaving the analyzer through columns of dried silica gel. On all occasions the result was a loss of weight of the silica gel columns.

The light source for the shoot during measurement was a quartz-iodide lamp mounted directly above the cuvette. Irradiance was altered, if necessary, by raising or lowering the light source. The constant flow of water through the water jacket reduced the amount of infrared radiation reaching the cuvette. The quantum flux (400-700nm) at the top of the shoot was measured with either a Licor sensor (Lambda Instruments) or a calibrated light

dependent resistor, constructed to fit within the cuvette.

Leaf area was measured daily by exposing each leaf over small pieces of light sensitive paper which, when developed in ammonia vapour, produced a positive image of the leaf. The leaf shapes were then excised, weighed and leaf area was estimated by comparing the cutout weight with that of known area of exposed and developed paper.

Xylem water potentials were measured by the pressure chamber technique (Dixon, 1914; Scholander *et al.*, 1965). Since the same shoot, where possible, was used for all gas exchange measurements during a particular phase of the drying cycle for a given plant,daily water potential readings were taken on similar shoots when net CO_2 uptake reached a steady state, usually 1-2 hours after beginning a gas exchange run and 7-8 hours after the main lights were turned on.

During a gas exchange run the quantum flux at the top of the shoot was 200 nE cm^{-2} s^{-1}. Leaf temperature was maintained at 30$\pm$ 0.5^{o}C (cf. Caldwell *et al.*, 1977). The relative humidity of air entering the cuvette was 55 per cent. Assuming that the intercellular spaces were saturated with water vapour the vapour pressure deficit between leaf and air was 15 mb.

After the rate of CO_2 uptake had remained stable for 45-60 minutes the light was switched off and the cuvette covered with a black cloth. The efflux of CO_2 in the dark was then recorded until a stable rate had been achieved. The water jacket temperature was not adjusted during the dark period and as a result leaf temperature dropped to a value 1-2^{o}C lower than that in the light.

At this point the shoot was removed from the cuvette and a reading was taken with the cuvette empty to account for any difference in CO_2 concentration of the air streams not due to net CO_2 exchange by the shoot. When this reading differed from the zero established during calibration it served as the reference point for the CO_2 differential used to calculate the rate of net CO_2 exchange.

5.3 Results

5.31 Gas exchange during rehydration

Individual shrubs were collected from the field during the summer and autumn (Dec - April) of 1979-80. Within 2.5 years from the time of their establishment as potted cuttings in the field these shrubs, despite restrictions to root growth (cf Richards and Rowe, 1977), had overall shoot dimensions comparable to those of many of the naturally established shrubs in the vicinity. The cuttings were more open and spindly than many of the surrounding shrubs, although not more so than some mature individuals. Leaf size, shape and general appearance were similar to those of leaves on nearby shrubs.

In the laboratory xylem water potential and CO_2 exchange rate of these shrubs were monitored until net CO_2 uptake in the light was reduced to zero by water stress. At this stage some of the shoots showed signs of wilting. The young non-woody stem on the most severely affected shoots had lost turgor, as judged by their appearance, and older leaves had begun to curl around the edges.

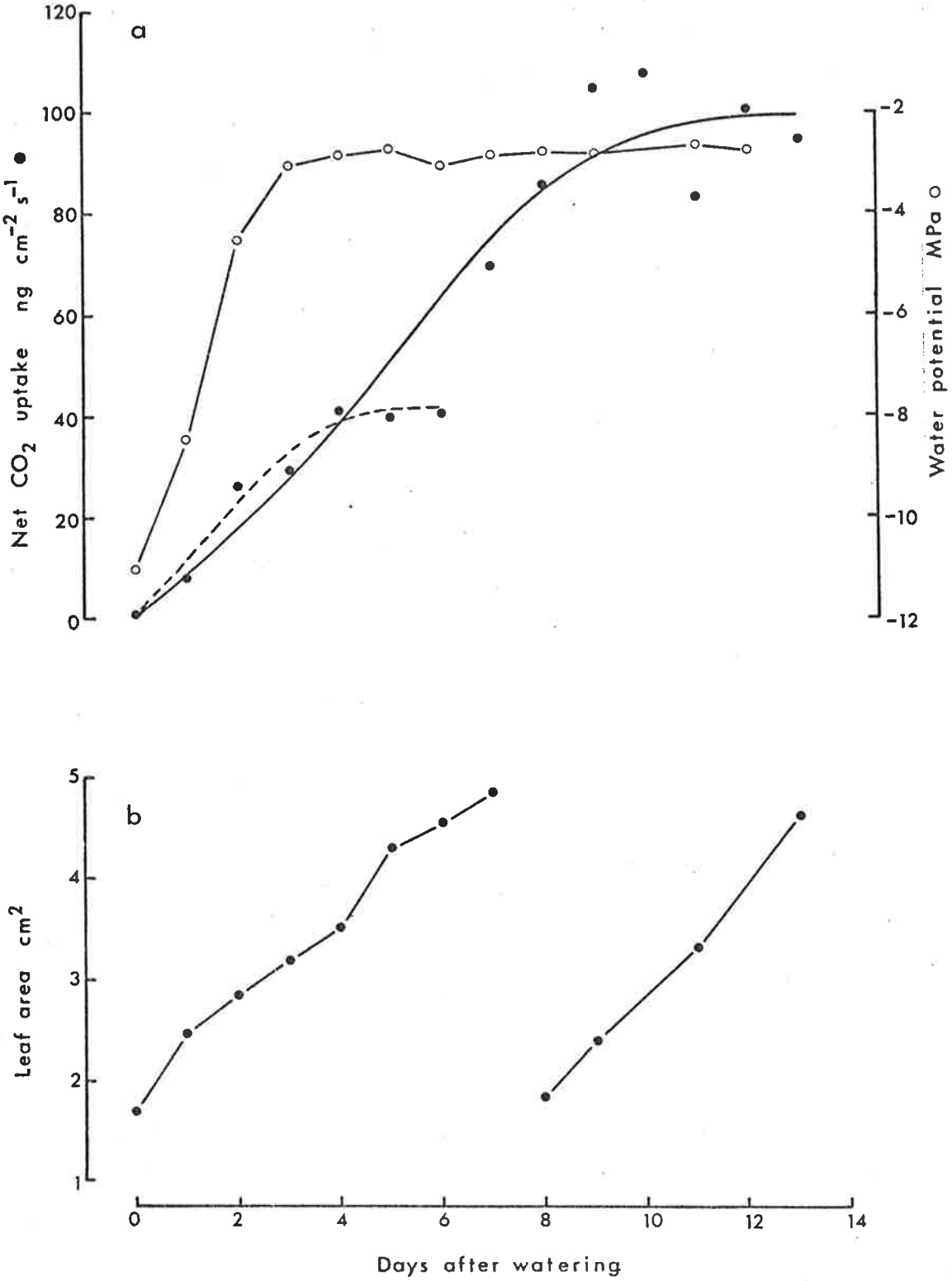
When xylem water potential had fallen to -11.0 MPa, or lower, the soil was watered to field capacity and CO_2 exchange was measured daily. Soil moisture was maintained at or near field capacity and measurements of CO_2 exchange were continued until there was no further increase in the rate of net CO_2 uptake in the light. The results of these measurements are presented in figs 5.3 - 5.6. The CO_2 exchange rates measured during the latter stages of the initial drying phase are presented in the next section (see fig. 5.9b).

Figure 5.3a illustrates some of the difficulties associated with the measurement of net CO_2 uptake rates by shoots bearing several small leaves. On day 7 the shoot used for gas exchange measurements on previous days was damaged while being sealed into the cuvette and was replaced by a shoot with fewer leaves. Net CO_2 uptake per unit leaf area had not increased since

Figure 5.3 Net CO_2 uptake, water potential and leaf area for shoots of field grown cuttings during and after rehydration in the laboratory.

(a) Net CO_2 uptake (closed symbols) for two shoots. Rates of net CO_2 uptake up to day 5 are based on shoot leaf areas estimated from the leaf area on day 6 and subsequent daily rates of leaf area increase measured for shoots on the same and other shrubs after watering. The dashed line represents the response of a shoot with a leaf area of 8.9 cm^2. After day 6 a shoot with a smaller leaf area was used. Xylem water potentials (open symbols) for similar shoots on the shrub were measured when net CO_2 uptake by the shoot in the cuvette reached a steady rate.

(b) The leaf area of two shoots on a second shrub during and after rehydration in the laboratory.


a
b
Net CO_2 uptake ng cm^{-2} s^{-1} ●
Water potential MPa ○
Leaf area cm^2
Days after watering
120
100
80
60
40
20
0
-2
-4
-6
-8
-10
-12
5
4
3
2
1
0
2
4
6
8
10
12
14

day 4, indicating that individual leaves had recovered, as far as possible, from the effects of low water potential. However, net CO_2 uptake rates calculated from measurements on the second shoot increased for a further 2 or 3 days. The apparent maximum rates measured on days 4 to 6 were attributed to uneven illumination caused by self shading within the shoot. An increase in the proportion of shaded leaf photosynthesising at less than maximum capacity, on a given day, apparently masked increases in the capacity for net CO_2 uptake by the whole shoot. Because of the relatively large initial leaf area of this shoot leaf expansion was not immediately obvious. Daily measurements of leaf area from day 7, however, showed that shoot leaf area was increasing by up to 17 per cent per day. The increase in leaf area was due partly to the expansion of existing leaves and partly to the production of new leaves. Such rapid production of leaf area was characteristic of all plants used in this series of experiments. Figure 5.3b shows the increase in leaf area with time for two shoots used for gas exchange measurements during and after rehydration. In this case total leaf area was more than doubling in five days. The initial rates of net CO_2 uptake presented in fig. 5.3a are based on estimates of leaf area calculated from the final leaf area for the shoot involved and average rates of leaf area increase measured on later occasions. All subsequent rates are based on leaf areas measured immediately after the gas exchange run. The final leaf area of the first shoot used (8.9 cm^2) was obviously far too high and later shoots used had an initial leaf area of less than 2 cm^2. These were discarded in favour of a smaller shoot when total leaf area exceeded 5 cm^2. Because of leaf growth at the apex and leaf production in the axils of existing leaves self shading could not be entirely eliminated. Slight differences in position of the shoot within the cuvette from day to day were also unavoidable. As a consequence variable shading may have contributed to the fluctuations in the rate of net CO_2 uptake observed after rehydration and photosynthetic recovery were complete,

although large fluctuations in photosynthetic rate of *A. vesicaria*, and other species, over much shorter periods (hours) have been recorded by others (Caldwell *et al.*, 1977; Chapman and Jacobs, 1979). Short term fluctuations were not observed during this series of measurements.

Recovery of net photosynthetic capacity under better light conditions is shown in figure 5.4. Leaf orientation and position on the shoot used were such that self shading was minimal, at least initially, except for the oldest leaves which were partly folded along the midrib. In this case maximum capacity for net CO_2 uptake was attained in 7-10 days whereas rehydration was complete within 2 days after an initial stress (less than -11.0 MPa) which was sufficient to kill a smaller plant growing in the same pot.

A small plateau in the curve for net CO_2 uptake, similar to that shown in figure 5.3a, developed on days 2-4. In this case the resumption of daily increases in the rate of net photosynthesis coincided with the onset of leaf production by both apical and axilliary meristems. Before day 5 there had been no evidence of new growth other than the expansion of existing leaves as the stress imposed during the drying phase had killed the young leaves enclosing the apex and presumably inhibited meristem activity.

The water potential of a third plant fell to at least -11.7 MPa before soil moisture was restored to field capacity. This resulted in the death of many of the apical meristems. On the shoot used, the apical meristem and those in the axils of the top three expanded leaves all died. The meristem in the axil of the fourth leaf did not begin rapid leaf production until day 16. Nevertheless expansion of the four remaining leaves began soon after the soil was watered and their capacity for net CO_2 uptake increased at a rate similar to that for shoots on other plants. In some instances the rate of net CO_2 uptake began to fall shortly after reaching a maximum. The rate of net CO_2 uptake by this shoot, in particular, began to fall immediately

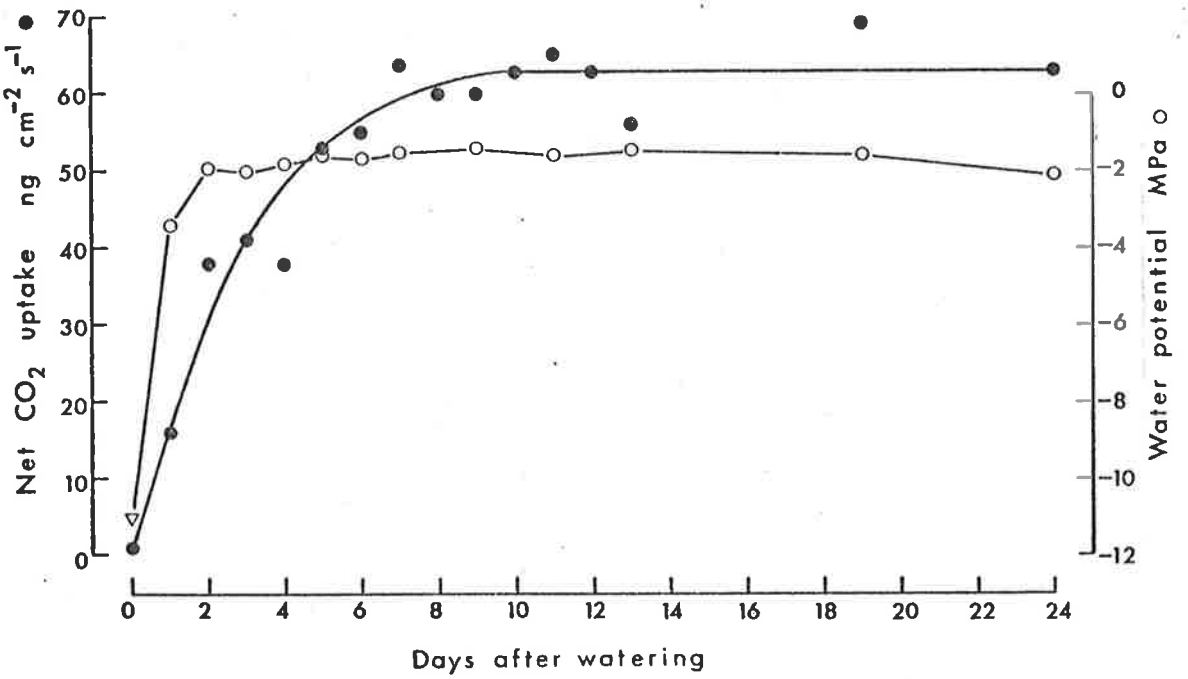


Figure 5.4 Net CO_2 uptake (closed symbols) and water potential (open symbols) for shoots of a field grown cutting during and after rehydration in the laboratory. Xylem water potentials were measured on similar shoots when net CO_2 uptake by the shoot in the cuvette reached a steady rate. The inverted triangle for water potential on day 0 indicates that water potential was less than -11 MPa, the limit of the pressure chamber.

after reaching a maximum on day 8 and was finally reduced to zero by day 60 (fig. 5.5). By that time all but one of the original leaves had fallen and the remaining leaf had apparently lost most of its chlorphyll. Nevertheless relatively high rates of net CO_2 uptake were maintained for most of this period despite visible symptoms of ageing.

The results of gas exchange measurements during rehydration, for six plants, are summarized in figures 5.6 and 5.7. Rehydration was, on average, complete by about day 4. Maximum rates of net CO_2 uptake, on the other hand, were not achieved until day 8 or 10 although 90 per cent of capacity was regained by about day 5.

Respiration rates, measured immediately after a period of net CO_2 uptake in the light, increased rapidly during rehydration and reached maximum, though fairly unstable, rates in 3-4 days. Large fluctuations were observed for some shoots (fig. 5.7). The rapid increase in respiration rate parallels the rate of increase of water potential and is consistent with the early enlargement of leaves during rehydration.

5.32 Gas exchange during dehydration

When no further increases in the rate of net CO_2 uptake by rehydrating shoots could be detected water was again withheld and gas exchange was monitored until net photosynthesis was reduced to zero. The conditions in the cuvette were similar to those during rehydration. In most cases a different shoot was chosen from among those which had not produced large numbers of lateral leaves near the apex during rehydration. All but one shoot, however, as a result of rapid growth during rehydration, consisted entirely of young growth not previously exposed to water stress. New shoots were chosen to avoid the problems associated with the presence of shaded leaves. However, some new leaves were produced during the first few days while turgor remained high. Shading was less of a problem during the drying phase as shoot leaf area decreased at low water potential. This

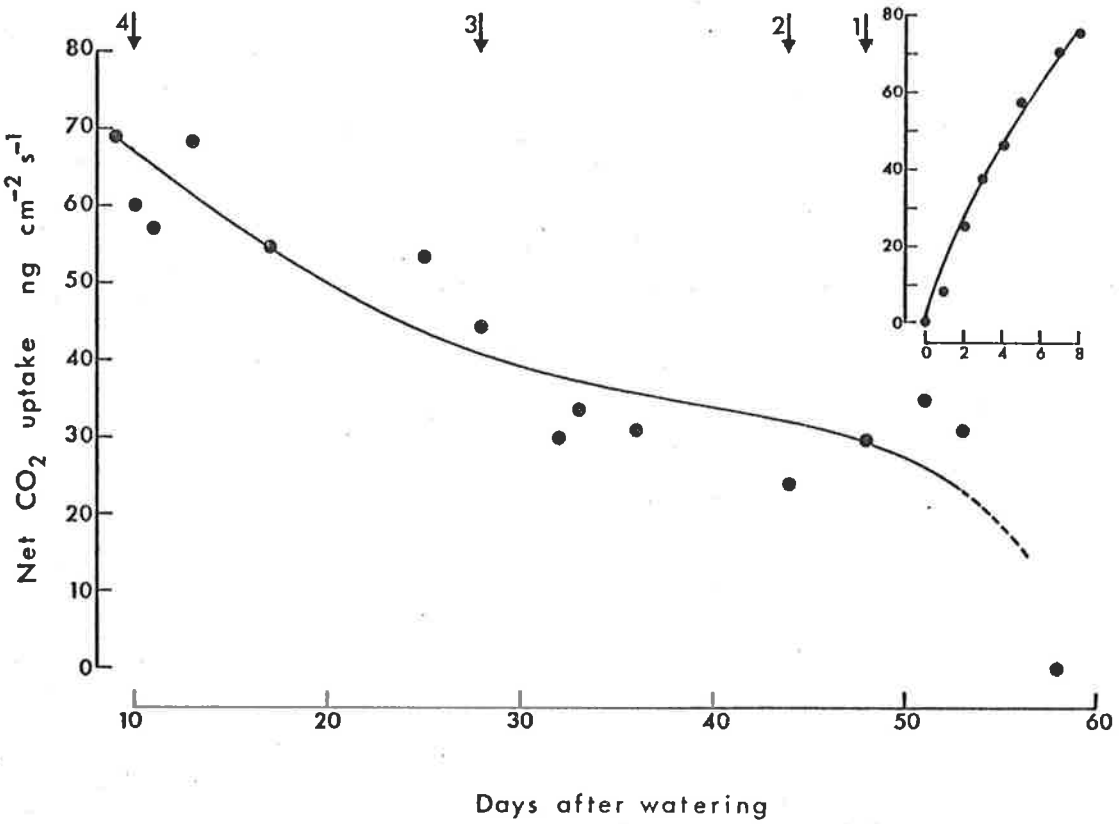
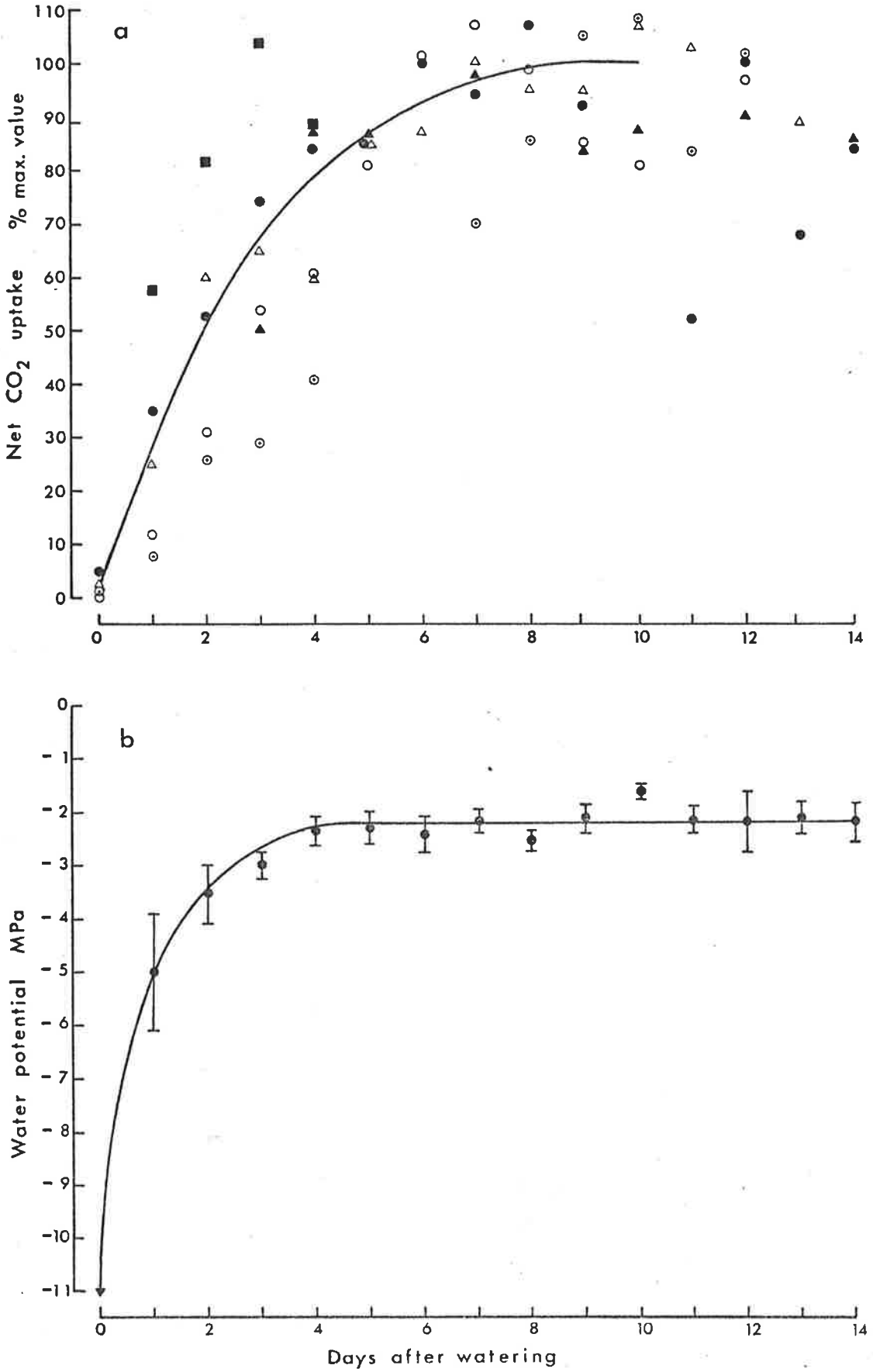


Figure 5.5 The decline in net CO_2 uptake measured for a shoot on a field grown cutting maintained at high water potential after rehydration in the laboratory. Initial water potential before watering was low enough to kill the apex and buds in the axils of the top four leaves, which all abscised during the period of measurement. The numbers along the top of the diagram show the number of leaves remaining on the days indicated by the arrows. The inset shows rates of net CO_2 uptake by those four leaves in the first 8 days after watering.

Figure 5.6 Relative net CO_2 uptake and mean water potential for shoots of six field-grown cuttings during and after rehydration in the laboratory.

(a) Net CO_2 uptake expressed as percentage of the mean maximum value reached after rehydration.

(b) Mean water potential on successive days after watering. The inverted triangle on day 0 indicates that water potentials were less than -11 MPa.


a
Net CO_2 uptake % max. value
110
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
b
Water potential MPa
0
-1
-2
-3
-4
-5
-6
-7
-8
-9
-10
-11
0
2
4
6
8
10
12
14
Days after watering

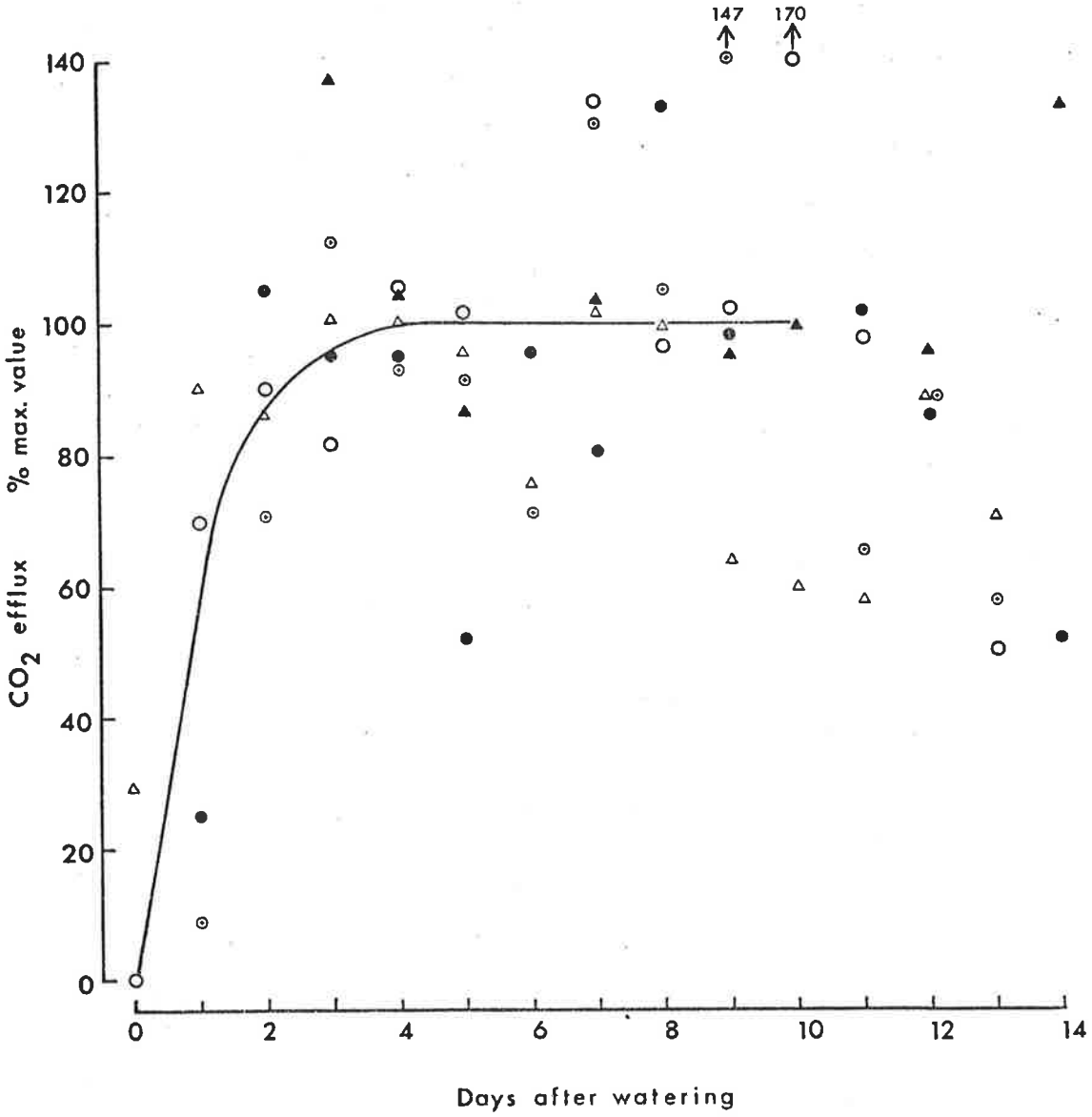


Figure 5.7 Relative CO_2 efflux in the dark for shoots of field-grown cuttings during and after rehydration in the laboratory. Measurements were made immediately after net CO_2 uptake in the light reached a steady rate. Values are expressed as a percentage of the mean maximum value assessed from curves drawn by eye through the data points for individual shoots.

was due to contraction of existing leaf area rather than loss of individual leaves. On the other hand, at very low water potential, poor leaf orientation due to wilting petioles and folding along the midrib did result in poor illumination for some leaves on all shoots.

The results of CO_2 exchange measurements during dehydration, expressed as percent of initial value, for shoots from five plants are summarized in figure 5.8. As xylem water potential fell below about -3.5 MPa the rate of net CO_2 uptake decreased rapidly. On average, less than 50 per cent of initial activity remained at water potentials near -5.0 MPa. At lower water potentials the decline was not as rapid and low rates of positive net CO_2 uptake were observed at water potentials less than -11.0 MPa. After an initial decline in the rate of dark respiration the relative rates were maintained at high levels until water potential had fallen to between -8.0 and -9.0 MPa. However, the errors associated with the calculation of CO_2 exchange rates at very low water potentials were such that it is not possible to say with any certainty, that net CO_2 uptake in the light and CO_2 efflux in the dark were positive at water potentials less than -9.0 MPa and -8.5 MPa respectively. These errors are depicted in figure 5.9 which shows the response to decreasing water potential for two of the shoots included in figure 5.8. The bars represent the percentage error associated with the calculation of absolute rates of net CO_2 exchange at low water potential.

Most of the error of measurement was due to low output from the analyzer when plant water potential was low. The output from the gas analyzer is independent of the flow rate only at normal flow rates of 30-60 litres per hour. In order to avoid complications the flow rate of sample and reference air streams were maintained at not less than 30 litres per hour. Air flow rates of 30-50 litres per hour were high enough to maintain a CO_2 differential of 20 ppm between sample and reference air streams when the small amounts of leaf used were photosynthesising at maximum rates. As net CO_2 uptake

Figure 5.8 Relative net CO_2 uptake in the light and relative CO_2 efflux in the dark for shoots of field grown cuttings during dehydration in the laboratory. Most leaves on these shoots were grown under laboratory conditions during a period of rehydration which preceded these measurements. For data plotted at -11 MPa water potential was in all cases less than -11 MPa, the limit of the gauge on the pressure chamber normally used.

(a) Net CO_2 uptake (percent.initial value) with decreasing water potential.

(b) CO_2 efflux (percent.initial value) with decreasing water potential. Measurements of CO_2 efflux in the dark were made immediately after net CO_2 uptake in the light reached a steady state.

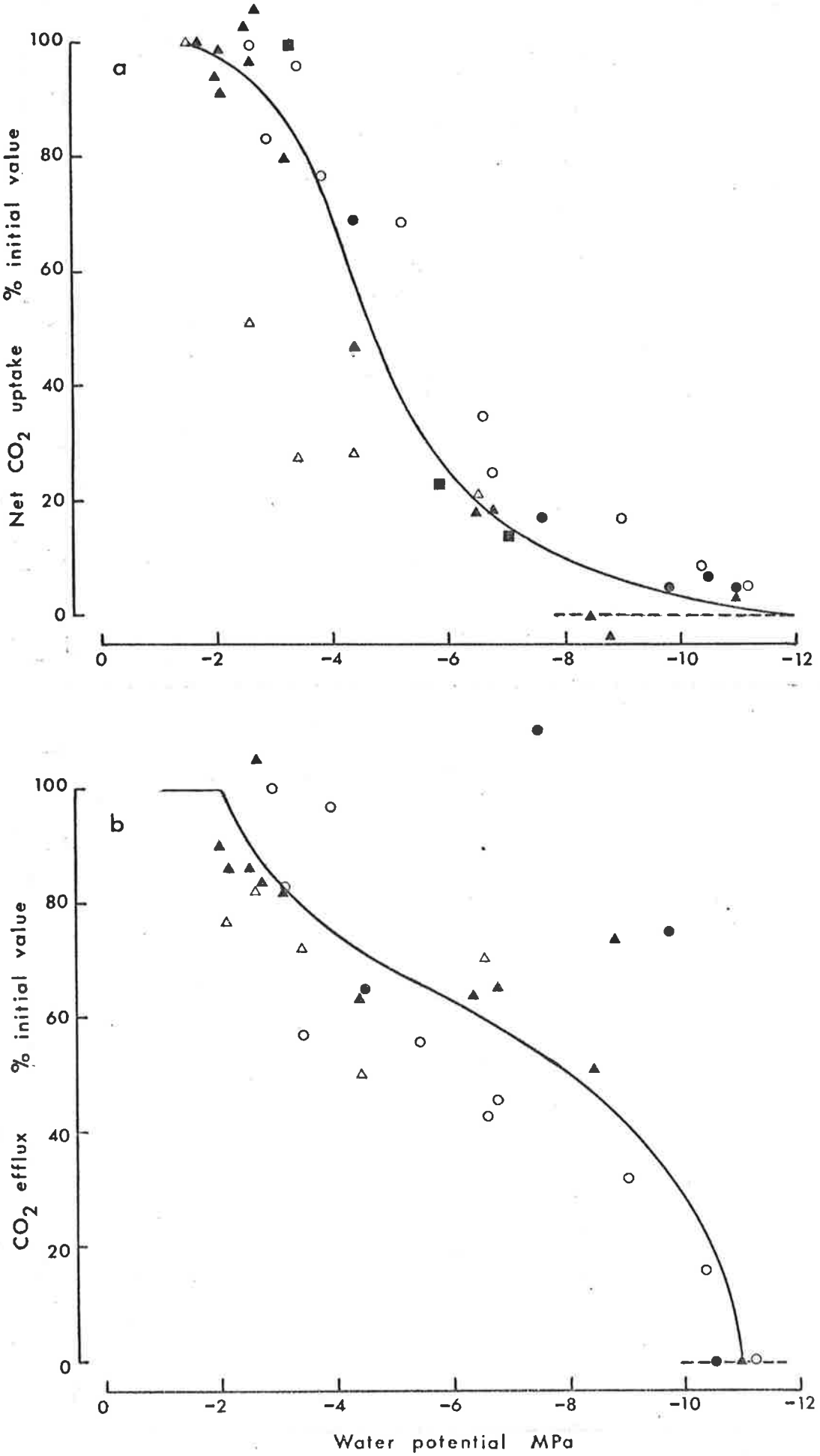
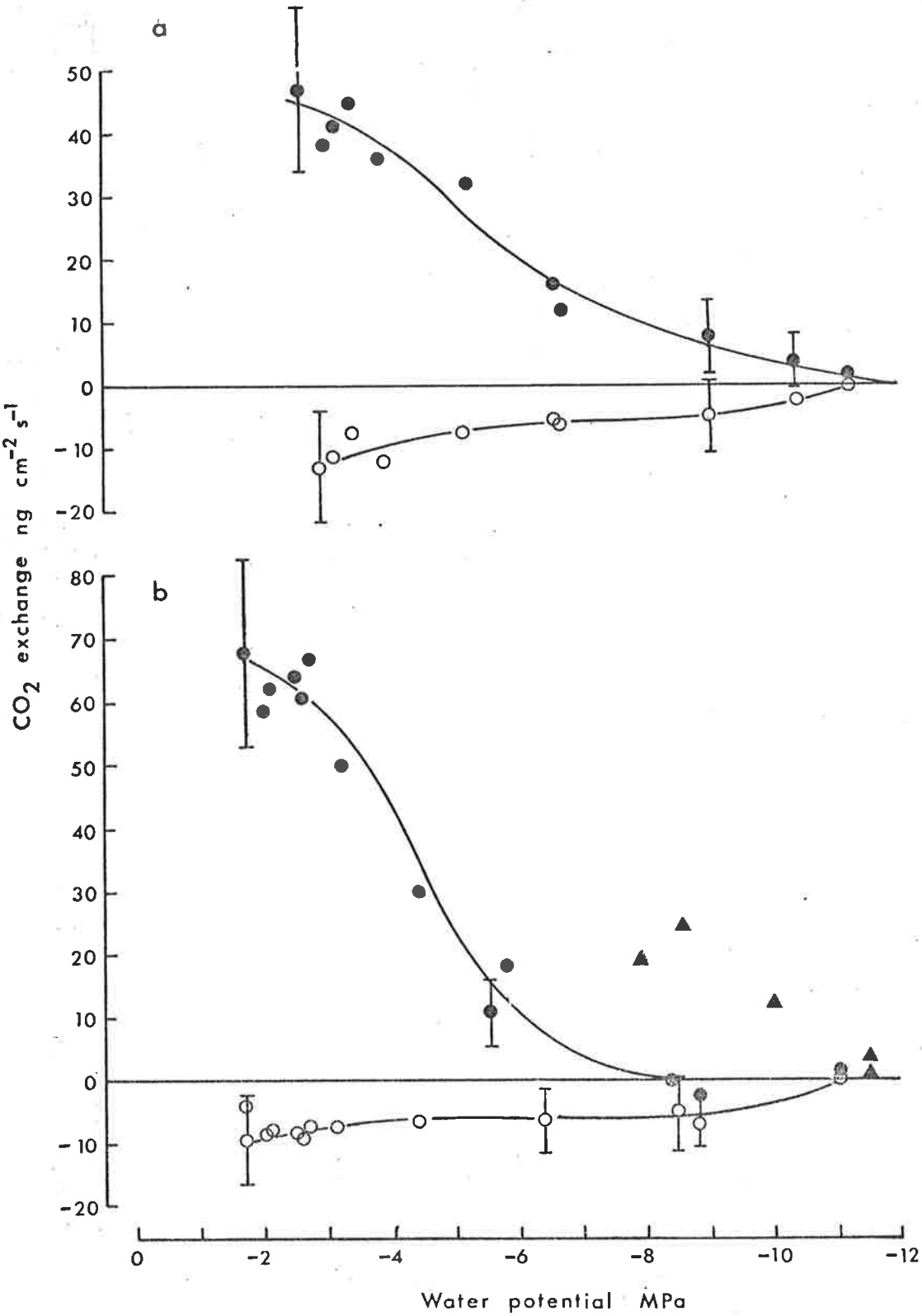

a
Net CO_2 uptake % initial value
100
80
60
40
20
0
0
-2
-4
-6
-8
-10
-12
b
CO_2 efflux % initial value
100
80
60
40
20
0
0
-2
-4
-6
-8
-10
-12
Water potential MPa

Figure 5.9 Net CO_2 uptake and CO_2 efflux for two shoots on field-grown cuttings showing the observed range of variability between shoots and percentage errors associated with measurements of CO_2 exchange during dehydration of shrubs in the laboratory. For data plotted at -11 MPa water potentials were in all cases less than -11 MPa, the limit of the gauge on the pressure chamber normally used. (a) CO_2 exchange for a shoot bearing leaves grown in the laboratory. Closed and open symbols represent net CO_2 uptake in the light and CO_2 efflux in the dark, respectively. (b) CO_2 exchange for a shoot with some field grown leaves of unknown age. The closed triangles represent data for shoots with all leaves grown in the field. These data were recorded during an initial drying phase shortly after shrubs were collected from the field and before they were watered. All the rates of net CO_2 uptake for these shoots were significantly greater than zero except for the smaller of the two rates at less than -11 MPa. Symbols are otherwise as in Figure 5.9a.


a
b
50
40
30
20
10
0
-10
-20
80
70
60
50
40
30
20
10
0
-10
-20
0
-2
-4
-6
-8
-10
-12
CO_2 exchange ng cm^{-2} s^{-1}
Water potential MPa

decreased at low water potentials, the CO_2 differential could not be maintained by decreasing the flow rate and the error associated with reading the chart became an increasingly greater proportion of the output from the analyzer, with the result that many of the calculated rates at very low water potential are not significantly different from zero.

However, I consider it probable that positive net CO_2 uptake by some shoots does take place at water potentials less than -11 MPa and that this could be demonstrated with well illuminated shoots. In all cases there was some shading of axilliary leaves as well as folding and poor orientation of older leaves which probably represented some unused photosynthetic capacity during measurements of net CO_2 uptake at low water potential. These leaves were not dead as they regained turgor when high water potential was restored. Their contribution to the total net CO_2 uptake by the shoot under better light conditions would increase the calculated rate of net photosynthesis and more importantly the output from the analyzer, thus reducing the percentage error associated with the calculation. The alternative to increasing irradiance to the lower leaves, which might be done by lining the cuvette walls with crinkled aluminium foil, is to increase the leaf area in the cuvette. The output from the analyzer could be increased in this way without reducing the flow rate to the measuring cells. A cuvette designed to accomodate several small shoots and lined with reflecting foil would provide more precise estimates of gas exchange. Increasing the leaf area on a single shoot would simply compound the problems of self shading described in section 5.31.

Figure 5.9 also illustrates differences between shoots due to natural variation or preconditioning. Although some leaves may be capable of positive net CO_2 uptake at low water potential, net CO_2 uptake by the shoot as a whole may be reduced to zero at higher water potentials, due simply to natural variation between shoots or plants. An example of such a rapid

decline is shown in figure 5.9b. This result was unexpected as the shoot represented was the only one used during the dehydration series which still retained a number of field-grown leaves. These were expected to maintain high rates of net photosynthesis at low water potential and thus contribute more favourably to net CO_2 uptake by the whole shoot. However, their age was not known and their photosynthetic capacity may have been reduced by the apparently rapid progress of physiological ageing at high water potential during prior rehydration (see fig. 5.5 and Section 5.4). The rates represented by triangles in figure 5.9b are for shoots bearing field-grown leaves only. These measurements were taken during the initial drying phase before rehydration. All were measurably greater than zero except for the two lowest rates at water potentials less than -11.0 MPa.

5.4 Discussion

5.41 Net CO_2 uptake at high water potential

The maximum rates of net CO_2 uptake recorded (ca. 50-100 ng cm^{-2} s^{-1}) are comparable to those reported for *A. vesicaria* by Caldwell *et al.* (1977) and Chapman and Jacobs (1979). Caldwell *et al.* measured maximum rates at 30°C of 50-60 ng $cm^{-2}s^{-1}$ for shoots of glasshouse grown specimens of *A. vesicaria* and *A. confertifolia*. The irradiance used for those measurements was half that used for the measurements reported in this chapter. Chapman and Jacobs reported maximum rates between 70 and 105 ng cm^{-2} s^{-1} for individual leaves of *A. vesicaria*. These measurements were made in spring, when xylem water potential was high, on plants growing in the field. In this case the measured rates were based on the amount of labelled CO_2 fixed by leaf segments during a 20-second exposure and therefore represent gross rather than net CO_2 uptake (Shimshi, 1969). These rates were maintained for only brief periods in the field.

The rates of net CO_2 uptake recorded for shoots are an average for all leaves and hence are not a good indication of maximum leaf rates, which are usually attained at the time leaves reach full expansion (e.g. Ludlow and Wilson, 1971). Field grown individuals of *Atriplex nummularia*, for example, were observed to photosynthesise at about 67 per cent of maximum leaf rates (Jones *et al.*, 1970) due, in part, to the presence of young and old leaves. Very high net assimilation rates (NAR) of 0.31 g cm^{-2} day^{-1}, equal to the highest rates achieved by *Helianthus annuus* (Warren Wilson, 1966) have been recorded for young plants of *A. vesicaria* (Jones *et al.*, 1970). Warren Wilson calculated that maximum rates of net CO_2 uptake, by whole plants, of 130 ng cm^{-2} s^{-1} could account for the high NAR recorded for *H. annuus* and estimated values of up to 180 ng cm^{-2} s^{-1} for the most active leaves. Similar high rates might perhaps be expected for mature leaves of *A. vesicaria* although they need not be as high if total respiratory losses are less than

those of *H. annuus*. If the highest rates recorded here are corrected according to the figures given for *A. nummularia* by Jones *et al.* (1970) values of about 150 ng cm^{-2} s^{-1} are obtained. These are within the range estimated for *H. annuus* and somewhat higher than a rate measured for apical leaves of a young seedling of *A. vesicaria* (135 ng cm^{-2} s^{-1}) while the gas exchange system built during this study was being tested. Even higher rates might be achieved in the field during periods of high water potential in summer. Wood (1932) observed a temperature optimum at air temperatures between 40-45°C for net CO_2 uptake by shoots of field grown plants. These temperatures are 10-15°C higher than leaf temperatures maintained during measurements in this study and mean air temperatures prevailing during measurements of NAR by Jones *et al.* (1970). Some degree of temperature acclimation, however, may reduce any differences in assimilation rate at the two temperatures (Strain, 1969; Mooney and Harrison, 1970). Caldwell *et al.*, for example, observed a temperature optimum for net CO_2 uptake at 30°C for shoots of *A. vesicaria* grown in a glasshouse at or near that temperature.

Since net CO_2 uptake was not measured at high water potential before stress was imposed it is not known whether photosynthetic capacity was fully regained on rehydration. Five woodland species studied by Davies and Kozlowski (1977) failed to regain original rates of net photosynthesis after a stress of less than -2.0 MPa. This result was attributed to accelerated leaf senescence. Ludlow (1975), however, considered that if photosynthetic recovery lagged rehydration any depression of final maximum rates of photosynthesis was due to normal decline with leaf age. Since, for *A. vesicaria* similar maximum rates were obtained for shoots both with and without a large proportion of new growth pre-stress rates of net CO_2 uptake were probably regained by stressed leaves.

The pattern of recovery is similar to that described by Ashton (1956)

for sugar cane recovering from repeated drying cycles between field capacity and 'permanent wilting percentage'. Recovery, as for saltbush, was characterized by an initial rapid increase of net CO_2 uptake followed by a slower increase to pre-stress levels. The times taken to regain full photosynthetic capacity ranged from 6 to 11 days after soil moisture was restored to field capacity. Shoots of *A. vesicaria*, in comparison, took 7-10 days to recover.

The slow recovery is clearly not due to persistent tissue water deficit as rehydration is relatively rapid. It is possible that part of the lag in recovery for some shoots is due to the production of young and shaded leaves in the axils of existing leaves and is therefore an artefact resulting from the use of shoots rather than single leaves for measurements of net CO_2 uptake during rehydration. The photosynthetic rate of young leaves increases with age until they reach full size (e.g. Ludlow and Wilson, 1971). In this series of measurements the leaf area of all leaves, irrespective of their age, was used to calculate the rate of net CO_2 uptake. Therefore until the proportion of leaf area contributed by young leaves becomes less as the new and existing leaves expand the calculated rates may be low and thus contribute to an apparent lag in recovery. However, judging by the equally slow recovery by one shoot (fig. 5.5, inset) on which no new leaves were produced, due to death of the meristems, apparent inhibition by low photosynthetic rates of young leaves is likely to account for only a small part of the observed response. Most studies of this kind are done on single leaves and therefore interpretation of the response is not complicated by the presence of previously unstressed leaves or leaves of different ages.

As a result of such experiments the after-effect of moisture stress has been attributed to inhibition of stomatal opening although, in some cases, a non-stomatal component has been detected (Boyer, 1971b; Kriedemann and Loveys, 1975; Davies and Kozlowski, 1977). One of the theories

on stomatal regulation during water stress is that an imbalance between abscisic acid (ABA) and cytokinin levels acts to promote stomatal closure via effects on cell permeability to inorganic ions (Itai and Benzioni, 1976). Massive accumulation of ABA during stress (e.g. Wright and Hiron, 1969) had been considered responsible for after-effects on stomatal opening. However, because endogenous ABA levels drop precipitously on rehydration Kriedemann and Loveys (1975) suggested and provided evidence that high levels of phaseic acid, a product of ABA metabolism, contributed to both stomatal and non-stomatal inhibition of photosynthesis by vine leaves after rehydration.

From the limited amount of evidence available so far it is not clear whether hormonal effects play a large part in the recovery of arid zone plants. Kriedemann and Loveys (1974) found that four arid zone species, including two chenopods, had low initial levels of ABA which increased only slightly during stress. They concluded that the low levels were an adaptation which would allow full use of intermittently available water but the slow recovery observed for *A. vesicaria* in this study suggests either a high stomatal sensitivity to low concentrations of ABA (or phaseic acid) or that high concentrations are accumulated in the leaves during stress. Allaway and Mansfield (1970) and Davies and Kozlowski (1977), on the other hand, considered that an after-effect delaying stomatal opening would be an advantage for species on dry sites in that water could be conserved following rehydration. For shallow rooted species such as *A. vesicaria* the benefits of such a response may be reduced because of water loss by direct evaporation from the shallow root zone during the period of photosynthetic recovery. In some circumstances, however, the loss of water by direct evaporation from the soil surface may be rapidly reduced if initially high rates of evaporation and rapid drying of the surface soil lead to the establishment of a zone of high resistance to water vapour diffusion in the upper layers

of the profile. (see Milthorpe, 1960). Most of the soil water would then be available for plant use after recovery of full photosynthetic potential. In this case an after-effect delaying stomatal opening may be an advantage especially if CO_2 fixing mechanisms are not inhibited. Relatively high rates of net CO_2 uptake might then be achieved in the days immediately after rain through generation of large CO_2 concentration gradients across partly open stomata. This applies particularly to C4 plants in which the rate of CO_2 fixation is saturated at low intercellular CO_2 concentrations. On this hypothesis, however, the slow recovery of *A. vesicaria* implies that stomatal opening, CO_2 fixation, or both, were strongly depressed by the presence of inhibitors.

A second possibility is that the stomatal apparatus or enzyme systems involved in CO_2 fixation sustained direct structural damage during the severe stresses imposed.

In the absence of data on stomatal and residual or mesophyll resistance to CO_2 uptake the exact reasons for the slow recovery of high rates of net CO_2 uptake by shoots of *A. vesicaria* remain unexplained. Possibly both stomatal and non-stomatal inhibition are involved. The relatively rapid recovery of high rates of CO_2 efflux in the dark gives some indication that the enzyme systems involved in respiration are less affected, if at all, by the presence of inhibitors. A possible reason for early increases in respiration, unrelated to water stress, is the daily handling of shoots and leaves during leaf area measurements. Gentle mechanical stimulation of leaves has been shown to almost double the rate of dark respiration, an effect which may last up to two days (pp.158-159 in Evans, 1972) These last remarks also apply to the interpretation of results on photosynthetic response during dehydration.

Before those results are discussed there is one further aspect of photosynthesis at high water potential observed following rehydration which

perhaps warrants some discussion. Figure 5.5 illustrates the photosynthetic response of the four topmost leaves on a shoot with damaged meristems. All those leaves died within 60 days after rehydration yet according to Charley (1972) the average life of leaves on field-grown *A. vesicaria* is about one year. The apparently premature death of the leaves in question may have been a result of damage to the meristems but this explanation is not consistent with the observation that experimental removal of apical meristems from individuals of some species has been shown to defer senescence of older leaves (Mothes and Baudisch, 1958; Woolhouse, 1974). Contrary to the commonly accepted belief that water stress accelerates ageing, Gates (1968) and Ludlow and Ng (1974) suggested that physiological ageing is suspended by water stress and have presented some evidence to support their view. If this is true then the death of leaves held at high water potential in the laboratory may be due to the normal process of ageing and the longer survival of leaves in the field a consequence of only intermittent periods at high water potential.

It is also of interest that the leaves represented in figure 5.5 maintained relatively high rates of net CO_2 uptake almost to the point of abscission, despite obvious symptoms of senescence. In this case the leaves were attached to a shoot with damaged meristems. On a normal shoot rates of CO_2 uptake by ageing leaves may be even higher if they are acting as a source of photosynthate for younger leaves during rapid growth. High demand from active sinks has been shown to increase the rate of photosynthesis by source leaves (e.g. the flag leaf of wheat; King *et al.*, 1967). On the other hand leaves attached to a normal shoot may age more rapidly because of the presence of an active apical meristem thus reducing any benefit gained from higher rates of CO_2 uptake stimulated by demand. Nonetheless if the behaviour illustrated in figure 5.5 is characteristic of ageing leaves of *A. vesicaria* it seems that leaves of this species continue

to contribute significant amounts of photosynthate to growth or storage until a few days before abscission.

5.42 Net CO_2 exchange at low water potential

The problems associated with measurement of photosynthesis by microphyllous shoots at low water potential have been outlined earlier and will not be discussed further here. It is clear, however, that leaves of *A. vesicaria* are capable of positive net photosynthesis at very low water potentials less than -9.0 MPa and probably, according to the data from plants before rehydration (figure 5.9b), less than -11.0 MPa. This capacity is comparable to that of other arid zone plants (Kappen *et al.*, 1972; Odening *et al.*, 1974). Net photosynthesis by shoots of *Larrea divaricata*, for example, was reduced to zero at water potentials around -7.5 MPa (Odening *et al.* 1974). These water potentials, however, were taken at dawn and therefore presumably underestimate that at the time of gas exchange measurements. According to Moore (1977) other chenopods are also capable of positive net CO_2 uptake at water potentials near -10.0 MPa.

The capacity for positive net photosynthesis at low water potential is also consistent with the reports by Wood (1932) of net CO_2 uptake in summer at Koonamore and by Chapman and Jacobs (1979) of fixation of labelled CO_2 in autumn at Fowler's Gap in N.S.W. In both cases water stress was likely to have been high although in the first case Wood was unable to measure water potential and in the second Chapman and Jacobs were able to indicate only that xylem water potential was less than -4.0 MPa, the limit of their instrument. The maximum rates recorded were 20 ng cm^{-2} s^{-1} (Wood) and 35-40 ng cm^{-2} s^{-1} (Chapman and Jacobs). The latter represent gross rather than net photosynthesis and were maintained only for brief periods in the field as was the case for measurements at high water potential, described earlier. It is notable that for two of the three days on which CO_2 uptake was recorded maximum activity occurred near the middle of the day when water potentials

were likely to have been at a minimum.

The figures given by Wood are probably underestimates of potential net CO_2 uptake. The photosynthetic rate observed, in conjunction with the low flow rate (10 L h^{-1}) and large leaf area (100 cm^2) used for measurement, would have been sufficient to reduce the CO_2 concentration around the shoot to at least 85 ppm. Since the air entering the enclosing glass vessel was not recirculated over the shoot, CO_2 concentration may have been much lower in the vicinity of the leaves. CO_2 concentration of this order are well below even the intercellular space CO_2 concentration (ca 150 ppm) observed by Caldwell *et al.* (1977) to saturate the rate of net CO_2 uptake by shoots of *A. vesicaria.* An intercellular concentration of 85 ppm would depress the rate of net CO_2 uptake by 15 per cent in which case the rates of net photosynthesis observed by Wood underestimated the potential rate by at least that amount. Self-shading in a shoot of the size used would also have contributed to a low estimate for net CO_2 uptake per unit leaf area.

Water potentials of the plants used were probably low as Osborn *et al.* (1932) had observed many wilting plants in the same area at the end of the previous year. Water potentials of about -10.0 to -11.0 MPa were required to produce such symptoms of water stress in laboratory grown shoots. Since Wood's measurements were made at the end of a sequence of years with below average rainfall and as most of the rain fell early in the year of observation xylem water potential was probably very low. For example, some plants with dawn water potentials of less than -11.0 MPa were observed in 1977, when rainfall was below average, in the middle of a sequence of years of high rainfall. Woods figure of 20 ng cm^{-2} s^{-1} for net CO_2 uptake per unit leaf area is similar to those reported here for laboratory grown shoots at water potentials of -4 to -5 MPa and for field grown shoots at water potentials of about -8 MPa (fig. 5.9). Because of the higher degree of self-shading and poorer ventilation, estimates of net CO_2 uptake, at any given water potential, made under the conditions used by Wood would be low in

comparison with those reported in this chapter. A combination of these results suggests that figures obtained for laboratory grown shoots under-estimate the photosynthetic capacity of shoots at low water potential in the field.

This view is supported by results for other species. Maize leaves are not normally capable of net photosynthesis at less than -1.5 MPa when grown at high water potential in controlled environments but have been shown to continue positive net photosynthesis at water potentials approaching -4.0 MPa in the field (Ludlow, 1976). The capacity for net photosynthesis at low water potential is increased by pre-conditioning leaves to water stress during and after growth, a process which decreases stomatal sensitivity to water stress (McCree, 1974; Brown *et al.*, 1976). This is not likely to be a benefit, however, unless non-stomatal aspects of photosynthesis are relatively unaffected at the lower water potentials developed before stomatal closure. Other effects of growth at mild or moderate levels of stress are an increase in leaf thickness and a more compact mesophyll, leaf characteristics which according to Cunningham and Strain (1969) increase the water use efficiency and allow positive net photosynthesis for longer, although at a reduced rate. The ability of stomata to remain partly open at low water potential is possibly a function of the size of guard cells. Cutler *et al.* (1977) suggested that there is an increased capacity for turgor maintenance in small cells, often developed as a result of growth during mild stress, due to their ability to maintain lower cellular osmotic potentials. These three responses to intermittent mild or moderate stress during growth interact to conserve water; they allow moderate rates of net CO_2 uptake at relatively high water potential further into the dry season and, since some turgor is maintained, some uptake at low water potential.

There is a good deal more work to be done before the limits to photosynthetic production by *A. vesicaria* are established. Some suggestions for further work are made in chapter 8. It is clear, however, that individual shoots are capable of positive net photosynthesis at very low water potential and that there is some inhibition of photosynthesis during and after rehydration.

CHAPTER 6

6 Growth responses to declining water potential

6.1 Introduction

One of the bases of the conceptual model of primary production in arid ecosystems presented by Noy-Meir (1973) is that growth occurs mainly in short pulses when conditions are favourable following rainfall. This is so particularly in warm arid regions where falls of rain may be separated by intervals much longer than the relaxation time of the system in response to individual events. A large proportion of net production by drought persistent species, which are considered to retain at least a residual ability to fix CO_2 throughout the dry season, must therefore be translocated to reserves. As far as can be assessed from the results presented in chapters 3 and 5 *A. vesicaria* behaves in the way expected of drought persistent plants in that net CO_2 uptake continues at low water potential and non-structural carbohydrates accumulate at times of low rainfall.

For a given species the time available for the accumulation of reserve carbohydrate will depend on the sensitivity of growth processes, as well as net CO_2 fixation, to decreasing water potential. In this chapter unless otherwise indicated, the term growth is intended to include those processes which result in an increase in plant size as distinct from an increase in dry weight. Such processes include root elongation, shoot extension and the production and irreversible expansion of leaves. In most circumstances the production of new structural material during growth is accompanied by an increase in total plant dry weight although if reserve materials are used to support new growth total dry weight may be temporarily reduced.

The response of the growth processes described above to decreasing water potential has been used as a guide to the timing of reserve accumulation in *A. vesicaria*. Reductions in growth rate have been taken as an

indication of the diversion of newly fixed assimilates to storage. While the diversion of all current photosynthate from synthetic processes does not necessarily coincide with the cessation of growth as measured by shoot extension for example, references cited in Hsiao (1973) show that metabolic activities such as protein and cell wall synthesis are markedly reduced by small water deficits. A comparison of various physiological processes reveals that synthetic activity, although less sensitive to decreasing water potential than growth, is substantially reduced at similar high water potentials.

Judging by the information given in various reviews (e.g. Slavik, 1975; Ludlow, 1976; Boyer 1977) studies on the dependence of growth on water potential are largely restricted to those of crop and pasture plants, many of which are mesophytes. From the data available it appears that growth of such species is highly sensitive to water stress. Acevedo *et al.* (1971) demonstrated a reduction in the rate of laminar extension in maize plants at water potentials as high as -0.28 MPa. Boyer (1970) found that water potentials of -0.4 MPa were sufficient to reduce the rate of leaf expansion of soybean and maize to about 33 and 25 per cent that of well watered controls respectively. Expansion of sunflower leaves was stopped completely by similar water potentials. Net photosynthesis was unaffected by the mild stresses involved during the early stages of dehydration and although leaves of soybean continued to expand at a very low rate until water potential had fallen to between -1.6 and -2.0 MPa net CO_2 uptake was observed at water potentials beyond -4.0 MPa.

The degree of water stress resulting in reduced growth rates in the crop plants referred to above is not likely to be common to all species. Boyer (1977) pointed out that leaves at the tops of tall trees and those of halophytes are able to expand rapidly even though water potential may always be less than -0.4 MPa. Xylem water potentials of *A. vesicaria* (Ch. 7)

have rarely been observed to approach the high values reported for crop species. However, since growth depends on turgor potential rather than directly on total water potential, cell growth at water potentials lower than the limiting values observed for mesophytes can take place if high turgor potentials are developed in response to low intracellular osmotic potentials. The low water potentials in *A. vesicaria* leaves are partly due to high concentrations of inorganic ions in the lamina (Osmond unpubl. p234 in Osmond *et al.*, 1980). The results of several series of measurements on growth responses of *A. vesicaria* to decreasing water and turgor potential are described in the following pages. Since growth following the relief of water stress may result in a demand for stored carbohydrate, as outlined in earlier chapters, data on growth responses, particularly leaf expansion, following irrigation of droughted plants have also been included here.

6.2 Methods

Two sets of measurements on leaf area are reported in this chapter. The leaf area of shoots selected for measurements of net CO_2 uptake (Ch.5) was recorded daily while shrubs were recovering from a period of water stress. After recovery was complete water was withheld and the leaf area of similar shoots was monitored as water potential declined. Details of the conditions at the time measurements were taken have already been described in Section 5.21. The shrubs used were 3-year-old cuttings grown for most of their life in the field. During the drying cycle they were kept under lights in a laboratory where temperature and humidity were not controlled.

In a second series of measurements the expansion of individual young leaves and shoot and root extension were monitored as xylem water potential decreased after water was withheld from young plants grown in containers with transparent walls. These plants were four-month-old seedlings grown in a temperature controlled glasshouse at Adelaide. Temperature regulation did not provide precise control but prevented air temperature from rising above 30^{o}C during the day and falling below 22^{o}C at night. Supplementary light, 100 nE cm^{-2} s^{-1} at the top of the canopy was provided by a Metalarc lamp (Sylvania GTE) for 16 hours per day. The young seedlings were grown in 2.5-litre square-sided glass containers holding about 5kg of air-dry sand from the Koonamore area. The plants were transferred to these containers two weeks before the start of the experiment and supplied with enough water to wet the soil to within 1 cm of the bottom of the container. The final weight was maintained by watering daily, if necessary, until the start of the experiment. Water was withheld when the first roots appeared at the walls of the container.

Similar measurements were later made on a single 2-year-old seedling grown under similar conditions in a perspex container of dimensions

50 x 30 x 30 cm. This shrub was transplanted one month before the start of routine measurements. The size of this shrub allowed more frequent sampling for xylem water potential during the course of the experiment. In all cases the sides of the containers were shielded from light between measurements of root growth, which took less than 15 minutes per day.

Most of the methods used to monitor growth have been described previously. The use of light sensitive paper to estimate leaf area, for example, was outlined in chapter 5. Shoot extension was measured directly with a millimetre rule rather than from photographs (cf Ch.4.) Root growth measurements were done in the same way as in observation pits in the field but in this case measurements were taken directly from the transparent faces of the containers in which plants were grown without transferring the pattern of growth onto plastic sheets. A temporary record of root growth was kept by marking the extent and pattern of root growth on the sides of the containers.

Xylem water potentials were measured with a pressure chamber. This instrument was also used to estimate osmotic and hence turgor potentials of shoots at intervals during the drying cycle. The average osmotic potential of the leaves on small shoots was estimated from a pressure-volume curve (Scholander *et al.*, 1965) a relationship between pressure applied to the shoot and the cumulative volume of sap expressed from the cut surface at successively higher pressures. The intercept on the ordinate formed by extrapolation of the linear section of a pressure-volume curve estimates the osmotic potential of the leaf cells at the time the shoot was removed from the plant. Turgor potential can then be calculated as the difference between the initial balance point and the estimated osmotic potential. Tyree and Hammel (1972) have provided a detailed account of the theory on which these estimates are based.

Net CO_2 uptake was also measured at intervals. The conditions during measurement were the same as those described earlier for field grown cuttings (see Ch. 5).

6.3 Results

6.31 Growth responses to decreasing water potential

The data presented in this section represent measurements on shoots from field grown cuttings and glasshouse grown seedlings. The terminal portions of shoots on cuttings were, however, grown entirely in the laboratory over a period of between 15 - 20 days while shrubs were recovering from a water stress of less than -11.0 MPa. After rehydration xylem water potential was, on average, about -2.2 MPa. Figure 6.1 shows the decrease in leaf area on several shoots during the following dehydration phase. As noted earlier (Ch. 5) no leaves were lost and therefore any decrease in leaf area was due to contraction of existing leaves. Leaf areas are expressed as percent. of maximum value and graphed as a function of xylem water potential. Because leaf areas were measured at intervals of several days, particularly during the early stages of dehydration, the maximum leaf area on which percentage areas are based may underestimate the true value slightly if leaf area peaked during one of the intervals. It appears that little if any leaf expansion occurred at water potentials less than about -2.0 MPa. The xylem water potentials associated with the values for percentage leaf area were measured during the light period and hence, because leaf growth is more likely to be correlated with dawn water potential, the limiting value may be even higher. It is not clear from these measurements, however, whether the growth of young leaves was completely inhibited at such high water potentials or whether the contractions of older leaves was sufficient to mask continuing growth of young apical or axilliary leaves. Nonetheless, growth rates during dehydration were substantially reduced in comparison with those measured during and after rehydration when water potential was maintained at values of -2.0 MPa or higher (see Section 6.32).

Figures 6.2 a-e illustrate the growth of roots, stems and individual

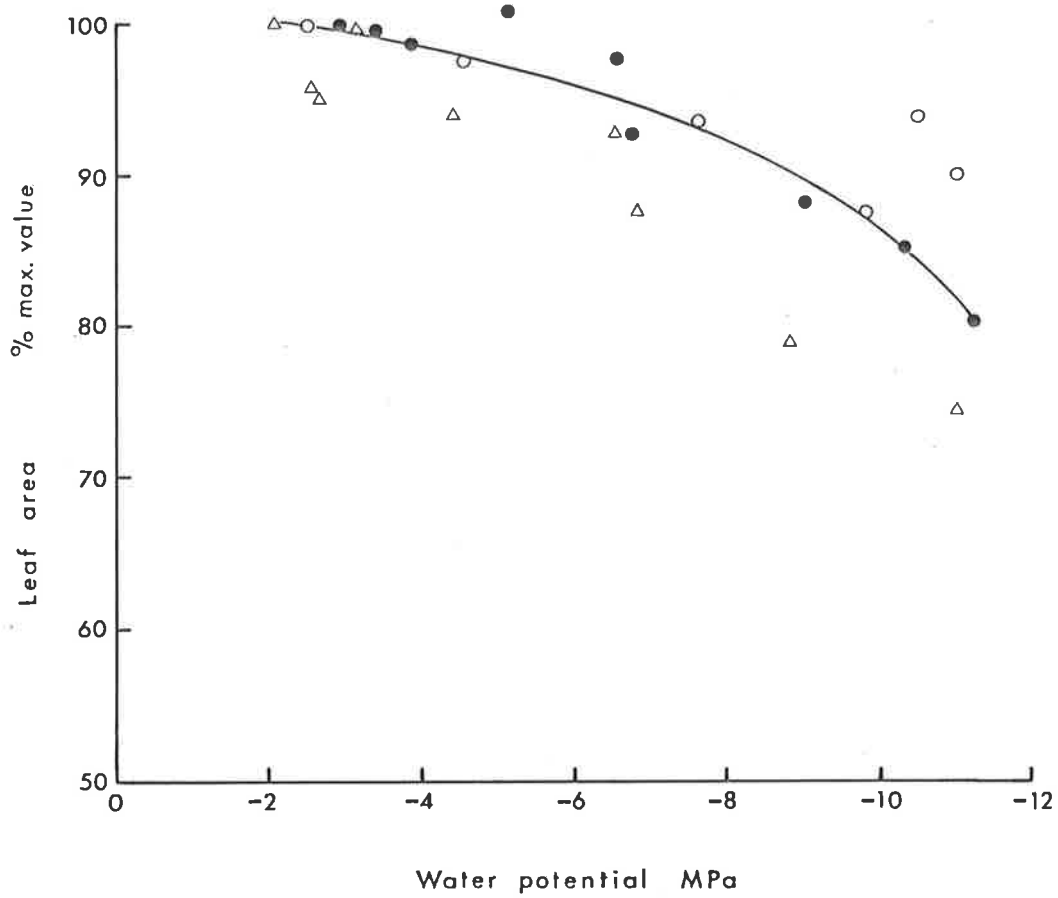


Figure 6.1 Leaf area (percent. maximum value) of shoots of field-grown cuttings during dehydration of shrubs in the laboratory. All leaves were grown under laboratory conditions during prior rehydration.

young leaves of the glasshouse grown seedlings as water potential decreased over a period of about 30 days. Figures 6.2 a-d describe the response of four 3-to 4-month-old seedlings. Although conditions were the same for all of these seedlings and water was withheld at the same time, dehydration proceeded at different rates. Because of the variation from day to day between plants the data from each plant are presented separately. Figure 6.2e shows the response of the 2-year-old seedling. Each figure consists of a set of four diagrams depicting root growth, shoot elongation, leaf expansion and water potential, numbered (i) - (iv) respectively. Water potentials (iv) for the younger seedlings (a-d) prior to day 14 were measured in mid morning. On all other occasions readings were taken at dawn. Water potential diagrams in figures 6.2a and 6.2e also incorporate data on net CO_2 uptake and turgor potential respectively.

The results of measurements on these seedlings confirm the conclusion drawn from the data in figure 6.1 that growth of saltbush is sensitive to relatively high water potentials. The vertical lines intersecting the diagrams in these figures show, approximately, the day when xylem water potential had fallen to -3.0 MPa, a value to which no special biological significance is attached. It is intended mainly as a reference point, although under the conditions used in this experiment growth ceased at water potentials near that value. Leaf expansion (iii), for example, stopped shortly before or shortly after water potential had fallen to -3.0 MPa. In figures 6.2 a-d rates of leaf expansion (mm^2 per day) for individual leaves are graphed separately. Values were calculated from mean areas based on 5 images per leaf. Those shown are for the second unfolded leaf below the apex on a given shoot and the inverted arrow indicates the day on which leaf area of the youngest unfolded leaf reached a maximum. Growth rates for the latter are not plotted. An inverted triangle replaces the symbol locating the rate of leaf expansion on the corresponding day for the older leaves. Beyond the

days denoted in that manner leaf area declined. The vertical bars represent the least significant difference ($P<0.05$) between rates of leaf expansion on successive days.

In figure 6.2e (iii) each graph depicts the mean rate of expansion of 5 leaves (5 images per leaf). The open symbols describe the response of the youngest unfolded leaf and the closed symbols that of the leaf on the node immediately below.

Apart from the high sensitivity of leaf growth to decreasing water potential the most striking feature of the data is the fluctuation in leaf expansion rate from day to day and the differences between leaves on the same plant. On some days the area of one leaf increased while that of a second declined. It should be noted, however, that in these diagrams a positive expansion rate immediately following a negative value does not necessarily imply leaf growth. The positive values on days 22 and 23 for leaf 1 in figure 6.2a, for example, represent only a partial recovery of the decline in leaf area from the maximum reached on day 18. Even earlier in the dehydration phase, 1-3 days were sometimes required for the previous peak in leaf area to be regained following a decline. In most cases the older leaves stopped expanding several days before the cessation of growth by the youngest unfolded leaves. Xylem water potentials at that stage were often higher than -2.0 MPa.

Fluctuations in stem length and differences between similar shoots on the same plant were also evident during dehydration. Stem lengths, expressed as percent. of initial length, for individual shoots of the four young seedlings are graphed separately in figures 6.2 a-d (ii) while the data in figure 6.2e are mean values based on 5 shoots from the two-year-old seedling. Stem growth was sensitive to relatively high water potentials and elongation ceased at water potentials similar to those which stopped leaf expansion and in some cases well before the nominal value of -3.0 MPa. The mean and

standard deviation of the increase in length for all shoots up to the time plants were rewatered was 13.3 $\pm$ 5.4 mm. Although shoots produced between 2 and 6 leaves during that time, as judged by the number of unfolded leaves, these remained clustered near the apex and most elongation was confined to the internodes immediately below.

The topmost diagram (i) in each figure illustrates the pattern of root growth as water potential decreased. In all figures the closed symbols represent the maximum daily rate of growth (mm) by an individual root and the open symbols, the total new root length (mm) produced since the previous day. The scales for these two variables differ in some of the diagrams.

Day to day variation in root growth rate and the amount of new growth was considerable even when water potential was high during the early stages of dehydration. As was the case for growth of above ground parts, root elongation was reduced to zero shortly before or shortly after water potential decreased to -3.0 MPa. There was, however, one exception. Root production by the 2-year-old seedling (fig. 6.2e) continued until day 22 when xylem water potential was slightly less than -6.0 MPa. The growth recorded between days 19 and 22 was restricted to a small area on one face of the perspex container and was preceded by several days during which no root growth was observed.

Whereas growth of both field grown cuttings and glasshouse grown seedlings was, in most cases, reduced to zero at relatively high water potentials the rate of net CO_2 uptake was unaffected at similar values and uptake, although at reduced rates, was observed at water potentials as low as -7.1 MPa. Figure 6.2a (iv) depicts the rate of net CO_2 uptake at both high and low water potential. Rates of net CO_2 uptake by the two-year-old seedling were also measured while the plant maintained a high water potential but these are not presented in graphical form. A fault, which could not be repaired immediately, developed in the gas analyzer on day 18 and hence no subsequent

Figure 6.2 Growth responses of glasshouse-grown seedlings to declining water potential measured for up to 30 days after water was withheld. Five full page diagrams (a) - (e) in sequence for four 3-month-old seedlings (a) - (d) and one two-year-old seedling (e).

(a)

(i) Maximum rate of elongation by an individual root and total new root length observed through the transparent walls of the container.

(ii) Shoot length (percent. initial value) for two shoots.

(iii) Rate of leaf expansion ($mm^2 d^{-1}$) for two leaves. Leaf area began to finally decline on the day indicated by the inverted triangles. The arrow shows the day leaf area began to decline for the youngest expanded leaf (data not plotted). LSD, $P < 0.05$, for comparison of rates of leaf expansion over time.

(iv) Water potential of representative shoots and net CO_2 uptake by a single small shoot. Water potentials recorded at dawn except those prior to day 14 which were measured several hours after dawn.

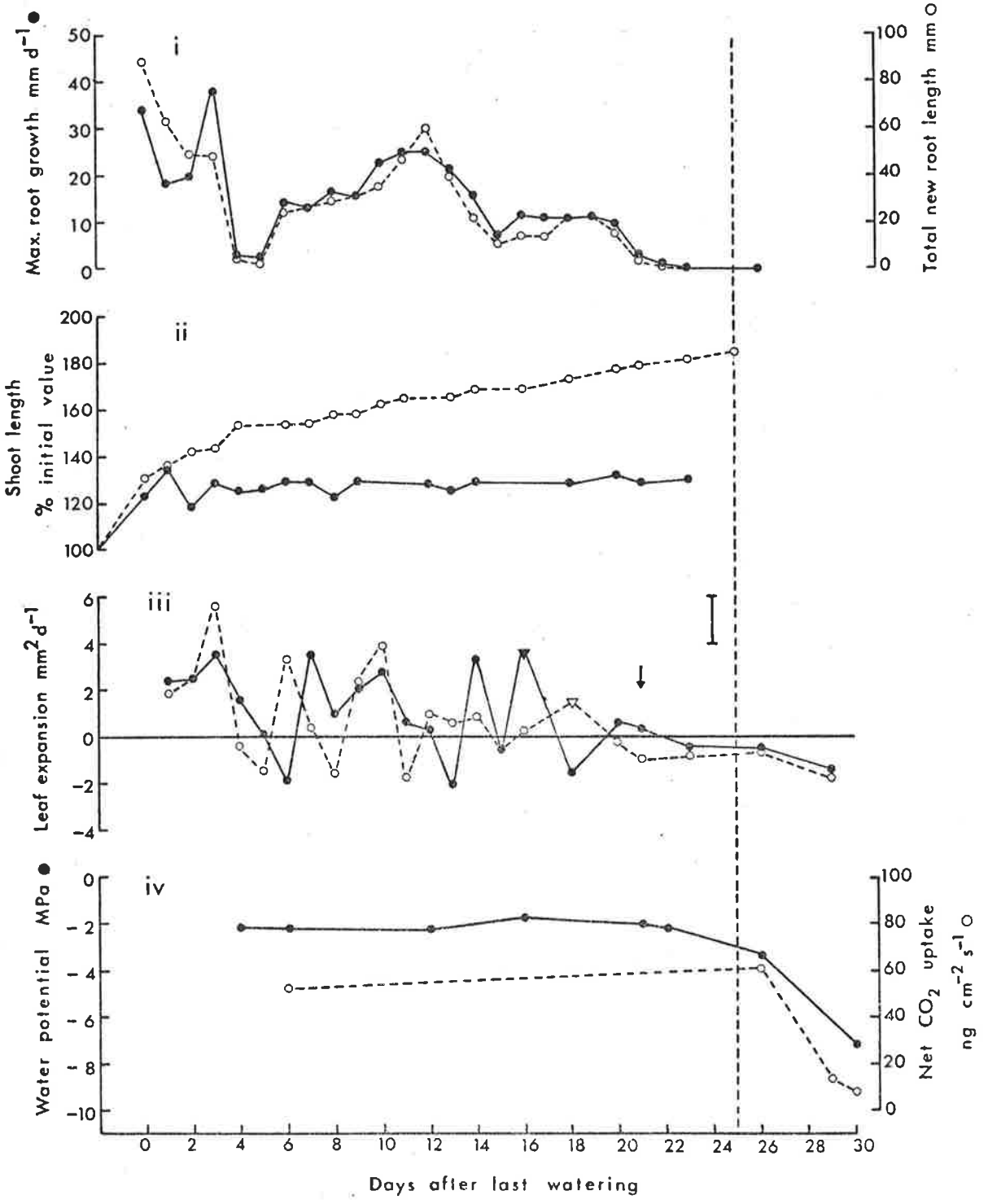

i
Max. root growth mm d⁻¹ ●
Total new root length mm ○
ii
Shoot length
% initial value
iii
Leaf expansion mm² d⁻¹
iv
Water potential MPa ●
Net CO₂ uptake
ng cm⁻² s⁻¹ ○
Days after last watering

Figure 6.2 continued

(b)

(i) Maximum rate of elongation by an individual root and total new root length observed through the transparent walls of the container.

(ii) Shoot length (percent. initial value) for two shoots.

(iii) Rate of leaf expansion ($mm^2 d^{-1}$) for two leaves. Leaf area began to finally decline on the day indicated by the inverted triangles. The arrow shows the day leaf area began to decline for the youngest expanded leaf (data not plotted). LSD, $P < 0.05$, for comparison of rates of leaf expansion over time.

(iv) Water potential of representative shoots. Water potentials recorded at dawn except those prior to day 14 which were measured several hours after dawn.

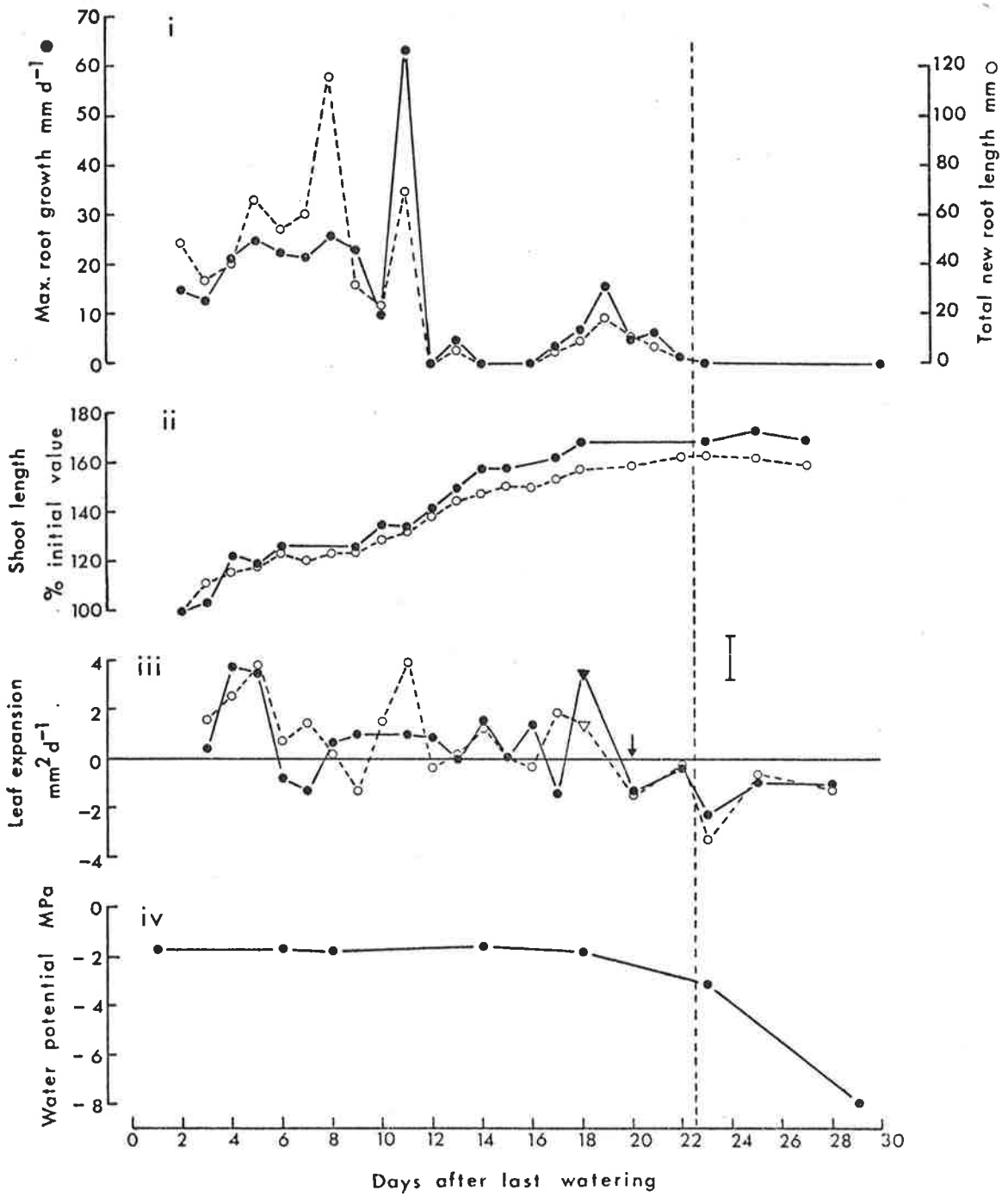

i
Max. root growth mm d⁻¹ ●
Total new root length mm ○
ii
Shoot length
% initial value
iii
Leaf expansion
mm² d⁻¹
iv
Water potential MPa
Days after last watering

Figure 6.2 continued

(c)

(i) Maximum rate of elongation by an individual root and total new root length observed through the transparent walls of the container.

(ii) Shoot length (percent. initial value) for two shoots.

(iii) Rate of leaf expansion ($mm^2 d^{-1}$) for two leaves. Leaf area began to finally decline on the day indicated by the inverted triangles. The arrow shows the day leaf area began to decline for the youngest expanded leaf (data not plotted). LSD, $P < 0.05$, for comparison of rates of leaf expansion over time.

(iv) Water potential of representative shoots. Water potentials recorded at dawn except those prior to day 14 which were measured several hours after dawn.

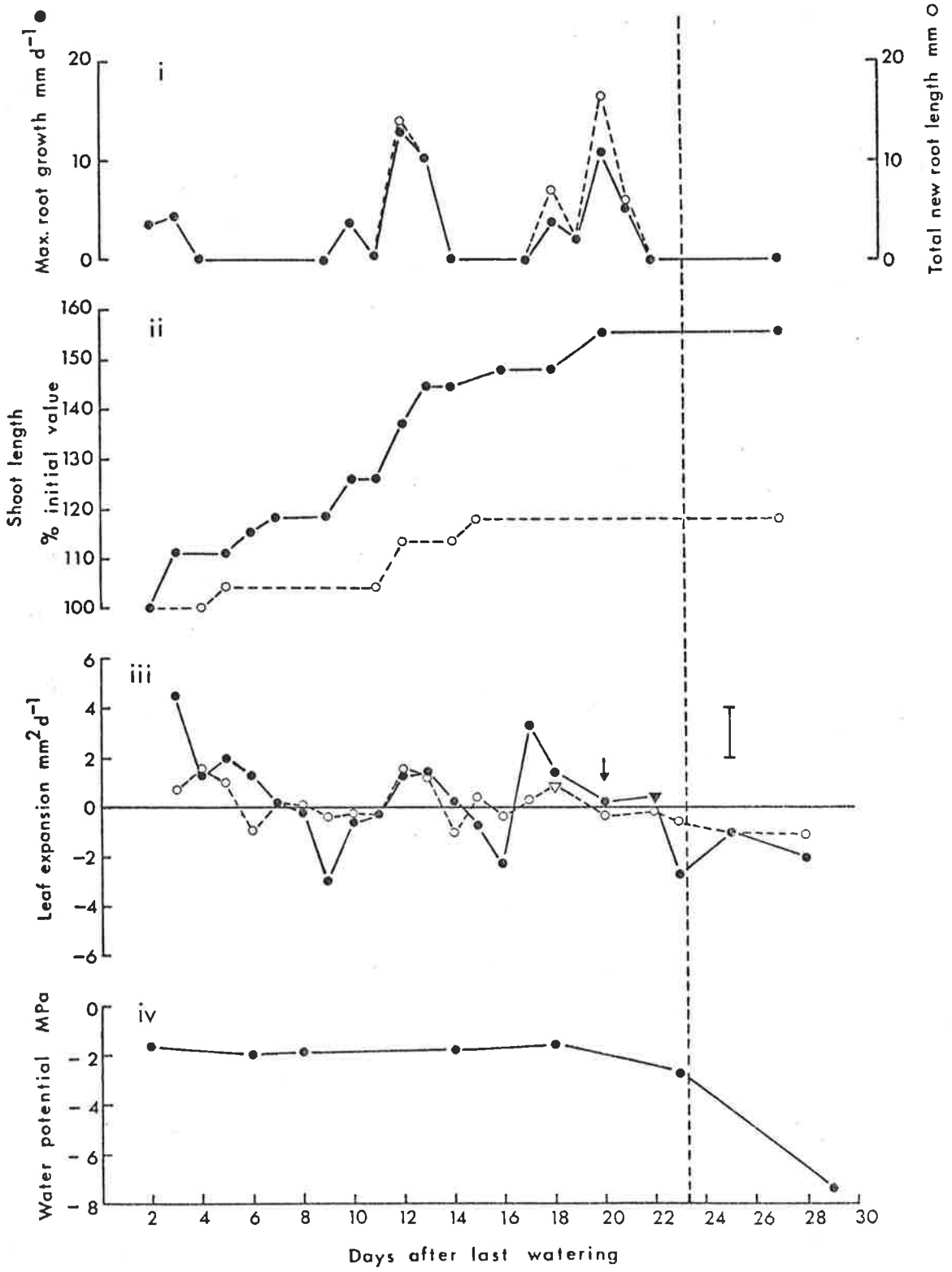

i
Max. root growth mm d⁻¹ ●
Total new root length mm ○
20
10
0
ii
Shoot length
% initial value
160
150
140
130
120
110
100
iii
Leaf expansion mm² d⁻¹
6
4
2
0
−2
−4
−6
iv
Water potential MPa
0
−2
−4
−6
−8
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
Days after last watering

Figure 6.2 continued

(d)

(i) Maximum rate of elongation by an individual root and total new root length observed through the transparent walls of the container.

(ii) Shoot length (percent. initial value) for two shoots.

(iii) Rate of leaf expansion ($mm^2 d^{-1}$) for two leaves. Leaf area began to finally decline on the day indicated by the inverted triangles. The arrow shows the day leaf area began to decline for the youngest expanded leaf (data not plotted). LSD, $P < 0.05$, for comparison of rates of leaf expansion over time.

(iv) Water potential of representative shoots. Water potentials recorded at dawn except those prior to day 14 which were measured several hours after dawn.

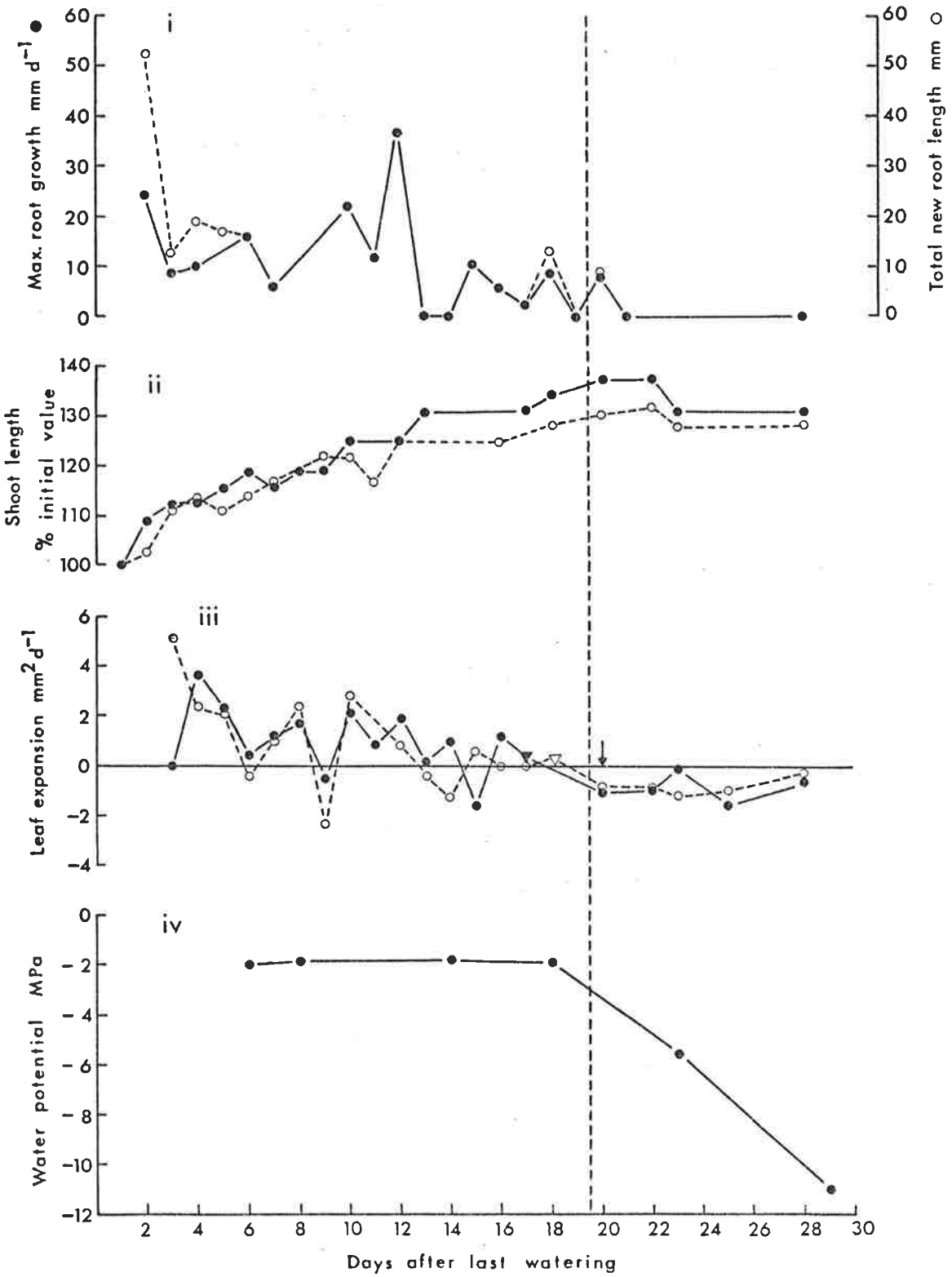

i
Max. root growth mm d⁻¹ ●
Total new root length mm ○
ii
Shoot length
% initial value
iii
Leaf expansion mm² d⁻¹
iv
Water potential MPa
Days after last watering

Figure 6.2 continued

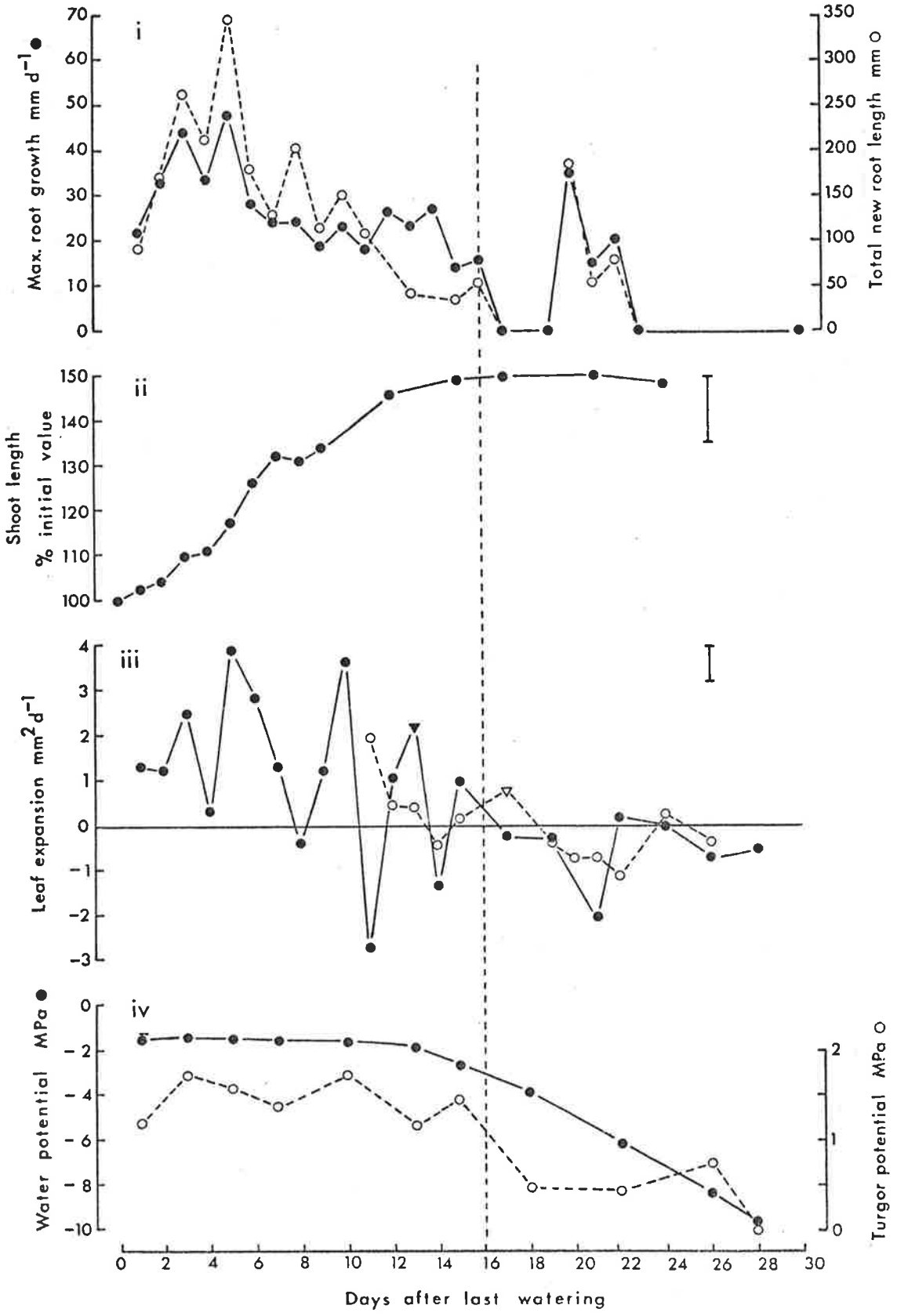
(e)

(i) Maximum rate of elongation by an individual root and total new root length observed through the transparent walls of the container.

(ii) Mean shoot length (percent. initial value) for 5 shoots. LSD, $P < 0.05$.

(iii) Mean rate of leaf expansion for 5 leaves. Open circles represent the youngest expanded leaf and closed circles the leaf on the node immediately below. LSD, $P < 0.05$ for comparison of means over time.

(iv) Total water potential and turgor potential of representative shoots recorded at dawn.


i
Max. root growth mm d⁻¹ ●
Total new root length mm ○
ii
Shoot length
% initial value
iii
Leaf expansion mm² d⁻¹
iv
Water potential MPa ●
Turgor potential MPa ○
Days after last watering

measurements were made. By day 15, however, when xylem water potential was -2.6 MPa and growth rates were markedly reduced, the rate of net photosynthesis was still 51.5 ng cm^{-2} s^{-1}, 80 per cent of the mean maximum value calculated from measurements made earlier.

As noted in the introduction, growth is more likely to be related to turgor potential than total water potential. Some data on turgor potentials during dehydration are shown in figure 6.2e (iv). There appears to be no consistent relationship between fluctuations in growth and turgor potential. It is clear, nevertheless, that turgor is not reduced to zero until well after growth stops. Turgor potentials of the younger seedlings were estimated less regularly for any given plant but are consistent with those presented in figure 6.2e. The highest recorded values (1.18 to 1.40 MPa) are within the range of those observed for the older seedling (1.17 to 1.75 MPa) However, while turgor was not reduced to zero until total water potential had fallen to between -8.4 and -9.7 MPa in the latter, one of the younger seedlings had lost all turgor by the time water potential had fallen to -7.3 MPa. Records for that seedling were not taken regularly enough to establish the water potential at which turgor was reduced to zero but it was probably close to -7.3 MPa as another seedling from the same group maintained a turgor of 0.55 MPa when total water potential was -7.1 MPa. In that instance also, some turgor was maintained well after growth had ceased.

6.32 Growth responses to irrigation

Some indication of the rapid production of leaf area during rehydration following a period of severe water stress was given in figure 5.3b which showed the daily increases in leaf area of two shoots from a field grown cutting. Those data are given in figure 6.3 which also includes data from most of the shrubs used for the series of measurements of net CO_2 uptake described in chapter 5. Because of the high degree of variability between

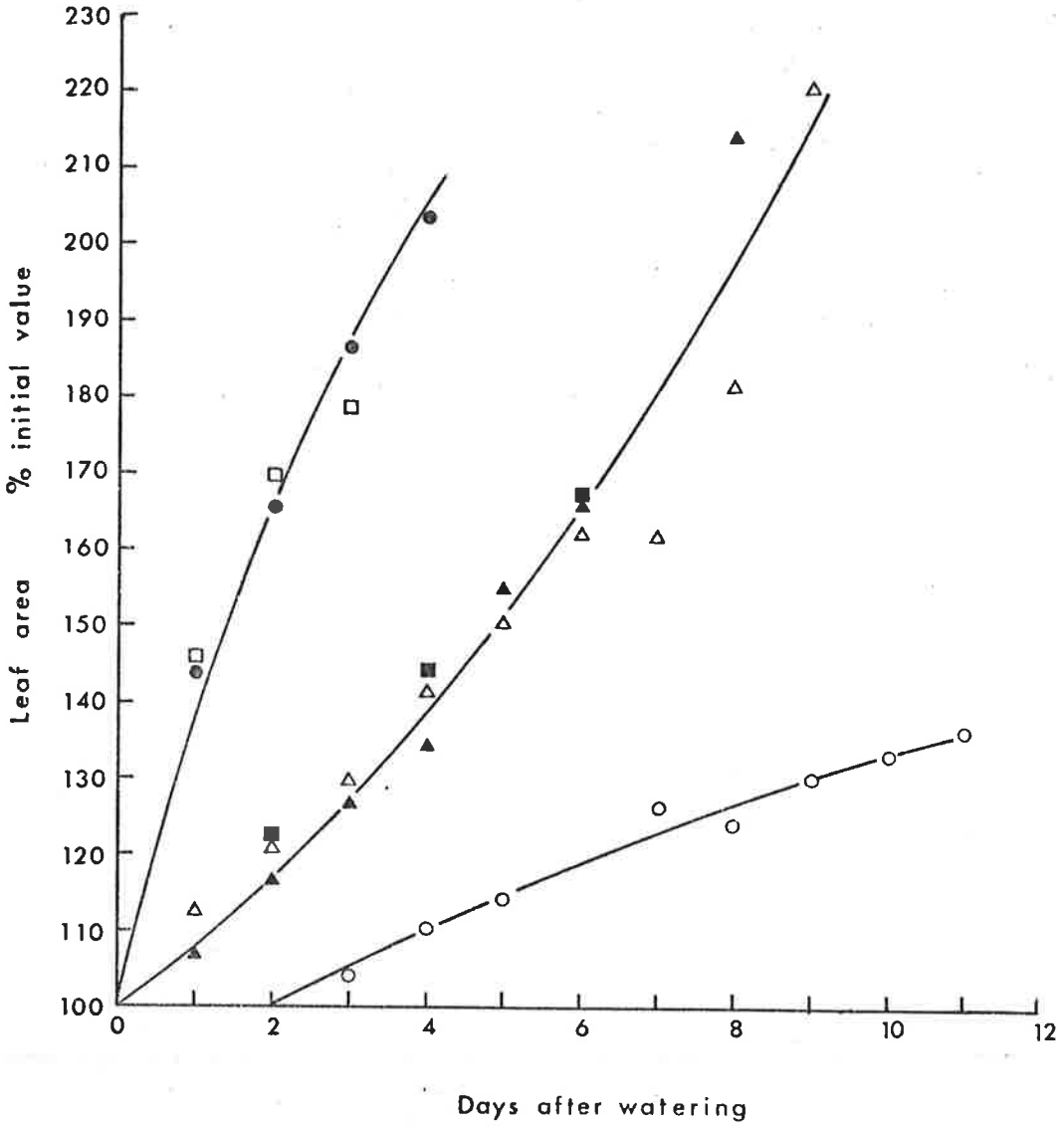


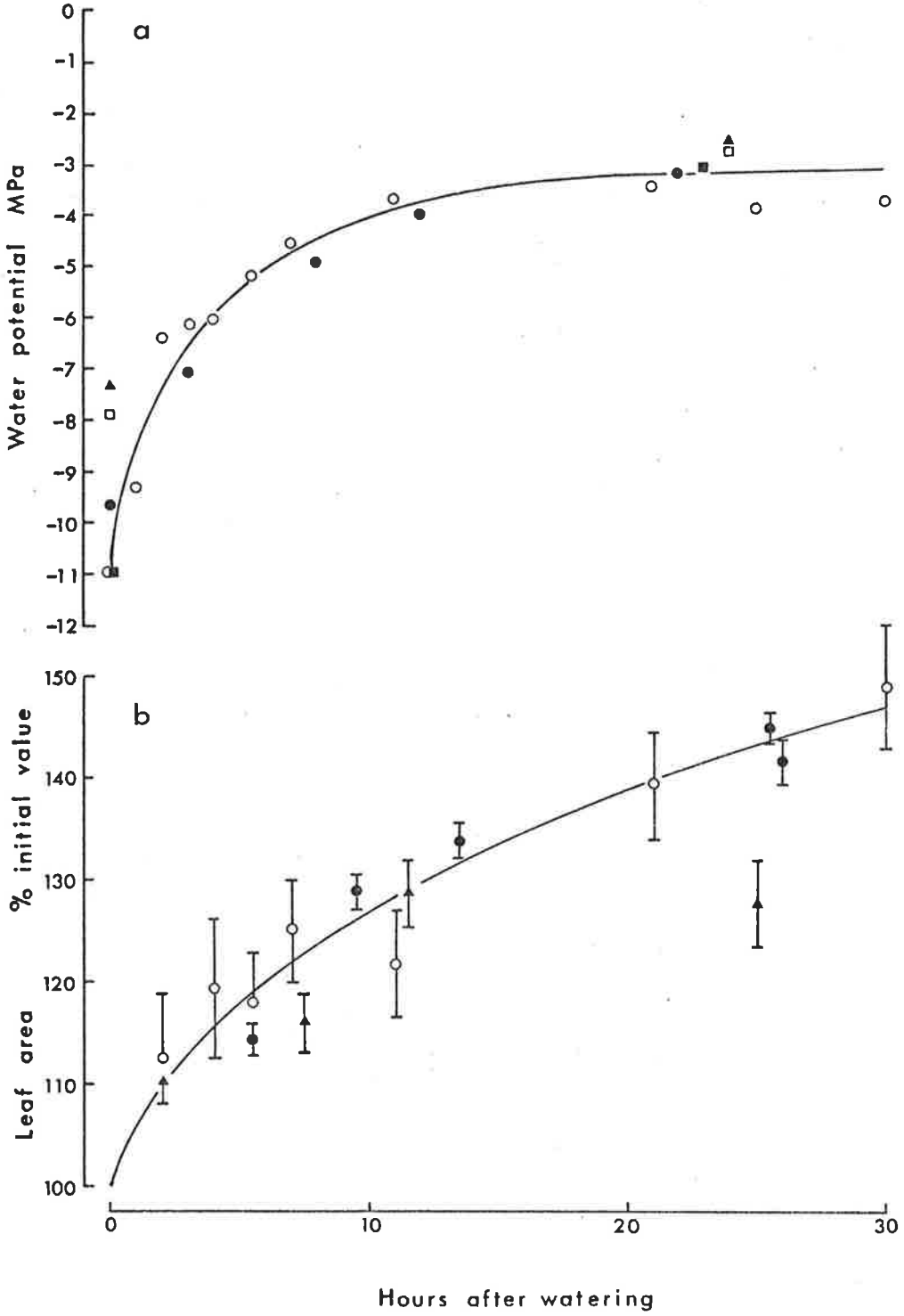
Figure 6.3 Leaf area (percent. initial value) for shoots on field grown cuttings during and after rehydration in the laboratory. The open circles show the increase in leaf area of a shoot with apical and axillary meristems killed by water stress and the open squares show that for a sample of 5 leaves from different shoots of a 2-year-old glasshouse grown seedling. The remaining symbols represent the increase in total leaf area of shoots due to the expansion of existing leaves and the production of new leaves.

shoots, leaf areas, expressed as percent. of initial value, have been plotted individually and a number of curves fitted by eye. For most shoots leaf area increase was due to expansion of both existing and new leaves. The open circles represent a shoot which did not produce any new leaves. The relatively low rate of increase in leaf area observed for that shoot is not necessarily characteristic of the response of individual leaves. The open squares depicting the mean increase in area of a sample of young leaves from the two-year-old seedling described earlier show that the rate of increase in area of those leaves is similar to that for a shoot which doubled its leaf area in 4 days. The area of one of the leaves in the sample doubled in 3 days. The central group may represent a more usual response. However, the tiny leaves enclosing the apices of at least one of the shoots in that group were damaged at the very low water potentials reached before irrigation. Other shoots in that group may also have sustained sufficient damage to inhibit their initial development immediately after irrigation.

Water potentials of the glasshouse grown seedlings fell to between -7.1 and -11.0 MPa during the dehydration phase. When these plants were rewatered both water potential and leaf area were monitored for up to 3 days. Figures 6.4a and b show the observed increases in water potential and leaf area respectively during the first 30 hours. Data on mean leaf area of a sample of 5 leaves and xylem water potential of one of the field grown cuttings (open circles) are included in this diagram. Both water potential and leaf area had increased by the time the first records were taken approximately 1 and 2 hours respectively after the soil around the plants was watered. On two earlier occasions when net CO_2 uptake was monitored immediately after irrigation there was no response in the first 5 hours before the light period ended although assimilation rate had increased by the following morning.

Turgor potentials also increase rapidly during rehydration and reach

Figure 6.4 (a) Water potential of glasshouse-grown seedlings during the first 30 hours after watering. The open circles show the response of a sample of leaves from a field-grown cutting dehydrated to a water potential of less than -11 MPa for the second time since collected from the field. (b) Leaf area (percent. initial value) during the first 30 hours after watering. Symbols as for (a).


a
Water potential MPa
0
-1
-2
-3
-4
-5
-6
-7
-8
-9
-10
-11
-12
b
Leaf area % initial value
150
140
130
120
110
100
0
10
20
30
Hours after watering

very high values. The response of two seedlings for up to 46 hours after rewatering is shown is figure 6.5c. Each estimate was based on a single pressure-volume curve. Examples of the pressure-volume curves used to calculate turgor potentials are shown in figure 6.5. The straight line in 6.5a represents a shoot at zero turgor while the deviation from linearity in 6.5b indicates a positive turgor potential.

Twenty hours after plants were rewatered the turgor potential of one of the young glasshouse grown seedlings had reached 2.95 MPa while that of another had risen to 2.48 MPa in only 12 hours. Leaf expansion of these two seedlings during the first 30 hours after rewatering is illustrated in figure 6.4b. It is notable that whereas the latter (closed circles in both figures) continued growing in the following 10 hours and turgor potential decreased to 1.72 MPa the leaf area of the former (closed triangles) did not increase during that time.

The high turgor pressures are probably a consequence of osmotic adjustment during the dehydration phase leading to high concentrations of ions in the vacuoles of leaf cells during the initial stages of rehydration. Osmotic potential fell from -2.8 to -9.7 MPa in one instance but to what extent active ion uptake contributed to the increasing ion concentration is not known. If there were no osmotic adjustment as water potential declined the osmotic potential at similar high water potentials before and after stress should be approximately the same. Pre- and post-stress values for the two seedlings referred to above, numbered for convenience, are compared in the following table. The second block of figures for seedling 1 are measurements taken during development of and recovery from stress.

Figure 6.5 Turgor potentials for two seedlings at various times after watering and examples of the pressure-volume curves used to estimate turgor potential (see Methods). V_e is the cumulative volume of sap expressed from the cut surface of the shoot at successively higher pressures.

(a) P-V curve before watering when total water potential was -9.8 MPa and turgor was zero.

(b) P-V curve 12 hours after watering when total water potential was -4.0 MPa and turgor potential was 2.5 MPa.

(c) Turgor potential for two seedlings in the first 50 hours after watering.

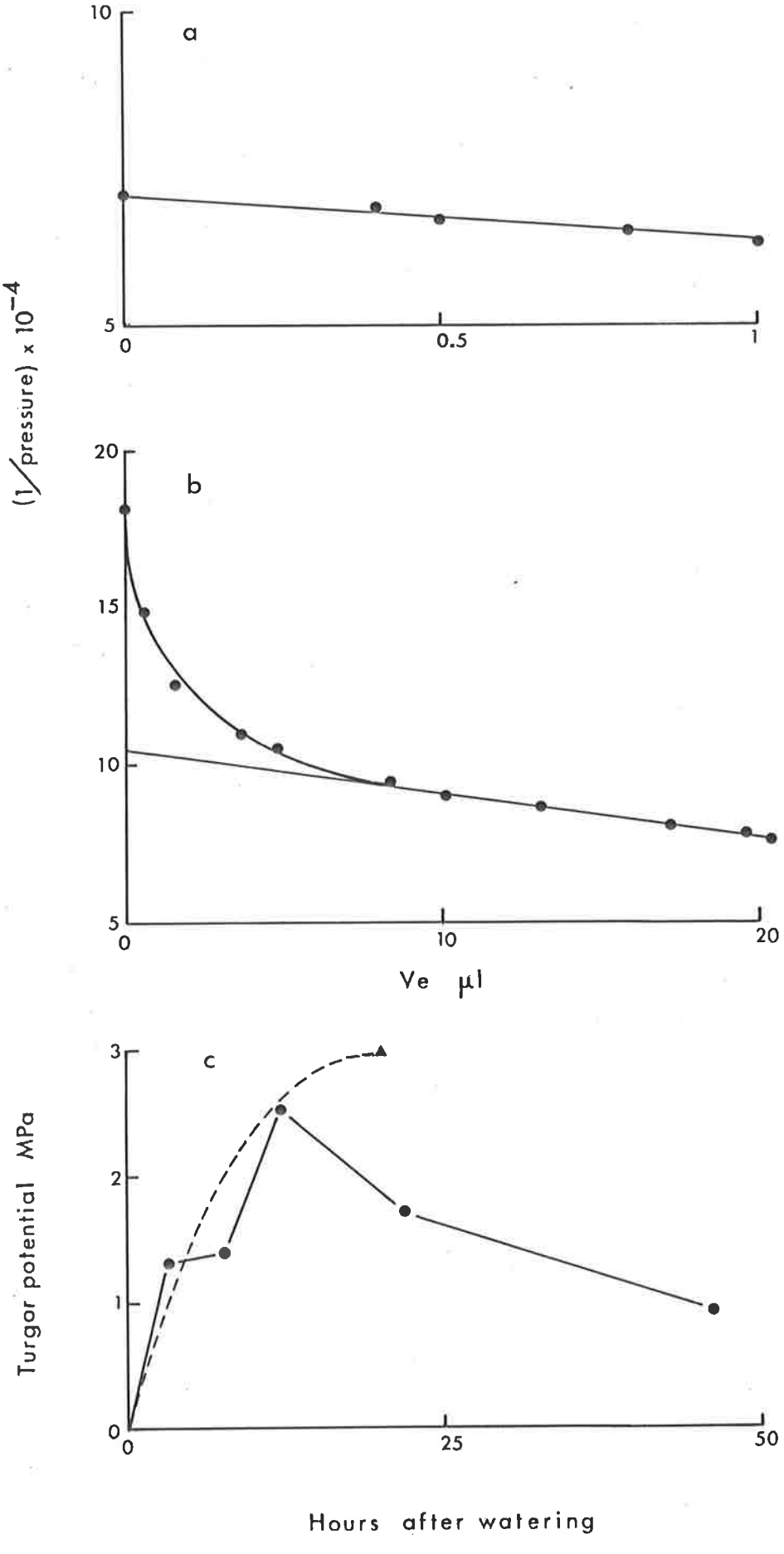

a
10
5
0
0.5
1
b
20
15
10
5
0
10
20
Ve µl
(1/pressure) x 10⁻⁴
c
3
2
1
0
0
25
50
Turgor potential MPa
Hours after watering

Table 6.1 Components of water potential (MPa) before and after stress

	Seedling 1		Seedling 1		Seedling 2	
	Before	After(46)*	Before	After(12)	Before	After(20)
Ψ	-2.59	-2.66	-3.79	-4.03	-2.19	-2.48
Ψ_π	-4.06	-3.62	-4.26	-6.51	-3.22	-5.43
Ψ_p	1.47	0.96	0.47	2.48	0.92	2.95

*The bracketed figures show the number of hours after watering.

The high post-stress osmotic potential in the leaves of the first seedling relative to that before stress was imposed might be taken as evidence of little or no osmotic adjustment to decreasing water potential in the substrate. The result is inconclusive, however, because of the possibility that the final concentration of osmotic solutes was reduced during rehydration by redistribution of inorganic ions to epidermal bladders and the use of organic solutes as substrate for synthetic reactions. The corresponding values for osmotic potential of the second seedling indicate considerable osmotic adjustment. It is probable that the leaves of the first seedling also adjusted osmotically during stress. At an earlier stage of rehydration, about 12 hours after watering, the osmotic potential was -6.51 MPa when total water potential was -4.03 MPa (table 6.1), more than 2 MPa lower than that at a similar water potential during the development of stress.

The following observations and speculation on leaf growth in saltbush may explain the observed results. As low water potentials developed in the seedlings used in these experiments the area of all monitored leaves decreased (figs 6.2a-e). The final area was on average 73.6 $\pm$ 3.6 per cent of the maximum value reached at high water potential. The contraction implies that peaks in leaf area were due to an elastic expansion of the cell walls. On rewatering early expansion of leaves may thus represent

only the recovery of prior cell volume to the point where further wall loosening and synthesis is required for continued irreversible growth (see Cleland, 1971). Given a mean decrease in leaf area of about 25 per cent it appears from figure 6.4b that 15-16 hours were required for leaf area to increase to pre-stress values (ca. 133 per cent of the area just prior to rewatering). The failure of cell walls to loosen after an initial increase in leaf area of about 29 per cent may account for the temporary halt in leaf growth of seedling 2 (closed triangles, fig 6.4b) 7-12 hours after watering, and both the high turgor potential and low osmotic potential 20 hours after watering (fig. 6.5c, table 6.1). Although similar high turgor and low osmotic potentials were recorded for seedling 1, 12 hours after watering, in this case cell wall loosening resulted in continued growth, an increased osmotic potential and a decrease in turgor potential. The rise in osmotic potential can be accounted for by the metabolic processes referred to earlier or may be due simply to the dilution of cell contents as cell volume increases during growth.

The cause of the temporary cessation of leaf growth despite the high turgor, in seedling 2, is not clear. This seedling was one of three which all behaved in the same way. The final record of leaf area was taken in the morning 25 hours after watering whereas turgor potential was estimated at dawn. At such high turgor only a small amount of cell water need be lost to result in a large drop in turgor and hence by the time leaf area was measured turgor may have fallen below some critical value and allowed leaf contraction. These concepts will be discussed in more detail shortly.

Whereas, in general, leaf expansion was immediate and rapid following irrigation root growth was not observed in the two weeks after the four young seedlings were rewatered. Some of the existing roots of the older seedling recommenced growth on day 2 but elongation rates were not as high as those observed after the initial watering. Maximum rates (14-25 mm/day)

were recorded on day 4. Over the next few days root growth rates declined to low values and since measurements were then discontinued the time taken for sustained high rates of growth to be reestablished is not known.

Shoot elongation rates, on the other hand, were similar to those observed in the period immediately after water was withheld and growth was recorded on the day after the seedlings were rewatered.

6.4 Discussion

6.41 Growth responses to irrigation

The depression of TNC concentration observed in field plants following a substantial rain in summer (Ch.3) implies rapid growth which cannot be supported entirely by current photosynthate. Results of measurements of net CO_2 uptake during rehydration, described in chapter 5, are consistent with that explanation although the stresses imposed in those experiments were severe and the observed slow recovery of high rates of net CO_2 uptake may not be representative of the response to water potentials normally developed in the field.

The rapid resumption of leaf production and expansion of existing leaves (figs 6.3, 6.4) is also consistent with the predicted sequence of events following the relief of water stress. Although the details of the response of individual seedlings were based on speculation about the properties of the cell wall it can be inferred from the results that osmotic adjustment did take place during stress with the consequent high turgor potential providing the driving force for the observed rapid growth. The turgor potentials recorded in the first 30 hours after rewatering were 2-3 times higher than the values quoted (0.5 - 0.9 MPa) for well watered plants in Hsiao (1973) but were much the same as those reported for *Triticum dicoccum* (1.3 - 2.5 MPa) by Turner and Jones (1980). Similar high turgor potentials have been recorded for *A. vesicaria* in the field. A group of undergraduate students from the Botany Department at Adelaide University calculated turgor potentials as high as 2.8 MPa from pressure-volume curves for saltbush growing on a sand dune at Koonamore. The corresponding osmotic potential was -5.0 MPa. These figures are comparable to those in table 6.1.

According to references cited in Turner and Jones (1980) osmotic adjustments of 0.4 - 0.7 MPa take 10-11 days to disappear after relief of stress

in some species. From the results for seedling 1 in table 6.1 it appears that an osmotic adjustment of about 2.0 MPa disappeared within 2 days of rewatering but considering the capacity for removal of ions from the mesophyll by *A. vesicaria* and the rapid growth observed with consequent dilution of cell contents the difference is not surprising.

However, while it is evident that leaves begin to expand almost immediately when plants are rewatered it is not clear whether cell wall synthesis is involved in the apparent leaf growth in the first few hours. As noted in the previous section the initial expansion may be wholly or partly elastic. Nevertheless it is unlikely that expansion can continue for any length of time without wall synthesis (Hsiao, 1973). It is also debatable, judging by models of cell wall extension outlined in Cleland (1971), whether a temporary halt in leaf growth is accompanied by a decrease in wall synthesis. According to one model, elastic extensions following metabolically controlled wall loosening are rendered irreversible by a second biochemical step, involving wall synthesis and hence the use of current or stored photosynthate.

The growth of stems and roots during and after rehydration will also constitute a sink for photosynthate from one or both of these sources. Although root growth after rehydration was apparently slow or non-existent, as judged from the response of roots already established at the transparent faces of the containers, there may have been considerable root production from the root crown soon after the soil was rewatered.

6.42 Growth responses to decreasing water potential

While high water potential was maintained at the beginning of the dehydration phase root growth was rapid and the peak rate of growth by an individual root exceeded 60 mm per day. In general the rate of root elongation was similar to that recorded in the field (Ch. 4) and the published

values of 23 - 50 mm per day (Jones and Hodgkinson, 1970; Williams, 1972; Hodgkinson and Baas-Becking, 1977). There was, nonetheless, considerable variation in the maximum rate of root elongation from day to day and between roots of the same plant on any one day. This variation may have been partly responsible for the fluctuations in leaf and stem growth at high water potential if roots were growing through patches of high soil moisture. The root growth observed when xylem water potential was about -6.0 MPa, for example, was apparently confined to a relatively small volume of soil which may have held water at a higher potential than the surroundings. Slatyer (1973) cited the work of Newman (1966), who found the rate of elongation of flax roots to be highly dependent on local soil water potential, and pointed out that roots in relatively moist soil may continue to elongate even though the plant as a whole is subject to severe internal water stress. Specifying a limiting water potential for root growth is somewhat difficult for this reason, especially when it is based on xylem rather than soil water potential. It is also possible that conditions for root growth at the walls of the containers were unrepresentative of those in the bulk soil.

Another difficulty associated with the interpretation of the root growth data is the treatment given the seedlings during the dehydration phase, namely the removal of shoots for measurements of water potential, a procedure which may have altered the root-shoot ratio enough to depress the rate of root growth before it was directly affected by falling turgor. This response would be especially true of the younger seedlings from which about one quarter to one third of the total number of shoots were removed during the course of the experiment. In such circumstances restoration of the dynamic equilibrium between root and shoot (Wareing, 1972) would favour shoot growth provided that water potential was adequate. However, a much smaller proportion of the total number of shoots, less than 10 per cent, was removed from the two-year-old seedling while water potential remained

high yet root growth still declined. Although this seedling did produce some root growth when xylem water potential was low the general pattern of growth was more consistent with the idea that root elongation is dependent on local soil water potential rather than inhibition by shoot removal. The fact remains, however, that xylem water potential is not a good indicator of root response.

Although there were some aspects of leaf expansion at high water potential which were unexpected, the data on leaf growth are not subject to the same uncertainties as those for root growth. Xylem water potential is the most conveniently measured variable likely to be associated with the rate of leaf expansion but as noted in the introduction to this chapter increases in cell volume are dependent on turgor potential rather than total water potential.

High turgor potentials were associated with intermittently high rates of leaf expansion during the initial stages of dehydration and relatively low turgor potentials with an overall decline in leaf area at lower water potential but there was no consistent relationship between individual pairs of values for growth rate and turgor potential. There is no question that leaf expansion is dependent on turgor potential but while a high mean value is expected at times of rapid growth there may be fluctuations in turgor potential if growth is reduced as a result of conditions other than a sub-critical turgor. Under these conditions, provided that osmotic potential is high, turgor potential will rise as the cell wall becomes more rigid and, unless osmotic potential is maintained, decline at times of rapid growth. On this basis the closeness of the relationship between the two variables will depend on the time interval between leaf expansion and estimates of turgor potential. A close correlation might be expected only if estimates are made during periods of steady state growth and if expansion is

proportional to turgor potential in excess of some critical value. The second condition may be met when soil water content is maintained at field capacity but apparently is not, even in the first few days, after water is withheld.

The fluctuations in the rate of leaf expansion at high water potential could be related to varying ambient conditions around the shoot or to changes in root growth although recorded fluctuations in the latter do not always correspond to changes in the rate of leaf growth (figs 6.2 a-e) possibly because observable root growth is not a good indication of overall root activity.

Of rather more interest are the fluctuations in leaf area (negative growth rates) which occur at high water potential. These are also likely to be the result of changes in turgor controlled by the relative rates of water absorption and transpiration or, according to Kozlowski (1972b), internal redistribution of water within the plant. Many observations of similar changes in diverse plant parts have been reviewed by Kozlowski (1972b), who attributed the shrinkage and swelling of tissues to changes in hydration and temperature. He considered that changes in hydration are responsible for much greater reversible changes in the size of plant tissues during their development than are direct thermal effects. Changes in size of plant tissues occur in response to diurnal and seasonal water deficits and often involve diurnal changes of decreasing amplitude superimposed on the trend of net shrinkage during a drought.

Diurnal changes in size may be quite large. The length of the apical shoot of *Pinus radiata*, for example, decreased by 1 cm during the daylight hours (Fielding, 1955), while changes in leaf thickness of between 4 and 7.5 per cent have been recorded following a change in 'leaf moisture content' of 1 per cent in the leaves of various other species (Meidner, 1952). Raschke (1970) measured a decrease of 32 and 18 per cent in the thickness

of the epidermis and entire leaf, respectively, of *Zea mays* during a drying cycle. The latter figures are comparable to the decrease in leaf area of about 25 per cent observed for *A. vesicaria* as xylem water potential decreased.

All the examples cited in Kozlowski (1972b) were reports of changes in leaf thickness rather than area. To what extent these reflect changes in area is not known. Apart from the work of Cremer (1976), who recorded diurnal contractions in both shoot and leaf length for *Pinus radiata* and *Eucalyptus regnans*, I am not aware of any reports of diurnal or seasonal contractions in the area of individual leaves. The absence of any such examples from the review by Kozlowski suggests that they are rare at best. Given that turgor dependent reduction in the area of individual leaves is a common occurrence, as it may well be judging by the numerous examples of changes in leaf thickness, the apparent rarity of reports on this subject is surprising. It is probably a consequence of the use of fixed leaf areas for many daily measurements of variables expressed on a leaf area basis and the use of dry weights in lieu of area for microphyllous species. Changes in leaf area during drought are usually assessed from successive samples of plants a procedure which is unlikely to detect the changes in area of individual leaves.

The fluctuations in leaf area while turgor potentials remain high (fig 6.2e) implies that there is a threshhold turgor potential associated with leaf expansion. Below the critical potential cells contract and can recover only through osmotic adjustment or an improvement in soil water availability allowing sufficient water uptake to account for the deficit. An approximate estimate of the critical turgor potential for *A. vesicaria*, of between 1.0 and 1.5 MPa, appears high when compared with that for other species. *Avena* coleoptiles, for example, have a threshhold value of about 0.6 MPa (Cleland, 1959) and leaves of sunflower require a turgor potential

of about 0.35 MPa before leaf enlargement begins (Boyer, 1977).

However, if the value suggested for *A. vesicaria* is accepted the small recovery in leaf area on day 22 (a small positive rate of expansion), when turgor potential was less than 0.5 MPa, is untenable. A possible explanation may be found in a model of cell wall extension outlined in Cleland (1971) which incorporates a shifting critical growth turgor. The model states that the critical turgor is determined by a balance between a metabolic process which lowers the yield point of the cell walls and a physical strain hardening caused by extension of the cell walls during growth. When turgor potential falls below the critical value strain hardening no longer occurs and the critical value is lowered by the metabolic process, until it is once again lower than the turgor pressure in the cell, whereupon growth resumes. The theory was developed to explain growth responses of the internode cells of the alga *Nitella* and at the time the review was written was untested. Nevertheless, positive expansion rates by the youngest leaves, depicted in figure 6.2e, at a time when turgor potential was apparently falling (days 16-18) may depend on a lower threshhold potential. On the other hand, there are several obvious objections to the necessity for invoking such a theory to explain the recovery of leaf area or irreversible growth at low turgor potentials. Small increases in leaf area may be independent of the critical potential and simply represent an elastic expansion in response to any change in turgor. Judging by the overall response of leaves to decreasing water potential it appears that at any given time a considerable proportion of the area of a leaf (25 per cent or more) is due to elastic expansion in which case most of the fluctuations at both high and low water potential may be independent of a critical turgor potential. It becomes more difficult, on this hypothesis, to specify a limiting water potential for growth but it will be higher than that indicated for leaf expansion in the diagrams (fig. 6.2a-c).

Turgor potentials derived from pressure volume curves are an average value for all the cells in the tissue. Attempts to relate values obtained in this way to rates of expansion or growth of individual leaves are confounded by the possibility of differing turgor potentials in different leaves. This point is well illustrated in figures 6.2 a-e where there are several examples of positive and negative expansion rates for leaves on the same plant, or even the same shoot, on the same day. A high turgor in young leaves when the recorded average is low would obviate the need to explain leaf area recovery by a low threshhold turgor potential. References cited in Wardlaw (1968) and Canny (1973) provide evidence of complicated patterns of vascular connections which may explain apparent differences in hydration of leaves of similar age on the same or different shoots. Observations and experiment have shown, that the pattern of translocation of organic solutes from a given source can be restricted to files of vascular tissue leading to a specific sink. The removal of the leaves from one side of a sunflower shoot, for example, leads to the failure of all the florets on one side of the head. Other examples include the greater development of swede tubers on the sunny sides of the plant and the supply of specific parts of beet root systems by various leaves. Xylem connections from the leaves on different shoots may therefore lead to different parts of the root system with access to water held at different potentials. Although water movement, when compared with solute transport, is likely to be more uniform, due to lateral movement in response to water potential gradients, the increased resistance associated with a more circuitous pathway may alter the balance between transpiration and water uptake by different leaves. Stocker (1960) cites an example where the tip of tomato shoots above the first node showed fairly uniform growth during both day and night while the lower internodes grew only at night and stopped or even shrank during the day.

In the preceding pages the discussion of decreases in leaf area have been based on the premise that cell wall contraction and a decrease in the volume of individual cells, as outlined in Cleland (1971), are involved in the observed response. An elastic contraction of the cell walls has been observed when *Avena* coleoptile sections are exposed to low external osmotic potentials. However, the loss of leaf area in *A. vesicaria* need not be due to reductions in cell volume if decreases in turgor potential allow a change in cell shape resulting in a closer packing of cells and a loss of air space in the mesophyll. The contraction of epidermal cells and cuticle may still be necessary unless there is some buckling of that tissue associated with decreasing projected area of the leaves.

The loss of leaf area in the way just described would have the disadvantage of reducing the internal surface area available for CO_2 absorption whereas the ability to reduce leaf by contraction of the cell walls, thus reducing cell volume, may have constituted a selective advantage over individuals with more rigid cell walls. Although some internal absorbing area would be lost as cell volume decreased the resistance to CO_2 or organic acid diffusion in the aqueous pathway may be reduced. Cell wall contraction may also play a significant role in maintaining turgor potential during periods of moderate stress, directly due to the contracting cell wall and indirectly by increasing the solute concentration. The loss of internal surface area in conjunction with the maintenance of turgor potential may result in some CO_2 fixation without a reduction in water use efficiency by reducing water loss through the transpiration stream. There is no evidence for direct effects of turgor potential on CO_2 fixation (e.g. Boyer and Potter, 1973) although there has been some speculation that changes in pressure differentials across the cell wall-plasma lemma boundary may result in conformational changes in enzymes located in the plasmalemma (Hsiao, 1973) and thus influence CO_2 fixation indirectly (Osmond *et al.*, 1980).

Hsiao (1973) has also pointed out that pressure within the cytoplasm is isotropic and changes in turgor potential cannot influence the electron transport systems of chloroplast membranes in the same way. The spatial relations and hence function of proteins in enzyme complexes in cell membranes may also be altered as membranes stretch or contract with turgor dependent changes in cell volume. Since organelles behave as osmotic sacs, taking up or losing water as cell water content changes, the function of enzymes in chloroplast membranes may be directly affected in this way.

Hsiao concluded, however, that the major effect of changes in turgor potential on cell metabolism is indirect via effects on cell expansion. Cell division may be reduced in turn if meristematic cells are unable to reach a minimum size before mitosis can occur. Hence because of a lack of demand for assimilates the rates of net CO_2 uptake and other synthetic processes may be reduced if cell growth stops for any length of time.

Turgor maintenance by cell contraction would retain neither the spatial relations and function of enzyme complexes in organelle membranes nor any demand for and hence production of current photosynthate and other cell constituents, as might occur if turgor was maintained by osmotic adjustment. Any advantage of turgor maintenance by cell contraction must therefore rely on the existence of a more direct effect of turgor on metabolic processes. Although the dependence of such processes on turgor is still obscure, in more recent reviews (Hsiao *et al.*, 1976, Osmond *et al.*, 1980; Turner and Jones, 1980) there appears to be general agreement that changes in metabolism during stress are most likely to be mediated by changes in turgor potential. According to Zimmermann(1978) there is sufficient experimental evidence for a direct control of membrane transport and the electrical properties of the cell membrane by turgor pressure. He suggested that during cell expansion turgor controls the uptake of solutes required for continuing growth. Taking this argument one step further, it is possible that a degree of turgor main-

tenance due to cell contraction may be of some advantage if it allows ion uptake to continue and hence increases the time available for osmotic adjustment during drought.

Cell contraction may also influence processes such as net CO_2 uptake indirectly by enabling stomata to open more readily. Since stomatal turgor is not closely linked to that in mesophyll, cell contraction in the latter is unlikely to affect stomatal opening but if guard cells and other epidermal cells also contract during stress a greater stomatal sensitivity may result from the higher turgor allowing some opening and hence CO_2 uptake at lower water potentials. Normal stomatal control would operate to reduce water loss.

Given that turgor is important in maintaining metabolic processes and if the effects of cell contraction on turgor potential, intercellular space and water use efficiency during leaf area reduction are as outlined earlier then diurnal fluctuations in leaf area at times of stress may allow a greater carbon gain than if cell walls were rigid. Nocturnal rehydration and leaf expansion would result in the maximum possible area for light interception in the early morning when the water status of the tissues is relatively high, while progressive leaf contraction during the day would allow some CO_2 uptake when perhaps leaves with rigid cell walls have lost sufficient turgor to induce a reduction in the rate of net photosynthesis.

However while the detailed response of leaves after water was withheld has raised more questions than it answered, and the fluctuations in leaf area make it difficult to specify the point where growth, as distinct from reversible expansion, ceases, it appears that growth of all plant parts is highly sensitive to decreasing water potential and, for plants grown in the glasshouse, stops at water potentials between -2.0 and -3.0 MPa. The use of photosynthate for elaboration of new cell wall and protoplasmic constituents is unlikely to continue for long after cell growth stops and hence,

apart from its use directly or indirectly in osmotic adjustment, maintenance respiration, suberization and similar processes, most current photosynthate will be diverted to storage.

Whether growth under field conditions is equally sensitive is not known. The ability to maintain turgor potential high enough to extend the range of water potentials over which growth is possible will depend on the time available for osmotic adjustment (Turner and Jones, 1980) which will in turn depend on the depth of rainfall penetration and the evaporative demand during growth. Since *A. vesicaria* has a shallow root system it is debatable whether the rate of dehydration will be any less than that for glasshouse grown seedlings. Some information on growth and rates of decrease in total water potential following rainfall or irrigation in the field is presented in the next chapter.

There are other factors which influence the turgor potential at a given water potential including the elasticity of the cell walls and cell size. Smaller cell size attendant on development of leaves under conditions of mild stress has been shown to result in an increased capacity for turgor maintenance (Cutler *et al.*, 1977) and increased elasticity of the cell walls (Steudle *et al.*, 1977 cited in Turner and Jones, 1980). Although changes in cell wall properties may allow continued growth at lower turgor potential Turner and Jones (1980) have also suggested that in some circumstances increases in the minimum turgor for growth or changes in cell extensibility may override any beneficial effects of osmotic adjustment in prolonging growth. The maintenance of turgor due to the development of cells of smaller maximum size appears more likely to be related to survival rather than growth despite the possible changes in cell wall properties.

The way in which development under field conditions affects the ability of *A. vesicaria* to continue growth as water potential falls is not known. The changes which result in growth at lower water potential are also expected

to allow greater carbon gain by existing leaves. Presumably the increase in leaf area will increase the whole plant carbon gain even further, although against this, possible increases in the rate of water loss must be considered. Hence even if growth does continue at lower water potentials in the field the hydration compensation point for net carbon gain may also be lower, in which case the range of water potentials over which the bulk of current photosynthate is channelled to storage will not differ markedly from that estimated in the laboratory.

CHAPTER 7

7 Water potential

7.1 Introduction

The observation that xylem water potential of saltbush may fall to -13 MPa (Anderson, unpubl., cited in Anderson *et al.*, 1972) was used earlier as an example of the very low water potentials recorded for this species. Others have recorded similar values during dry conditions in summer. Sharma (1976) estimated water potentials of about -11 MPa from relative leaf water content (RLWC) of leaves harvested early in the mornings in a year with rainfall close to the long-term average. Evapotranspiration had reduced soil water potentials in the profile beneath this community to -80, -25 and -12 MPa at depths between 7.5 - 15 cm, 15 - 30 cm and 30 - 45 cm respectively. Excavation of a few individuals revealed that most of the root system (ca. 93%) was concentrated in the top 15 cm, and all but 0.2% in the top 45 cm of the profile. Under these conditions it is unlikely that the small amount of root mass with access to water stored at high potential could maintain the observed xylem water potentials for long unless stomatal resistance was very high. From the relation between relative leaf water content and pressure chamber readings (Sharma, 1976) it is evident that, at low water potentials, very little water need be lost to lower water potential markedly. A fall from 72 per cent to 62 per cent RLWC corresponds to a reduction in xylem water potential from -4 MPa to -11 MPa. However, while xylem water potentials may regularly fall to low values, and on occasion remain low for lengthy periods, for much of the time water potentials are likely to be higher than the minimum values quoted above. Williams (1972), for example, measured xylem water potentials for saltbush at a number of topographic sites where minimum dawn readings in summer were as high as -5.0 MPa in a run on area

and -8.5 MPa at the driest site.

At the other end of the scale, readings as high as -0.1 MPa have been recorded for *A. vesicaria* in early spring (Chapman and Jacobs, 1979) but maximum water potentials recorded for this species are usually below -1 MPa even for well watered plants. (e.g. Osmond, unpubl., p256 in Osmond *et al.*, 1980).

Because water availability has such an overriding influence on the physiology and growth of arid zone plants, regular measurements of plant water status are useful in that, given other information on plant response to water potential, they allow some idea of the physiological condition of individuals under various conditions in the field. Seasonal trends and absolute values of water potential will vary from year to year and from site to site. Studies on water use, hydrology, root distribution and weather conditions, for example, may eventually be combined to produce models capable of predicting soil, and possibly plant, water potential for a given site or soil type but until this is done direct measurements taken more or less regularly remain the only indication of plant water status at various times of the year. The most comprehensive account to date for *A. vesicaria* is that by Williams (1972) who measured dawn water potentials for saltbush over a period of twenty months, although during this time readings were taken on only seven occasions.

In the following pages data from a similar series of measurements at Koonamore are presented and compared with published values of water potential for *A. vesicaria* at other sites. Seasonal values for plants given supplementary water at regular intervals are also shown.

In earlier chapters the high potential rate of evapotranspiration and its importance in the water relations of *A. vesicaria* and other shallow rooted species have been emphasised. A rapid rate of rehydration was considered necessary for *A. vesicaria* to make full use of intermittently

available water. For plants in the laboratory it has been shown that rehydration is rapid during recovery from low water potential. There is no reason to expect that under similar conditions rehydration in the field would be any less rapid. However, at times of low water potential in the field environmental conditions are likely to be much harsher than those in the laboratory. Apart from the possibility of rapid loss of water from the soil, which may result in a lower maximum water potential after recovery, rehydration may be initially delayed because of slow infiltration. Some indication of the immediate response to substantial falls of rain can be gleaned from the seasonal records of water potential. This information is supplemented with data from a few short experiments in which water potential of field plants was monitored for a few days after irrigation with varying amounts of water.

7.2 Methods

Seasonal values for xylem water potential were measured with a pressure chamber (Scholander *et al.*, 1965). On the days when water potential was measured dawn and midday readings were taken to estimate the maximum and minimum water potential respectively. Measurements were made on a random sample of 4-8 plants, growing within an exclosure of 0.5 ha, on each occasion. Two of these, chosen randomly at the beginning of the series of measurements, were sampled on every sampling date. For most readings, particularly those at midday and when water potential was low, shoots were wrapped in plastic film, before excision, to minimise transpiration. In hot dry weather the relative humidity of the gas around the shoot was increased by lining the inside of the pressure chamber with damp blotting paper.

On several occasions xylem water potential was monitored for a few days following irrigation. These plants were irrigated with rain water collected from a large underground storage tank at Waukaringa, 24 km south of Koonamore. The water was pumped directly onto the soil surface. Since up to 25 mm was applied within a few minutes runoff was prevented by means of a steel collar enclosing the plant, or plants, and 1-4 square metres of the surrounding area. Because the soil surface was rarely flat the use of four square metre collars, enclosing a central group of plants resulted in uneven infiltration over the area enclosed. Applied water was more evenly distributed within the smaller collars. Damage to surface roots was more likely in the lattercase but, as the collars penetrated the soil to a depth of less than 3 cm, the damage was probably negligible.

An individual saltbush bears an array of shoots of varying age and condition, some relatively well foliated and others partly withered or damaged by insect galls. In all cases, whether for irrigated or control plants, shoots for water potential readings were chosen from among those with the healthiest appearance in a given bush. Intensive sampling of a

single bush, on a few occasions showed that when this procedure was followed the standard error was within 2 per cent of the mean. Therefore unless a given bush had a water potential which varied markedly from others on the same date no more than two shoots were sampled. In most cases duplicates had a balance point within 100 kPa.

7.3 Results and discussion

7.31 Seasonal water potentials

The results of water potential readings taken over a period of 30 months are shown in figure 7.1. On most occasions these readings were taken in consecutive months, with only a few instances of longer intervals between readings. Each point is the mean of measurements on 4-8 plants. The standard error is shown for those points where it exceeds the diameter of the symbol locating the mean. Closed symbols represent dawn, and open symbols midday water potential. The accompanying bar diagrams depict two sets of rainfall data. The upper diagram shows the rainfall accumulated between readings at the site where water potential measurements were taken, while the lower shows the daily rainfall figures for Koonamore homestead about 7 km to the north. As before the latter are included to indicate the probable distribution of rainfall at the experimental site during the intervals between readings.

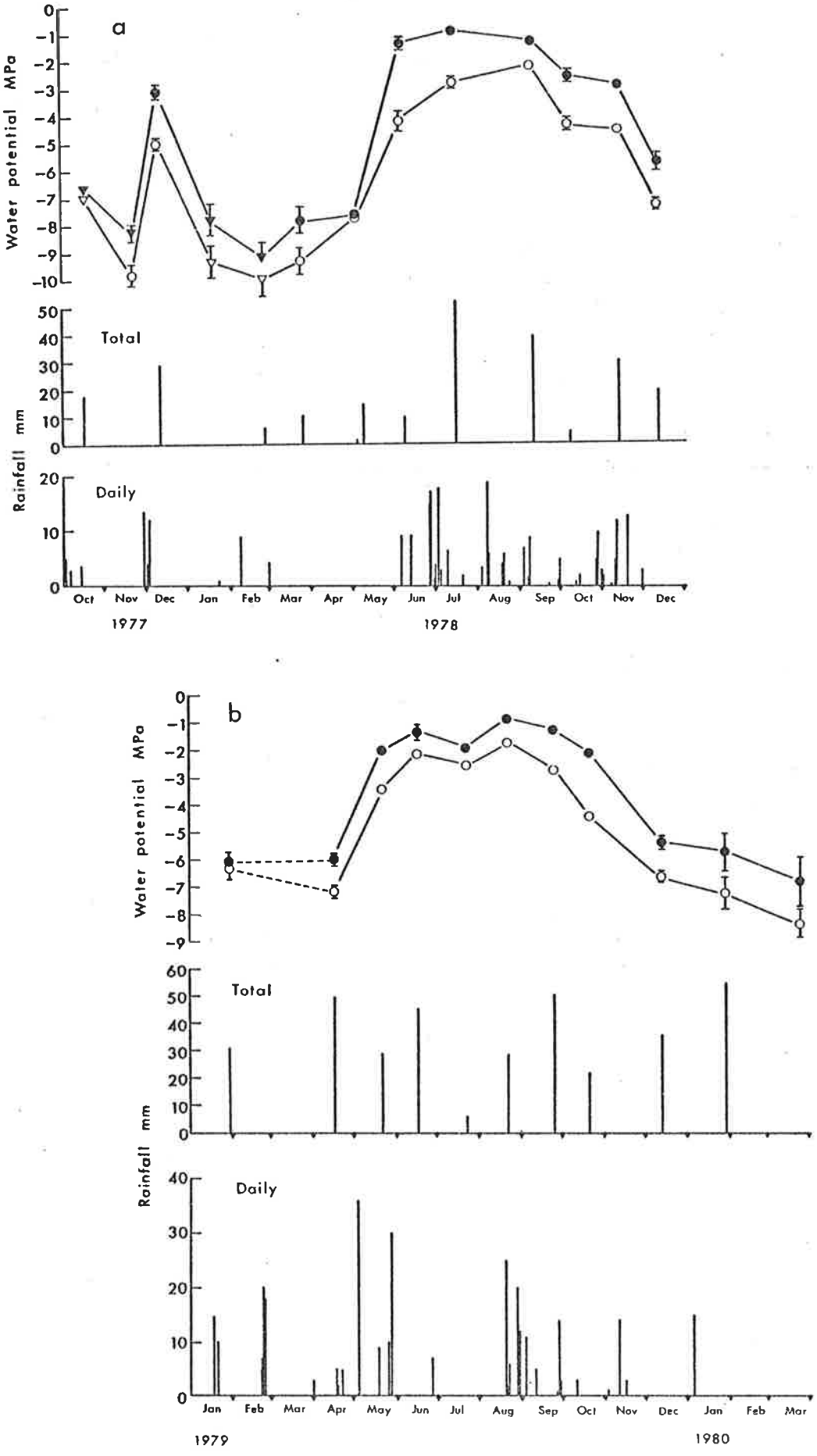
Xylem water potentials, as expected, were low in summer and high in winter. The lowest values were recorded during the summer of 1977-1978 at the end of a year of well below average rainfall. Total rainfall in 1977 (86.5mm) was less than half the long-term average (214 mm) for Koonamore. At the end of that summer (February, 1978) the mean value for dawn water potential was less than -9 MPa and some individual readings included in the mean were less than -11 MPa. The mean for the February sample is located by an inverted triangle which signifies that the balance point for one or more of the sampled plants was not reached by the time the pressure applied to the shoot equalled the limit of the gauge on the pressure chamber (11 MPa). The limiting values (-11 MPa), which were included in the calculation, yield a mean and standard error which were higher and lower, respectively, than the true values. A few earlier sample means were also overestimates but in

Figure 7.1 Mean water potentials for samples of 4-8 shrubs in the field. Closed symbols represent dawn water potential and open symbols midday water potential.

(a) Recorded between October, 1977 and December, 1978.

(b) Recorded between January, 1979 and March, 1980.

The two sets of rainfall data beneath each plot of water potential show total rainfall accumulated at the site between samplings and daily rainfall at Koonamore Homestead. Standard errors are shown for potentials where they exceed the diameter of the symbol locating the mean. Inverted triangles indicate that the balance point for some shoots in the sample could not be measured either because the limit of the gauge on the pressure chamber was reached or because of low gas pressure in the supply cylinder. In November, 1977, gas pressure was too low at dawn but rose high enough as temperature increased during the day to allow balance points to be recorded at midday.


a
Water potential MPa
0
-1
-2
-3
-4
-5
-6
-7
-8
-9
-10
Total
Rainfall mm
50
40
30
20
10
0
Daily
20
10
0
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
1977
1978
b
Water potential MPa
0
-1
-2
-3
-4
-5
-6
-7
-8
-9
Total
Rainfall mm
60
50
40
30
20
10
0
Daily
40
30
20
10
0
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
1979
1980

those cases the balance point of some of the shoots in the sample was not reached because of low gas pressure in the cylinder supplying the pressure chamber.

Water potentials did not fall to such low values in the early months of the following years due to substantial summer rains. In the interval between January and April 1979 no readings were taken but water potentials were probably maintained at relatively high values by heavy rains totalling 50 mm, the major part of which apparently fell in the middle of that period. Even when plants were highly stressed substantial falls of rain were able to reverse the downward trend in water potential during summer. Falls of rain totalling 29 mm in December 1977, for example, resulted in a large increase in water potential. According to the daily rainfall figures this rain probably fell 8-10 days earlier, in which case the maximum value may have been higher than that shown. In comparison, the response to 10 mm of rain in the following June illustrates the greater effectiveness of rain during the cooler months of the year, although part of this increase may have been due to the second and larger of the two falls of rain recorded in May. This fall was recorded a few days after the May water potential reading, thus raising the total rainfall for the May-June interval (26mm) to a value similar to that recorded in December. Two such falls if well separated would probably have been less effective in the summer months due to higher evaporative losses from the soil surface.

Most of the changes in water potential can be adequately accounted for by the figures for rainfall accumulated between readings but the small rise between February and May, 1978, which was significant at the 5 per cent level, was unexpected because of the low rainfall during that period. The significance of this difference is based partly on a standard error (February) which, as noted earlier, is an underestimate but judging by standard errors in preceding months it is not a gross underestimate. Part of the response was due to mild and overcast conditions on the days leading up to, and

including, the sampling date in May. Rain (14mm) fell a few days after. These conditions were reflected in the small diurnal fall in water potential on that day. As will be shown later heavy cloud during the day, even in a hot dry period, can result in large changes in dawn water potential.

The water potentials observed at Koonamore were similar to those recorded elsewhere. The lowest values observed during the summer of 1977-78 were as low as, or lower than, those estimated by Sharma (1976) and Williams (1972) for shrubs on the Riverine Plain in New South Wales and the range of water potentials observed at Koonamore during the following comparatively wet summers (-4 to -8 MPa) was similar to that observed by the latter author (-5 to -8.5 MPa).

During a diurnal series of measurements in spring Osmond (unpubl. see Osmond *et al.*, 1980) measured a dawn water potential of -6.5 MPa, close to the lowest recorded at a similar time at Koonamore. Osmond also measured a diurnal range of 1.5 MPa for an irrigated plant on the same day but was unable to establish the extent of the afternoon depression for unwatered plants because water potentials fell below the limit of the pressure chamber. From the tone of the discussion in Osmond *et al.* (1980) in which this work was described (p. 256) it was implied that because plants were still losing water they retained some physiological activity at such low water potentials. On this basis plants at Koonamore were active at much lower water potentials. Midday depression of water potential (represented by open symbols in fig. 7.1) and subsequent rehydration at night continued at water potentials less than -9 MPa and on one occasion at less than -9.8 MPa. Results summarizing the extent of the midday depression at various times of the year and for the whole period of observation are presented in table 7.1.

Table 7.1 Midday depression of xylem water potential (MPa)

Time	Number of observations	Mean ± s.e.
Summer (Dec - Feb)	23	1.54 ± 0.07
Autumn (Mar - May)	16	1.18 ± 0.10
Winter (June - Aug)	12	1.41 ± 0.25
Spring (Sept - Nov)	12	1.59 ± 0.12
Total (year)	63	1.43 ± 0.07
Irrigated (year)	21	1.23 ± 0.14

An analysis of variance showed that under the conditions prevailing during this set of observations there were no significant differences in the magnitude of the midday depression at different times of the year, nor between irrigated and control plants over all seasons. The values shown in table 7.1 are similar to that reported by Osmond (1.5 MPa) for an irrigated plant on the Riverine Plain. Water potential of the control plant in his experiment, for which the change was unmeasurable, probably also fell by approximately the same amount.

During the cooler months xylem water potentials at Koonamore reached higher maximum values (fig. 7.1) than those recorded at the same time of the year elsewhere; Williams (1972) reported maxima of between -1.6 and -2.3 MPa and Sharma (1976), a maximum of -2.5 MPa. The values reported by Sharma are probably less than the potential maximum as plants were sampled in the early morning (6am-9am) rather than at dawn or before. Chapman and Jacobs (1979) recorded a maximum of -0.8 MPa in spring at Fowlers Gap in New South Wales. This value is similar to the highest observed (-0.69 MPa) for unwatered plants at Koonamore but the very high value of -0.1 MPa which they observed for a plant at Hay (N.S.W.) was not achieved at Koonamore even

by plants irrigated with 25 mm per month in addition to natural rainfall. Although too few records of water potential have been taken to assess whether water potentials as high as -0.1 MPa are common it is probable that some of the values recorded during that diurnal series of measurements at Hay were overestimates. A spurious balance point was often observed at Koonamore due to gas entering xylem vessels through holes left by recently fallen leaves or, in some cases, those removed to allow the shoot to be fitted in the lid of the pressure chamber. This caused bubbling at the cut surface well before the true balance point was reached. The large fluctuations in water potential at Hay, -0.1 to -1.5 MPa within an hour in the middle of the day, were probably due to this source of error.

7.32 Water potential of irrigated plants

Figure 7.2 shows seasonal dawn water potentials for irrigated and control plants. The open symbols represent the average for two plants irrigated with 25 mm of water every month, in addition to natural rainfall, while the closed symbols show the average for the two control plants which were sampled regularly (see Methods). Readings for irrigated plants were taken about one month after water was applied. On three occasions during summer and autumn water potential was monitored for 1-4 days after irrigation. These results are shown in the inset in figure 7.2.

During the cooler months (June - September) water potentials of control and irrigated shrubs were similar. The latter may have reached higher water potentials briefly following irrigation but no readings were taken to verify this. However, in June, several pairs of plants on a nearby sand dune were irrigated with 25 mm of water for undergraduate students taking a course in plant-water relations. During a diurnal series of measurements they found no difference between the maximum water potentials of a pair of unwatered

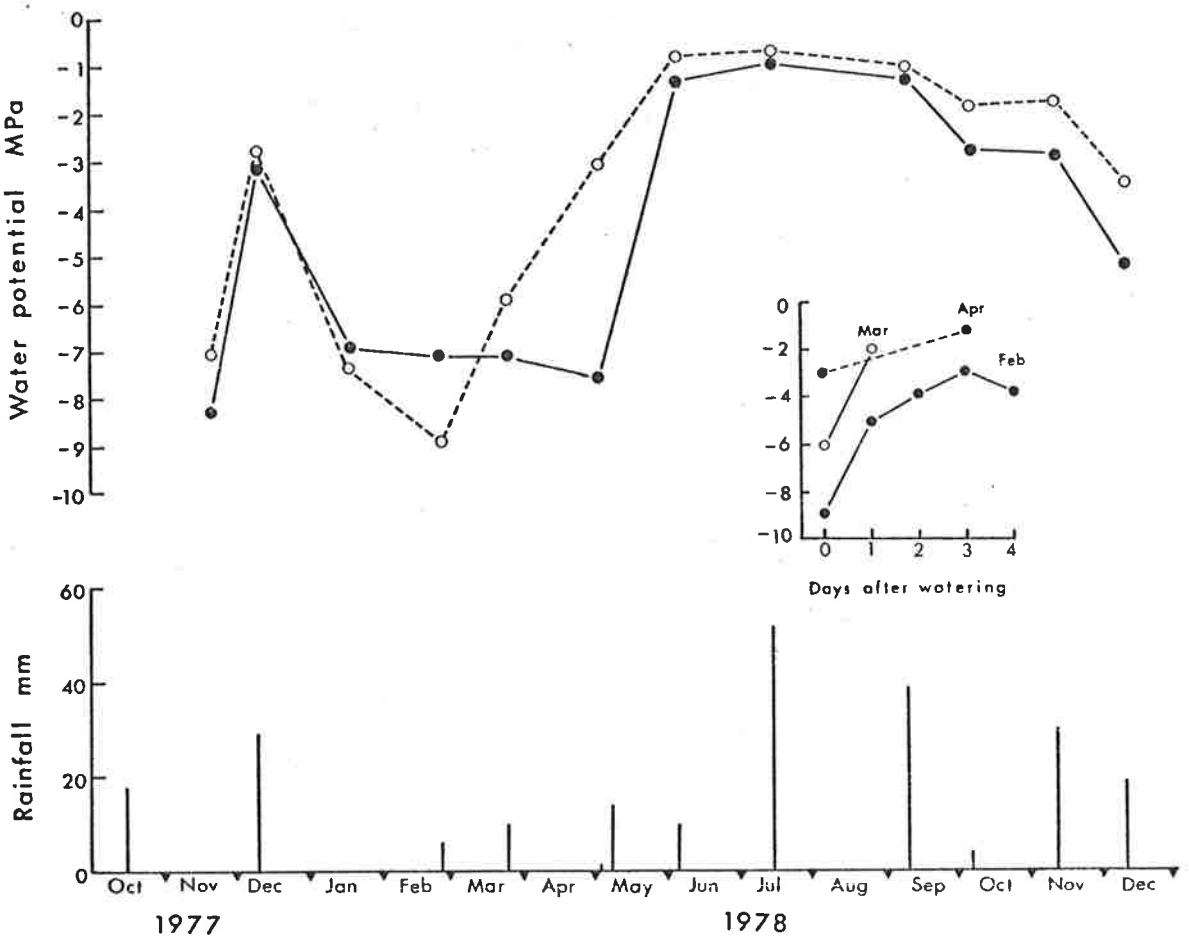


Figure 7.2 Water potential of control and irrigated plants in the field. The open symbols represent the mean dawn water potential of two shrubs irrigated with 25 mm of rainwater each month in addition to natural rainfall and the closed symbols that of two nearby unirrigated shrubs. Records were taken between November, 1977 and December, 1978, the day before irrigation. The inset shows the water potential of irrigated shrubs for 1-4 days after irrigation on the dates shown. The amount of rainfall accumulated at the site between readings is shown in the accompanying bar diagram.

plants and those irrigated 0 - 3 days earlier. Differences werc reduced because of a 9 mm rain a few days earlier but it seems that a high minimum concentration of osmotic solutes and possibly a reduced turgor pressure during rapid cell expansion in relatively mild winter conditions imposes an upper limit on the maximum water potential of saltbush on these soils.

An unexpected observation (fig. 7.2) was the comparatively low water potential of irrigated shrubs in January and February of 1978. Water potential was similar to the mean for all control plants (fig. 7.1) at that time but, as shown by the representative controls in figure 7.2, water potentials were higher in some unwatered shrubs. In both cases water loss from irrigated plots must have been much greater than that from unirrigated areas. Later in the year as weather conditions become less harsh the water potential of irrigated plants exceeded that of the controls and remained high for the rest of the year.

The data, in the inset, depicting the immediate response to irrigation show that water potential increased rapidly, but in February water potential began falling three days after water was applied. If this is a characteristic response pattern water potential evidently does not remain high for long after rehydration in summer. On the other hand, evapotranspiration is likely to be less after substantial summer rains than after irrigation. An irrigated plot will lose water rapidly to the dry surroundings, which constitute a very large sink for water vapour, whereas rainfall as well as affecting a much larger area than that irrigated may be associated with cloud, lower air temperatures and higher relative humidity both before and after the event.

The rapid increase in water potential observed for these few plants may not be representative of the response to summer rains by plants in the field. Regular irrigation may have allowed the shrubs to retain active roots during

the dry season and hence rehydrate more rapidly than unirrigated plants. The latter shed tertiary roots during the dry season (Osborn *et al.* 1932) and water absorption may be slow when the soil is rewetted if the remainder of the root system is suberized before an effective rainfall. According to Drew (1979) if the root systems of some species are subjected to severe water stress they show a lower average permeability to water for several days after rewetting.

For plants rehydrated in the laboratory (Chapters 5 and 6) there was no evidence of a slow recovery of water potential but the extent of suberization may depend on the length of the drought period and the activity of roots during that time (Slatyer, 1973), factors which may differ between field and laboratory. The other possibility is that, if surface roots have been shed, slow infiltration may delay rehydration simply because active or potentially active roots do not have immediate access to water.

A slow recovery by previously unwatered field plants would allow qualified support for the view that such factors are important under field conditions. Two short experiments on the immediate response to a single irrigation are described in the following pages.

7.33 Rehydration of field plants

In early summer (9th December) 1979 seven shrubs were irrigated with 25 mm of water. Water potentials were measured at dawn and at intervals during the day for the following few days. A few unwatered plants were also monitored over the same period. The results from this experiment are presented in figure 7.3. Dawn water potentials for individual shrubs are shown in figure 7.3a. For convenience the results for irrigated plants are separated into two groups. The data in figure 7.3a (ii) are from plants treated by watering 1 m^2 of surrounding soil and figure 7.3a (iii) represents two groups of two plants irrigated with an equivalent amount of water spread

over 4 m^2 of the surrounding soil surface. Diurnal water potentials are shown in figure 7.3b.

It is apparent from these figures that recovery of high water potential following irrigation in the field is at least as fast as that observed for potted cuttings in the laboratory. The latter reached maximum water potentials, on average, within 4 days. In most cases dawn water potential of field plants showed signs of levelling off by about day 3. The water potential of control plants decreased during this time and probably would have continued to do so but for a sudden change in weather conditions. Shortly after midday on day 3 a dense, complete cloud cover reduced irradiance markedly. Air temperature fell to 15^{o}C and strong winds blew for most of the afternoon. A small amount of rain fell but did not register in any of the gauges. Irradiance which approximated that at approaching dusk remained low for the rest of the day. On previous days there had been little cloud and air temperatures had reached maxima of 30 - 40^{o}C. The peaks of the graphs of diurnal water potentials in figure 7.3b are dawn maxima. From the trend of these peaks for the control plot (represented by open symbols) it appears that the overcast conditions had allowed the unwatered plants to rehydrate to a level beyond that expected for the following dawn by 1800 hrs in the afternoon. A similar rise in water potential during that afternoon was evident for irrigated plants. Further rehydration during the night resulted in elevated dawn water potentials on day 4 for both control and irrigated plants. The effect is most clearly seen in figure 7.3a. However by the following day water potentials of the controls resumed their downward trend and those of the irrigated plants had begun to fall. Nevertheless, in most cases water potentials on day 5 were higher than on day 3. The effect of this longer than normal period for diurnal rehydration was therefore not transient. This result implies that the decline in water potential of the control plants was due to a progressively greater discrepancy between

Figure 7.3 Water potential of shrubs irrigated in the field.

(a) Dawn water potentials of controls and individual shrubs

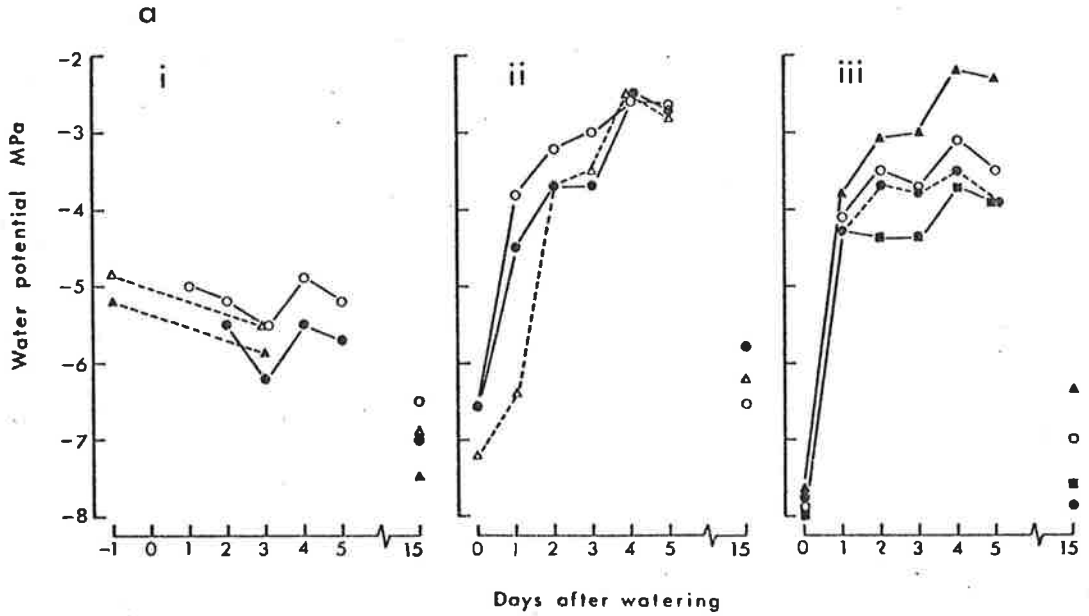
(i) Unirrigated

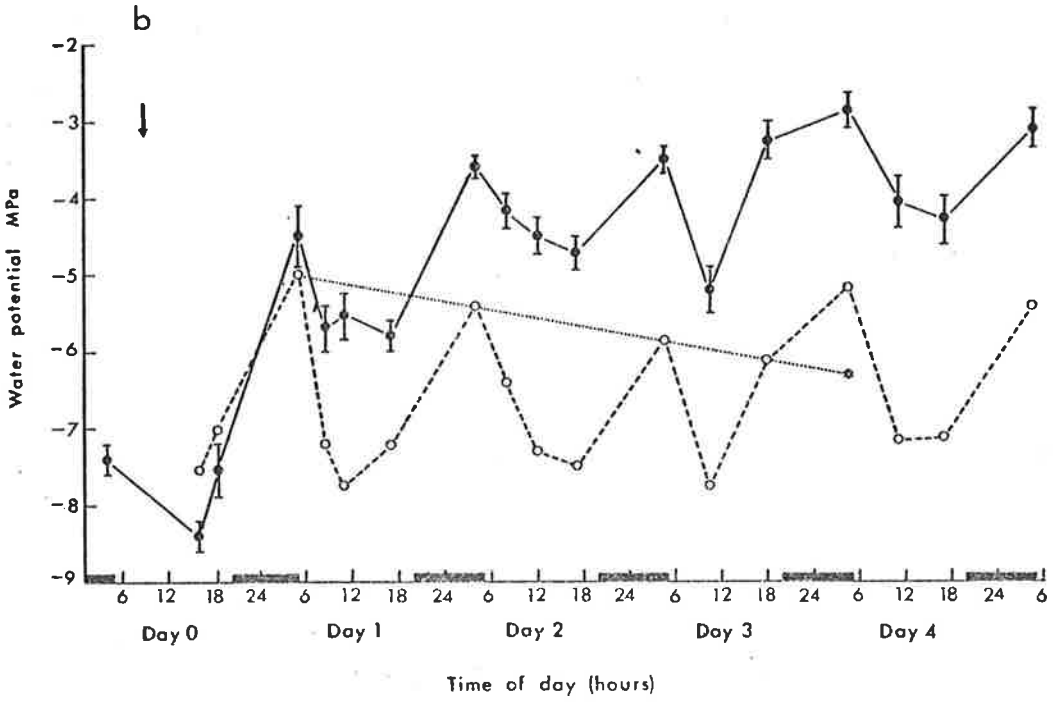
(ii) The surrounding 1 m^2 of soil irrigated

(iii) The surrounding 4 m^2 of soil irrigated

The last two groups are graphed separately for convenience.

(b) Mean diurnal water potentials for irrigated (closed symbols) and unirrigated shrubs (open symbols) in the days after irrigation. The shading on the abscissa indicates the time between sunset and sunrise. The arrow shows the time of watering on day 0. The dotted line passes through the points representing dawn water potential for unirrigated shrubs and that for 1800 hours on the afternoon of day 3 when overcast conditions had allowed water potential to rise to a value higher than that expected for the following dawn. The value that was expected is indicated by a star located directly below the recorded dawn water potential for day 4. The experiment was done in December, 1979.


a
i
ii
iii
Water potential MPa
-2
-3
-4
-5
-6
-7
-8
-1 0 1 2 3 4 5 15
0 1 2 3 4 5 15
0 1 2 3 4 5 15
Days after watering


b
Water potential MPa
-2
-3
-4
-5
-6
-7
-8
-9
6 12 18 24 6 12 18 24 6 12 18 24 6 12 18 24 6 12 18 24 6
Day 0
Day 1
Day 2
Day 3
Day 4
Time of day (hours)

equilibrium and actual dawn water potential rather than an upper limit imposed by falling soil water potential. If the rate of water absorption at night is limited by the number of active roots and equilibrium with the soil is not attained by dawn, water loss during the following light period will further depress water potential and as a consequence the plant may fall short of equilibrium with the soil by an even larger amount the next morning. The extra time for rehydration provided by overcast conditions during the light period allows the plants to approach the equilibrium water potential more closely. Since the maximum water potential of irrigated plants was also apparently limited by the time available for eliminating the diurnal water deficit it is possible that water absorption by these plants depended on a few active old roots rather than new root growth. However, Cowling (1969) observed new root growth for *A. vesicaria* 1-4 days after irrigation so part or all of the increase in water potential on day 4 for irrigated plants may have been due to root growth. This is unlikely to have been the reason for increasing water potential in the controls.

If overcast conditions rather than root growth were responsible for the elevated water potentials of irrigated plants on day 4, and this seems probable considering the simultaneous increase for the controls, then the observed response demonstrates that rehydration following a substantial rain in summer is likely to be more rapid than after an experimental irrigation in hot dry conditions. A large fall at the beginning of an overcast rainy period would be particularly effective. It is also evident that heavy cloud during the day in summer can have an appreciable effect on water potential even if no rain falls.

As well as illustrating the response to irrigation, figure 7.3 shows that the water potential of shrubs growing in the field varies widely at any given time. The extent of this variation can be gauged from the dawn water potentials of the controls and those of the irrigated plants on day 0,

a few hours before irrigation. Water potentials were as high as -5 MPa and as low as -8 MPa at that time presumably because the microtopography of the area leads to an uneven distribution of soil water. As predicted in Section 7.2 (Methods) this effect was reflected in the post-irrigation water potentials when water was applied to a large area of soil (4 m^2) around a group of plants. Uneven infiltration resulted in a wide variation in maximum dawn water potential (fig. 7.3a (iii)) for groups of plants watered in this way. The water potentials of individually irrigated shrubs were much less variable. Such variation might perhaps be reduced progressively during a drought if plants in drier sites restrict water loss earlier than those in run on areas but in Dec. 1979 insufficient time had elapsed since recent rains. Total rainfall at the site since mid October was 36 mm. Judging by the daily rainfall records for Koonamore (fig. 7.1) most of this fell in mid-November, only a little over 3 weeks before the start of the experiment.

Because the control plants had high initial dawn water potentials relative to those of irrigated plants the data for this group provide no information on the diurnal pattern of water loss at very low water potential. Measurements taken later, on days 14 and 15, when water potentials of most plants had fallen to pre-irrigation values, confirm that diurnal changes were still occurring. On average water potential on the evening of day 14 was 1.2 MPa less than at dawn the following day.

The rate of decline in water potential for the controls when compared with that for irrigated shrubs does allow some speculation on the overall pattern of dehydration following irrigation in the field. The mean rate of dehydration between day 5 and 15 for control plants was 0.13 MPa/day whereas that for irrigated plants was 0.37 MPa/day. If the rate of dehydration of irrigated plants at low water potential is similar to that of control plants then this implies that the initial rate of dehydration of irrigated plants was much higher, which in turn implies that high post

irrigation water potentials are not maintained for long. There is not enough data from the controls to confidently establish the significance of the difference between these rates of dehydration but the latter prediction is consistent with the pattern of water loss from a saltbush plantation on the Riverine Plain (Sharma, 1976). Evapotranspiration from this community averaged 2 mm per day over 55 days but initial rates calculated for the first 10 days were over 5 mm per day. Greenwood and Beresford (1980) measured rates of 0.7 - 1.3 mm per day from an *A. vesicaria* plantation but in this case the soil surface was moist and encrusted with salt a condition which according to Milthorpe (1960) can result in a lower initial rate of evaporation from the soil. However, even the average rate of 2 mm per day is sufficient to explain the drop in water potential of irrigated plants at Koonamore. Fifteen days after irrigation with 25 mm the water potential of some plants was close to that measured on day 0. Assuming that a return to pre-irrigation water potential implies the loss of all the applied water, an average rate of 1.7 mm per day, or less for some plants, over the 15-day period, could account for the observed response. Sharma (1976) noted that most of the water stored in the profile beneath a saltbush community on the Riverine Plain following a 30 mm rain in mid-summer was depleted within 15-20 days. Considering the possible differences in prevailing weather conditions and other factors affecting water loss at the two sites, this close correspondence between the inferred rate of water loss from small irrigated plots and the calculated rate from a naturally watered plantation is doubtless a coincidence. The comparatively low rate of water loss from a community at Koonamore following a similar rain in early summer (29 mm in December, 1977, see fig. 7.1) was probably a result of such differences. In this case depletion of soil water, again inferred from changes in xylem water potential, was not complete until about 50 days after rain.

A second short irrigation experiment was started in late December 1980, when 3 groups of two plants were irrigated with 6, 12 and 25 mm of water. As before dawn water potentials were monitored over the following few days. For the first three days midday water potentials were measured but these readings were discontinued to conserve the gas supply to the pressure chamber. The results for individual shrubs are shown in figure 7.4.

On this occasion the mean dawn water potential of the plants before irrigation (8.43 $\pm$ 0.48 MPa) was lower than in the previous year (6.94 $\pm$ 0.34 MPa) despite rainfalls totalling 52 mm since mid-October. Over half this rain fell in December according to the distribution of rainfall at Koonamore Homestead during the same period. Conditions during the course of the experiment were uniformly hot and dry except for a rain of 4.1 mm late on day 5. Maximum and minimum air temperatures were as high as 44.5^{o}C and 25^{o}C respectively. On most days maximum temperature was higher than 43^{o}C.

Shrubs irrigated with 25 mm of water behaved as in the earlier experiment. All had rehydrated by day 3. The third shrub in this group was watered one day later than the others, hence the shift of the arrow indicating rain. Only one other shrub of the remainder showed a positive response to irrigation; the water potential of one of the pair irrigated with 12 mm of water also increased to a maximum by day 3 but in this case did not rise much above -5 MPa. The water potential of this shrub had fallen to -6.4 MPa by day 5. The depth of penetration of applied water into the soil around the remaining shrubs was presumably too small to allow much of a response before significant losses occurred as a result of evaporation.

For the first four days the water potential of one of the unwatered plants declined more rapidly than that of unwatered plants in the previous summer but thereafter was maintained at the same level. The mean midday depression of water potential of all the plants before irrigation was 1.4 $\pm$ 0.08 MPa and that for the controls over the first 3 days 1.32 $\pm$ 0.17 MPa,

Figure 7.4 Dawn water potentials of pairs of shrubs irrigated with different amounts of rainwater in December, 1980.

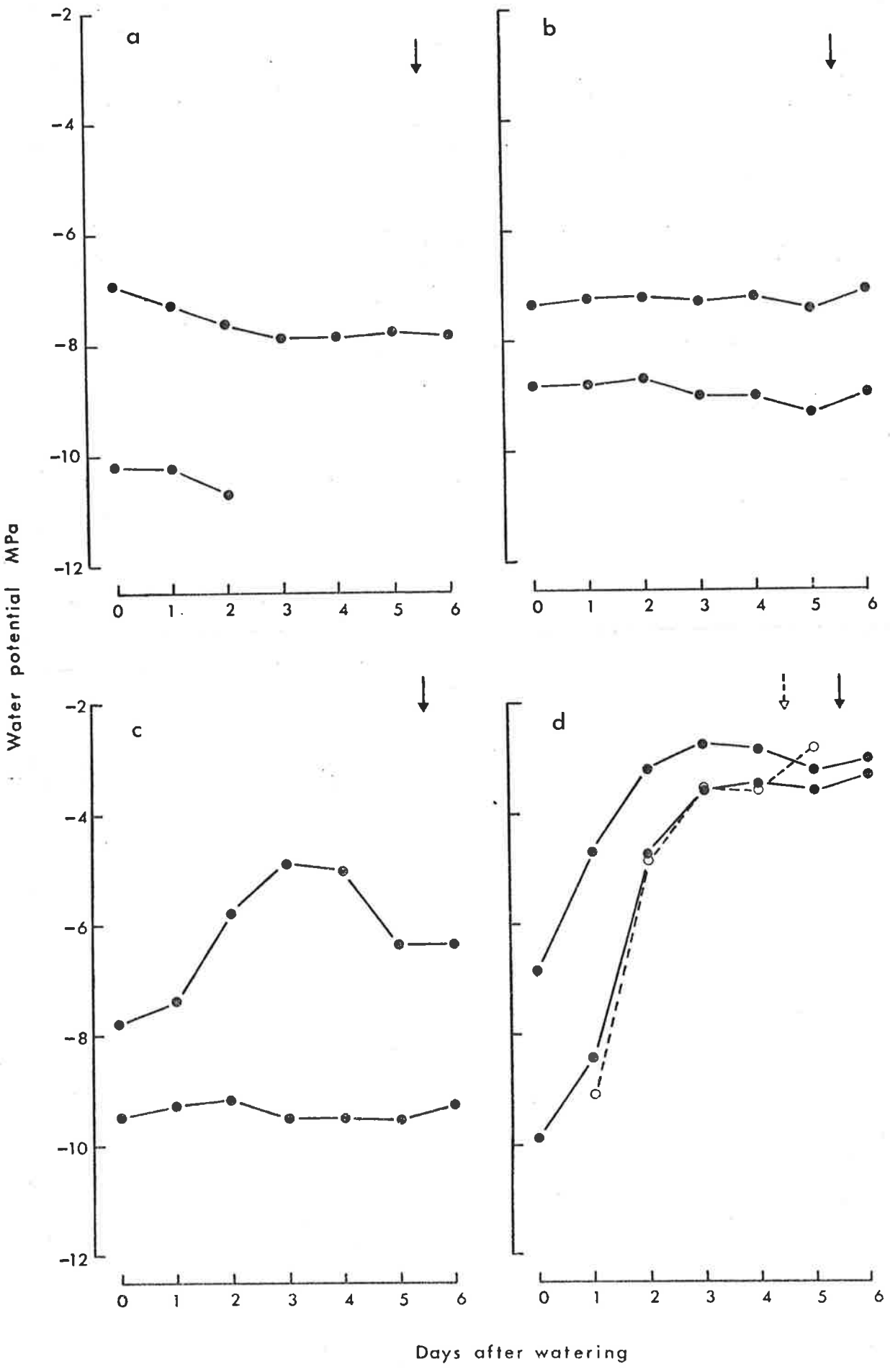
(a) unirrigated

(b) 6 mm

(c) 12 mm

(d) 25 mm

The arrow indicates a fall of 4.1 mm of rain. The third shrub in (d) represented by open symbols was irrigated one day later than the others, hence the shift in position of the arrow.


a
b
c
d
-2
-4
-6
-8
-10
-12
0
1
2
3
4
5
6
Water potential MPa
Days after watering

so it is obvious that the plants were losing water at that time. It is not known whether there was a diurnal change in water potential on the last few days when dawn water potential remained steady. If there was it is possible that early stomatal closure allowed the plants to rehydrate by the following morning. Some species have been shown to respond directly to low relative humidity by reducing stomatal aperture (Schulze *et al.*, 1972). These shrubs may have been responding to the hot dry conditions in this way although Whiteman and Koller (1967) found no evidence of such a reaction for well watered individuals of *A. vesicaria* at least not for vapour pressure deficits (between leaf and air) over the range 10-30 mbar. However, given a mean minimum humidity of 35 per cent in January (Osborn *et al.*, 1935) and assuming the intercellular spaces to be saturated with water vapour, the vapour pressure deficit may have been closer to 40 mbar. Stomates may also be more sensitive to humidity when leaf water potential is low.

The other possibilities are that the decline in water potential was halted by stomatal closure in response to hormones or low leaf water potential but since other plants have been shown to continue water loss at much lower water potentials than those recorded for the control in this experiment (e.g. fig. 7.1) this is perhaps unlikely. On the other hand, while uneven infiltration of rainfall has been suggested several times as a reason for the wide variation in pre-irrigation water potential, it may be that differences in stomatal sensitivity between individuals with different genotypes is partly responsible for the variation in water potential.

Osborn *et al.* (1932) suggested that 25 points (6 mm) was the minimum effective rainfall for saltbush during a dry period at Koonamore. Irrigation with 6 mm at best stopped water potential from declining further. Under the conditions of this experiment 12-25 mm of water were required to produce an appreciable increase in water potential. As discussed earlier, an

equivalent fall of rain may be much more effective, although the 4 mm rain on day 5, in this instance, had little effect on the controls by the following morning.

In both irrigation experiments described in the preceding pages it appeared that water potential began to decline after reaching a maximum on about day 3, a decline which was arrested or reversed either by overcast conditions or rain. Judging by the response of glasshouse grown plants to declining water potential, growth following irrigation in summer may therefore be limited. Some information on shoot elongation of irrigated plants in the field is presented in the next section.

7.34 Growth responses to irrigation

At various times between November, 1977 and October, 1978 pairs of shrubs were irrigated once with 25 mm of water and the growth of five shoots on each was recorded in the following 1-2 months. During this time, beginning in October, 1977, a group of four shrubs was irrigated regularly, and a series of measurements were made on these plants. Just before irrigation in each month the length of twenty tagged shoots from this group was recorded. In both cases measurements were made from photographs as described in Chapter 4. The mean water potential of two of the individuals from the group is illustrated in figure 7.2.

The results of growth measurements are shown in figures 7.5 and 7.6. The height of the bars represents the mean increase in length of the shoot as a whole (solid bars) and foliated stem (cross-hatched) except in figure 7.6a where the height of the stippled bars shows the mean increase in length of laterals of tagged shoots on male plants in the regularly irrigated group. The abscissa in figure 7.5 shows the month of irrigation and the number of subsequent monthly measurements on plants irrigated once only. For shrubs irrigated in December, 1977, for example, readings were

taken in January and February and the increase in length is shown for those two intervals. On two occasions no record was taken in the second month. Least significant differences ($P < 0.05$), from left to right where applicable, refer to increases in overall and foliated length, respectively.

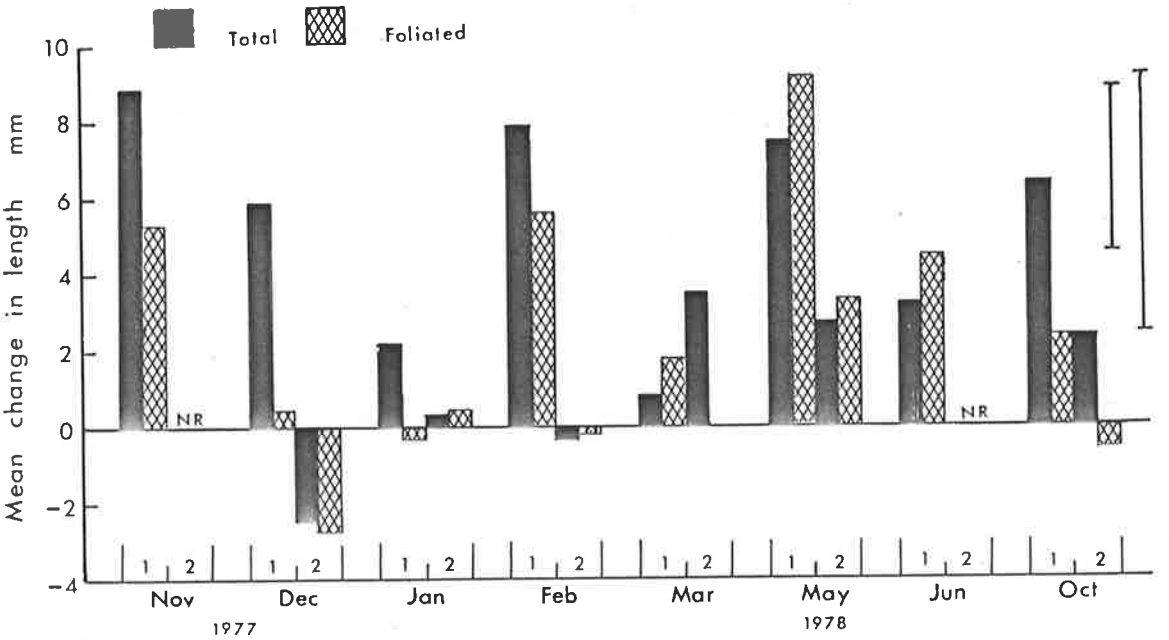
It seems from figure 7.5 that little growth occurs in the second month after irrigation, especially in the hot, dry months of the year. On two occasions shoots of shrubs watered in the summer months (Dec-Feb) decreased in length after a flush of growth during the first interval. This was also true of shrubs receiving natural rainfall only. The mean increase in shoot length for 47 shoots on control plants after a rain of 29 mm in December 1977 was 5.2 ± 1.2 mm while in the following month, when no rain fell, shoot length decreased by 1.4 ± 0.5 mm. Figures for 10 shoots from a pair of shrubs irrigated later that month were 5.9 ± 1.8 mm and -2.6 ± 0.9 mm, respectively. The LSDs also illustrate the large variation between shoots of this species and their response to rehydration. In some cases, as found in earlier chapters, the variation was such that while some shoots grew, others on the same plant contracted. Standard errors were so large that, while differences between growth on the first and second month after rainfall or irrigation, were usually significant, increases in length in the first month, at different times of the year, were not significantly different in most cases. Later in the year growth in the second month was initiated by rainfall and the data thus provide little further information on the length of the growth period except to demonstrate the obvious point that frequent rains or mild conditions are required to maintain continued growth. The amount of rainfall associated with growth in those instances can be assessed from rainfall data presented earlier.

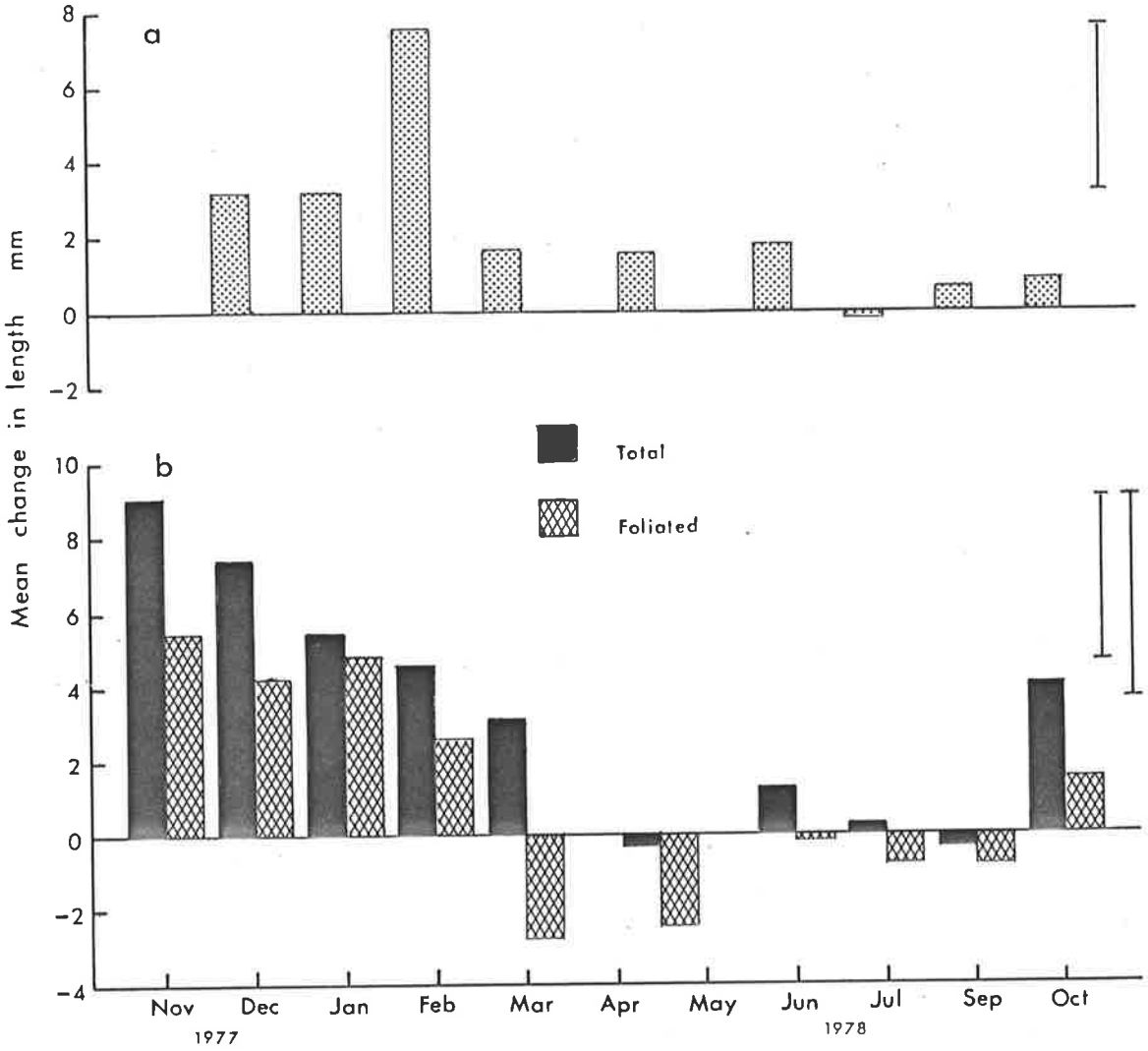
Figure 7.5 Mean change in total and foliated shoot length for a total of 10 shoots on pairs of plants irrigated once with 25 mm of rainwater between November, 1977 and October, 1978. Shoot length was recorded at the time of irrigation and at the end of the first and second month after irrigation. Changes in length for the first (1) and second (2) intervals are shown. NR = no record. LSDs, $P < 0.05$, are from left to right for total and foliated length, respectively.

Figure 7.6 Mean change in total and foliated shoot length for a total of 20 shoots on two female and two male shrubs irrigated regularly with 25 mm of rainwater.

(a) Growth of laterals on tagged shoots of male plants.

(b) Growth of tagged shoots. LSDs as in figure 4.5 above.


Total
Foliated
Mean change in length mm
10
8
6
4
2
0
−2
−4
NR
NR
1 2
Nov
Dec
Jan
Feb
Mar
May
Jun
Oct
1977
1978


a
b
Total
Foliated
Mean change in length mm
8
6
4
2
0
−2
10
8
6
4
2
0
−2
−4
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Sep
Oct
1977
1978

In some months the increase in foliated length was greater than the increase in shoot length, an apparent anomaly which was explained in Chapter 4. To recap.briefly, if basal leaves have been shed before the first measurement, leaving viable axillary buds, the latter can sprout when the shrub rehydrates. In conjunction with apical production of leaves the increase in foliated length can exceed that of the shoot.

Regular irrigation (fig. 7.6b) allowed some growth in each of the summer months. It was shown earlier that this treatment (25 mm of water per month) was not adequate to maintain high water potentials during summer (fig. 7.2) which implies in conjunction with the data in figures 7.3a and 7.5 that growth occurred in pulses while water potential remained high for a short period after irrigation.

The seasonal pattern of shoot growth in figure 7.6 is inconsistent with that shown by unirrigated plants (ch. 4) which grew rapidly in spring. The growth of irrigated shrubs was significantly less at that time despite regular supplements of water. In fact, irrigated shrubs began to appear unhealthy after several months of watering. Possibly the repeated ponding of water on the surface of the soil resulted in leaching of nutrients away from the root zone, or had some other detrimental effect.

Hodgkinson *et al.* (1978) found that although a 23 mm rain in mid-summer increased the dawn water potential of *Atriplex confertifolia* (Curlew Valley, N. Utah) from about -3.0 to -1.6 MPa in 1-2 days, a weekly watering treatment later in the summer, while reducing the decline on water potential recorded for unirrigated shrubs, did not maintain water potentials at the value measured shortly before regular irrigation was begun. Water potential was maintained above that of the controls but fell from -2.4 to -3.1 MPa over a three-week period. A single application

of 25 mm to previously unirrigated shrubs, on the other hand, resulted in an increase in water potential within 24 hours.

Their data on water potential do not imply that regular watering was detrimental, as suggested for *A. vesicaria*, but shoot growth by *A. confertifolia* in summer could not be initiated by either watering or watering plus nitrogen, a response which was attributed, with some qualification, to partitioning of photosynthate to the root system rather than the shoot, since root growth was observed. The authors, Hodgkinson *et al.* (1978), discounted summer dormancy as a reason for the lack of growth. The partitioning of photosynthate to roots may be a response to, rather than a cause of, the lack of shoot growth. This possibility was implicit in their final comment where it was suggested that evolution of tolerance to a cold winter environment had resulted in the loss of ability to initiate shoot growth in summer. In other words, the response is due to changes in shoot rather than root metabolism. The observed activity of the root system suggests that metabolites originating from the root which promote shoot growth ought not be lacking.

Considering the high sensitivity of shoot growth to water stress in glasshouse grown seedlings of *A. vesicaria*, the lack of growth of *A. confertifolia* in summer may have a physical rather than a metabolic cause. The latter species may also be unable to maintain turgor above a critical value for cell expansion in the leaves and stem during summer. However, dawn water potentials were as high as -2.0 MPa and since the species has been shown to exhibit considerable osmotic adjustment during summer (see Hsiao *et al.* 1976) *A. confertifolia* would have to be particularly sensitive to water stress for this explanation to account for its failure to respond to irrigation.

In contrast, shoots of *A. vesicaria* grew in response to either rainfall or irrigation in summer (ch. 4, fig. 7.5). In the irrigation experiment in summer 1979 growth of shoots was recorded over the first 5 days after irrigation. The mean increase in shoot length (n = 19) was 3.0 $\pm$ 0.3 mm. The increase in length of unirrigated shrubs, which had water potentials as high as -4.9 MPa during that period, was 0.5 $\pm$ 0.3 mm (n = 10). The difference between the two groups is significant ($P < 0.001$) and the increase in shoot length of unirrigated plants is not significantly different from zero. Since water potential (fig. 7.3) apparently began to decline shortly after reaching a peak on day 3 it is also interesting to compare the above figures with the growth of other irrigated shrubs in the field. The mean increase in length of 50 shoots in the first month after irrigation in the dry conditions of summer and autumn, 1978 was 4.8 $\pm$ 0.8 mm which is significantly greater than that over the first 5 days in 1979. Nevertheless, given the same initial rate and pattern of growth over 60 per cent of the increment in length over a month might be expected in the first few days after irrigation.

The mean increase in length of 47 control shoots in December, 1977 which grew in response to rainfalls totalling 29 mm, 8-11 days earlier, was 5.2 $\pm$ 1.2 mm also significantly higher than the 5-day total for irrigated shrubs in 1979 but not significantly higher than that over a month for other irrigated shrubs. As noted earlier stems, on average, had contracted some time between irrigation or rainfall and the second sampling date, and possibly had stopped growing even before the first measurement when the increment was recorded. Growth was probably not much more than that shown.

If growth is assumed to be negligible at water potentials near -5.0 MPa, as shown for the controls in 1979, and since shrubs appear to be most active in the first 5-10 days after rainfall or irrigation then, unless water potentials decline very rapidly to a low critical value for cell expansion, that value must be quite high, perhaps not much lower than for glasshouse grown plants. However, depending on conditions during previous shoot production and on the rate of dehydration after a given input of soil moisture the ability of cells to maintain turgor may vary. Direct measurements of growth following irrigation or rainfall shoud be done at different times of the year to establish the range of water potentials over which growth occurs in the field.

Further comments on the data in this chapter are made in the next chapter where the results are briefly discussed in relation to other work described in this thesis.

CHAPTER 8

8. Summary and conclusions

In the preceding chapters the main emphasis in the discussion of results has been placed on conditions during the experiment, experimental methods and on possible physiological mechanisms for the observed responses. In the following pages the results of measurements of CO_2 exchange and growth in the laboratory are compared with those on growth, water potential and the accumulation of TNC in the field. Some conclusions about the known reaction of *A. vesicaria* to heavy grazing are made from the data on the accumulation of TNC and its distribution within the shrub.

The results are also discussed in relation to the amount and pattern of rainfall which is the most important feature of the physical environment of *A. vesicaria*. Hall *et al.* (1964) suggested that there was an increase in the incidence of summer rainfall at Koonamore over the two decades preceding their study compared with that in earlier years. The possible consequences of such an increase are outlined. It is not known whether there is a definite trend toward a higher frequency of heavy summer rainfalls at Koonamore but the predictions made about the growth and survival of *A. vesicaria* in a grazing system after heavy summer rains may apply to shrubs in summer rainfall areas.

The chapter is concluded with some suggestions for further research on the physiology and ecology of *A. vesicaria*.

8.1 Rainfall and physiological activity in the field

Most of the seasonal records of xylem water potential were taken during years of average to above average rainfall and hence for much of the time were well above the lowest values recorded for *A. vesicaria* at

Koonamore and elsewhere. In the years preceding the experiments reported here, there were also some with high total rainfall. In 1974, for example, rainfalls totalling 850 mm, four times the long-term and 4.6 times the median, were recorded at Koonamore Homestead. Nevertheless, very low water potentials were developed during the summer of 1977-78 at the end of a year of below average rainfall. The sequence of wet years leading up to 1977 probably resulted in some water storage low in the profile but in the main *A. vesicaria* has limited access to water stored at depth and relies on frequent rain to maintain high water potential. Fluctuations in water potential were observed even in winter when rainfall was low.

Rehydration of *A. vesicaria* is rapid following heavy summer rains, judging by the response to irrigation, but high water potentials are not maintained for longer than a few days. For some shrubs maximum post-irrigation water potential in the field was less than that at which leaf expansion and shoot extension ceased in the glasshouse but it is evident that conditions during rehydration can markedly influence water potential; for example, when skies were overcast during the day a higher maximum water potential was reached by the following dawn. It is also likely that a direct comparison of water potential and growth in the field will show that the threshold is slightly lower for field grown shoots. The different conditions associated with irrigation and rainfall in summer also influence the rate of rehydration during subsequent rainless periods. The mean water potential of a sample of shrubs 8-10 days after a heavy summer rain was not significantly different from the peak water potential of shrubs irrigated with a similar amount of water (ca. 25 mm) at the same time of the year. The water potential of irrigated plants reached a maximum

by about day 3 and then began to decline. In the hot, dry conditions prevailing when shrubs were irrigated with less than 25 mm (fig. 7.4) water potential did not reach the threshold for shoot extension measured for glasshouse-grown seedlings.

Rainfalls greater than 25 mm are more common in summer than in winter (see Hall *et al.*, 1964) but it is unlikely that saltbush will have many opportunities for long periods of rapid growth in the warmer months. In contrast, water potentials in the field were generally much higher than the hydration compensation point for net CO_2 uptake measured for shoots in the laboratory. There were some individuals which developed very low water potentials by the end of the driest interval during this study presumably because run-off from the sites occupied by these particular shrubs was high, or perhaps because their roots were unable to penetrate a layer of limestone rubble or a hardpan lying close to the soil surface. Conditions in the field probably allow positive net CO_2 uptake by individuals on more favourable sites, in all seasons, even during years of below average rainfall. Provided that monthly rainfall during the warmer months is not substantially less than average, net photosynthesis may be reduced to zero only at the end of a sequence of dry years.

There are, of course, objections to the use of measurements made in the laboratory for predictions of physiological limits in the field. Most measurements of net CO_2 uptake at low water potential during this study were made on shoots grown under laboratory conditions. The evidence from other studies, in which net CO_2 uptake of various species in the field and controlled environments has been compared, points towards a lower hydration compensation point when plants are grown under field conditions. The only evidence to support the view that the same is true for *A. vesicaria* are the measurements of net CO_2 uptake made on shoots

of field grown cuttings, shortly after they were returned to the laboratory, before they were watered. Whereas the rates of net CO_2 uptake calculated for laboratory grown shoots of *A. vesicaria* were not significantly higher than zero at water potentials less than -9 MPa, two of the measurements made on field grown shoots at -10 MPa, or lower, were greater than zero. On one occasion a positive rate of net CO_2 uptake was recorded at a water potential less than -11 MPa. It was suggested that, with modifications to the gas exchange cuvette, positive net CO_2 uptake could also be demonstrated for laboratory grown shoots at water potentials of that magnitude. It is likely that individual shoots on shrubs in the field are capable of positive net CO_2 uptake at such low water potentials but it is possible that the hydration compensation point for the whole plant is higher than that for shoots, due to respiratory losses by non-photosynthetic tissues. However, since leaf and young stem make up by far the largest proportion of live tissue (ch. 3) the hydration compensation point for the whole plant may not be very different from that of the individual shoots.

The overall trend toward higher TNC concentration in the leaves during intervals with little or no rain (ch. 3) suggests that net CO_2 uptake continued at low water potential, but with increasing difficulty in loading the phloem (e.g. Wardlaw, 1968). Part of the increase may be due to withdrawal of carbohydrate from lower leaves before drought-induced abscission but why, if this were true, the transfer would be from leaf to leaf rather than to stem or root is not clear. Lower leaves on annual plants, at least, are thought to export metabolites primarily to the root (e.g. Canny, 1973). The rapid response of shoot growth (ch. 6) and root growth (Cowling, 1969) on rehydration of saltbush suggests that carbohydrate is required quickly at both shoot and root apices. If low water potential does affect loading of the phloem the

withdrawal of carbohydrates from older leaves, if this occurs after they develop water deficits severe enough to eventually induce leaf abscission, is presumably associated with altered membrane characteristics due either to hormones or damage.

The need for stored carbohydrate to support the rapid growth during and after rehydration is illustrated by the slow recovery of high rates of net CO_2 uptake and relatively rapid recovery of respiratory activity measured for shoots of field grown cuttings in the laboratory (ch. 5.). In this case the shoots used were grown in the field and, apart from the fact that shrubs were kept under laboratory conditions during and between measurements after they were watered, the observed response is probably similar to that following summer rains in the field. Shoot growth was so fast that, towards the end of the recovery period, a high proportion of the numbers of leaves present had developed under laboratory conditions, but the response immediately after watering, at least, is likely to be representative of normal behaviour in the field.

In summer, when mild conditions associated with rainfall do not last long, recovery may not be complete but judging by the immediate increase in photosynthetic activity observed for rehydrating shoots in the laboratory, rainfalls sufficient to induce an increase in water potential probably also result in higher rates of carbon gain, even if no structural growth occurs. If the fluctuations in TNC following heavy summer rains can be used as a guide, the photosynthetic recovery of shrubs in the field is sufficiently slow that substantial amounts of TNC are required to support growth. The high post-stress turgor potentials (ca 3.0 MPa) recorded in the laboratory suggest that leaf expansion and shoot elongation will be rapid as considerable osmotic adjustment can be expected to occur in the field during dry periods.

The decline in TNC concentration at such times may have been responsible for the eventual death of irrigated shrubs defoliated by sheep at Deniliquin, N.S.W. (Leigh and Mulham, 1971). Water equivalent to a rainfall of 75 mm every 3-4 weeks was applied to the soil around these shrubs throughout the grazing experiment. This treatment probably lowered the TNC concentration in all plant parts (cf. figs 3.4-3.6). The first water was applied two weeks before the start of the grazing experiment so that growth had probably reduced TNC concentration even before sheep were given access to the plants. Further reductions in TNC concentration, due to regrowth during the 12 days sheep were confined to the experimental areas, may have occurred leaving little substrate for regrowth after sheep were removed. However, repeated removal of regrowth by sheep may also have reduced the number of available buds. At Koonamore regrowth from epicormic buds began within two days of a mid-summer defoliation. The increasingly poor growth of regularly irrigated shrubs at Koonamore (fig. 7.6b) suggests that the large amounts of water applied to defoliated shrubs at Deniliquin may also have contributed to their death in some way.

The loss of some leaf and young stem during a dry period will not have an immediate effect on TNC concentration as regrowth is unlikely to occur when turgor potential is low. The loss, in this case, may even ameliorate the effects of drought since total water loss by the shrub may be reduced or the water supply to the remaining leaves improved. On the other hand, the loss of roots and temporary cessation of root growth observed by Hodgkinson and Baas-Becking (1977) on well watered defoliated shrubs suggests that water absorption could be adversely affected (ch. 2) although the less severe treatments, clipping for example, in that experiment did not result in much root loss. It

would be interesting to know the effect of defoliation on the growth and survival of roots in initially dry soil. Moderate grazing during dry periods may also affect shrub productivity for some time considering the seven years of reduced vigour recorded for some North American arid species by Cook and Child (1971). In *A. vesicaria* about half the TNC is located in the edible parts accessible to grazing animals.

If TNC concentration is a critical factor controlling the regrowth of saltbush grazed by sheep then the increase in occurrence of heavy rains during summer, suggested by Hall *et al.* (1964), may have unfavourable consequences for shrub survival. Sheep make most use of saltbush in the dry season. Hence given a rain heavy enough to produce growth of saltbush and a large fall in TNC concentration, especially in the root and inedible stem fractions, then continued grazing by sheep may severely restrict the ability of the shrubs to regrow if defoliation has been severe. Thus summer rains which in the absence of grazing animals might be expected to improve the overall carbon-balance of individual shrubs, due to the partial replacement of the existing leaf population with younger and perhaps more drought resistant leaves, may in fact be detrimental in a grazing system. On this basis, even without more frequent summer rains, the most critical period may be when TNC concentration is reduced dramatically after the first heavy rains at the end of the dry season, before the germination and establishment of herbaceous species which sheep prefer. The chances of shrub survival may be higher in the ensuing cooler months but shrub productivity in the longer term if it depends partly on the amount of TNC available, may be supressed as much, if not more, by grazing immediately after late autumn or early winter rains than after heavy summer rains. In winter the grazing pressure on the shrubs around some watering points may be reduced due to the growth of herbs and the formation of casual waters, allowing a wider dispersal of the flock.

The relatively high concentration of TNC in the tissues of saltbush in summer and autumn may also account for the observations on long-term changes in edible biomass by Noble (1977). On the basis of data from an analysis of photo point records for Koonamore Vegetation Reserve Noble suggested that while rain in cool seasons contributed to the growth of *A. vesicaria* the pattern of growth was essentially that of a pulse most frequently triggered by summer rains and followed by a slow decline. The pattern of growth of tagged shoots over two years in this study suggests that while summer rains initiate some growth, most occurs in late winter and spring. However, as stated by Noble (1977) the data from photo point records represented standing non-woody biomass and the estimated change between any two records may have been the sum of a number of periods of production and loss. Since estimates were made from records taken at about the same time each year, it is not possible to say with certainty when plant growth took place. Nevertheless, the peaks in biomass were built up over a period of several years in most cases, a pattern which Noble suggested was associated with the life span of individual leaf-bearing shoots. It is not clear to me whether the description given for those shoots referred to small laterals on primary branches or the primary branches themselves but a relatively high concentration of TNC in the tissues in summer may be responsible for the initiation of primary shoots from the base of the plant after summer rather than winter or spring rains. In the cooler months when shoot elongation and leaf production is rapid the demand for photosynthate from all sources by existing shoots may be high enough to inhibit the development of epicormic buds near the base of the plant. In summer, when TNC concentrations are initially higher and growth of terminal apices in full sun is restricted by falling turgor, the supply of stored photosynthate may be sufficient to allow some development of new primary branches from epicormic buds.

Initiation of buds may also be associated with higher temperatures as well as higher TNC concentration in summer. Their partial development, after initial expansion, may be facilitated by better light penetration, due to the sparser canopy in summer, and that development in turn may result in a higher demand for the available TNC in competition with existing shoots. Full development is no doubt deferred until later in the same, or succeeding years, as suggested by the growth patterns shown in Chapter 4, but nevertheless this explanation could account for the observation by Noble (1977) that long-term fluctuations in productivity are initiated by summer rains.

The only information on bud growth from this study is that after complete defoliation. Overall, bud expansion was not significantly higher in the warmer months but some bud growth was recorded on each occasion in summer and autumn. In contrast, the only times no bud activity was recorded after defoliation, for either individuals or the whole sample, were in winter and spring when TNC concentrations are relatively low.

8.3 TNC content of *A. vesicaria* and other arid zone species

In chapter 3 the TNC concentration in the tissues of *A. vesicaria* was shown to be low compared with that in various storage organs of diverse species which regularly shed much of their above-ground tissue in cold or dry seasons. Those species included herbs and drought-deciduous trees.

The most useful comparison is that with similar shrubs from an arid area. Coyne and Cook (1970) described the seasonal pattern of TNC accumulation and depletion for five shrub species, including two from the genus *Atriplex*, from a semi-arid area of northern Utah. Leaves were not analyzed for TNC. On the basis of the data presented for *Atriplex*

confertifolia and *A. nuttallii* * the main difference between these two species and *A. vesicaria* is the concentration of TNC in the roots. The roots of these species had maximum TNC concentrations between 2.5 and 3.5 times that in the roots of *A. vesicaria*. The figure for *A. nuttallii* was the highest recorded by Coyne and Cook for any of the five shrub species, all of which had higher concentrations of TNC in root tissue than did *A. vesicaria*.

The difference in allocation of TNC to roots of *A. vesicaria* and *A. confertifolia*, for example, becomes even more pronounced when root-shoot ratios are compared. The root-shoot ratio for *A. confertifolia* is about 7, whereas that for *A. vesicaria* is about 0.3 or less. The quantity of TNC stored in roots of *A. confertifolia* when both differences in dry weight and concentrations are taken into account may be 60-70 times that stored in roots of *A. vesicaria*. The greater part of the TNC in *A. confertifolia* is thus stored below ground. *A. vesicaria* on the other hand, as shown in figure 4.8b, stores most of its TNC in the leaves and a large proportion in old stem. Even if *A. confertifolia* stores little non-structural carbohydrate in its leaves it is evident that the quantity of carbohydrate stored in *A. vesicaria* is much less than that in the cold winter desert species.

The greater allocation of both structural and non-structural assimilates to the root system of *A. confertifolia* is probably also related to the climate in which it lives. Melting snow in spring, is likely to wet the soil to a much greater depth than the sporadic rainfall of warm arid areas. While extensive root systems among arid zone species are not restricted to those in cold winter areas selection for a deeper

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* Coyne and Cook used the binomial *A. falcata* in association with the common name "Nuttall saltbush". Apparently this is now a synonym for *A. nuttallii* (see p. 28 in Osmond *et al.* 1980).

root system would probably operate in a system where the soil is regularly wet to a great depth. The increase in TNC concentration of roots, observed for *A. confertifolia* is probably associated with lower air temperatures around the shoot with the approach of winter. Assimilate partitioning towards the root is increased when shoot growth is restricted by low temperatures relative to those around the roots (e.g. in maple seedlings, Richardson, 1956). While root extension of three desert species in that climate, especially *A. confertifolia*, continued for several weeks after cessation of shoot growth (Fernandez and Caldwell, 1975) the reduced rate of root growth appears to have resulted in storage of large quantities of photosynthate. As well as apparently supplying assimilates for shoot growth in spring these high concentrations may also allow increased resistance of the root system to the freezing temperatures in the upper soil layers during winter. Conversion of starch to sugars has been associated with increased frost hardiness in some plants (e.g. Siminovitch *et al.*, 1953).

A similar increase in allocation to the root system of shrubs in warm arid areas might also be expected as shoot growth is restricted by the onset of water stress, although in this case, as noted earlier carbohydrates may be retained in the leaves for other reasons.

Maximum TNC concentrations (ca 80 mg/g) in the leaves from 3 of 4 shrub species from the Colorado desert in California (Strain, 1969) were lower than those recorded for *A. vesicaria* (ca. 150 mg/g). The drought-deciduous species *Encelia ferinosa*, however, had concentrations as high as 450 mg/g in early spring. The values for these species were based on the dry weight of samples taken for starch extraction after alcohol soluble sugars had been removed. Since sugars, included in the final figure, were sometimes about 70 per cent of TNC the high values are quite large over-estimates relative to those calculated for *A. vesicaria*.

TNC concentrations for the latter were also based on dry weight consisting of up to 20 per cent salt, if it is assumed that the cation providing electrical balance for chloride ions is sodium. The use of ash-free dry weights, perhaps a more suitable figure for comparison with species which do not accumulate large amounts of salt, would increase the calculated TNC concentrations for saltbush leaf by up to 25 per cent. The percentage increase would be higher if heavier ions such as potassium were assumed to contribute to the electrical balance. Similarly TNC concentrations in the young stem fraction of *A. vesicaria* are increased by up to 11 per cent when dry weights are adjusted to account for salt content.

The greater allocation of TNC to roots of North American shrubs, at least for the cold winter species, may also be associated with the longer grazing history of animals native to the region. Individuals with most TNC above ground may have been selected against in those circumstances. Ellis *et al.* (1977) did find evidence that macropods native to Australia (kangaroos and euros) do eat flat-leaved chenopods at times, *A. vesicaria* for example, but these animals are mobile enough to seek out preferred herbaceous plants in other areas when drought reduces their availability.

The storage of high concentration of TNC in leaves of drought-deciduous species (e.g. *Encelia farinosa*, *A. vesicaria*) would also seem to be a disadvantage. Except in severe droughts, leaf fall presumably does not involve a significant loss of TNC or, alternatively, reserves stored in other plant parts are adequate to support regrowth. Withdrawal of carbohydrates from abscissing leaves may also be important. Oechel *et al.* (1972) suggested that, although *Larrea divaricata* loses most of its leaves in severe droughts, it can be considered an evergreen. In most years the species retains a high proportion of its leaves. This is also

a reasonable description of *A. vesicaria*, at least of those at Koonamore. The advantage of large stores of TNC in young shoot tissues may be that photosynthate is more accessible at times when rapid growth is possible.

Losses due to leaf fall apparently do not pose a threat to survival of shrubs protected from grazing. However, I suspect that the distribution of TNC in *A. vesicaria* is a prime factor in the response of individuals to heavy grazing. Undoubtedly shrubs such as *A. nummularia* and *Maireana sedifolia* (ch. 2) survive after complete removal of the shoot because they have adequate stores of TNC in the root system. These shrubs have extensive root systems which should allow much greater storage of TNC than does the small root system of *A. vesicaria*. The low overall content of TNC in the latter may simply reflect an ability to maintain positive net photosynthesis throughout the year.

8.4 Further research

As noted in Chapter 2 the information on TNC accumulation for species in the arid areas of North America was collected with the expressed intention of using it as a basis for management policies. The work there has been supplemented with data on the response to defoliation at different times of the year. The response of *A. vesicaria* to grazing in different circumstances has also been studied but whereas in North America work on the effect of grazing on TNC concentration has been done and related to the subsequent vigour of shrubs, little is known of physiological responses by shrubs of the Australian arid zone to the removal of young leaf and stem tissue. The information on seasonal patterns of TNC accumulation presented in this thesis provides a basis for planning grazing experiments of this kind.

It is possible that grazing experiments will show that the greatest reduction in TNC concentration and shrub vigour will occur after heavy

grazing in the wake of late autumn or summer rains. Grazing of saltbush in early spring when TNC concentration is at a minimum may also be detrimental but under normal circumstances saltbushes are not heavily grazed in the cooler months because preferred herbaceous species are available. However, the response of shrubs is likely to be complex and should be tested by clipping or grazing at various times of the year. Recommendations for management may be possible from the results of such studies, although such recommendations may be difficult to implement.

Judging by various reviews (Perry, 1967, 1974a, 1974b; Moore, 1969, Newman, 1974) the management of chenopod shrublands in the past has been relatively inflexible. The main policy has been one of year-long set stocking and the main option that of reducing or increasing stock numbers. Decisions are generally made on the basis of animal condition rather than pasture condition. This may have to change in the future, particularly if pasture continues to deteriorate, but it is probable that policy will continue to be directed by economic factors for some time. High stocking rates are often maintained despite suggestions that economic return may be higher in the long term if paddocks are stocked with a smaller number of animals. The problem is complicated by the presence of a mosaic of plant communities in most paddocks and pastures have to be managed for the conservation of the species most susceptible to grazing. The presence of such mosaics may also reduce the effectiveness of rest-rotation grazing systems if the season of defoliation is important, since some populations of susceptible species would still be exposed to grazing at critical times.

Apart from the more practical aspects of research into the problems of shrub productivity and survival in arid zone grazing systems, which are of prime importance, the broad basis of this thesis has naturally left many other questions about the biology of *A. vesicaria* unanswered and raised others.

The assembly and construction of parts of the gas exchange system described in Chapter 5 left insufficient time to explore some of the relevant questions on gas exchange of *A. vesicaria*. The possibility of a higher hydration compensation point for whole plants has already been mentioned. A large assimilation chamber designed to accommodate young shrubs was built during the course of this study but was not used. Ideally some measurements on both whole plants and shoots should be done in the field although this approach is both difficult and costly.

The response to dehydration at higher leaf temperatures would be of interest as increases in respiration at higher temperatures may cause a more rapid decline in the rate of net photosynthesis (Depuit, 1979) unless substantial temperature acclimation occurs (Strain, 1969).

Since xylem water potentials are more often maintained at higher values than the severe stresses imposed during this study the response of net CO_2 uptake to rehydration at higher minimum water potentials may help establish the level of stress at which the after-effect becomes apparent. *A. vesicaria* has several different forms and hence a comparison of the physiology of two or more of these would be useful. A comparison of CO_2 exchange measurements during drying cycles would serve the purpose of checking the results reported here and, if combined with measurements of stomatal resistance and transpiration, may allow some insight into the reasons for the relatively slow recovery of net CO_2 uptake on rehydration.

Some hypotheses on net CO_2 uptake at low water potential formulated before experimental work was begun were also left untested. One of these was the possibility that leaf abscission in drought-deciduous species, as well as reducing overall water loss and postponing stress in the younger leaves, could result in some increase in net CO_2 uptake by the

remaining leaves. I was led to considering this possibility by the work of Wareing *et al.* (1968) who concluded that the observed increases in net photosynthetic rate of maize and bean plants, partially defoliated by the removal of lower leaves, was due to the effects of an improvement in the cytokinin supply on carboxylation capacity in the remaining leaves. The roots of droughted plants supply less cytokinin to leaves (Ben Zioni *et al.*, 1967; Itai and Vaadia, 1971) and hence the reduction in the number of sinks for this metabolite, by leaf fall in water-stressed saltbush, may contribute to the maintenance of positive net photosynthesis at low water potential.

A further possibility for research arose during the discussion of turgor maintenance in Chapter 6. It was suggested, as an extension to the theory that ion uptake is controlled partly by pressure on the plasmalemma (Zimmermann,1978), that if cell walls contract as leaves of *A. vesicaria* shrink with declining water potential, then some turgor maintenance by this means may allow a longer time for maintenance of turgor by osmotic adjustment. I suspect, however, having considered ways in which this might be explored that the hypothesis is untestable. An examination of the environmental and internal controls of the reversible changes in leaf area for *A. vesicaria*, and perhaps other species, on the other hand, is warranted.

One of the reasons for selecting both male and female shrubs for studies of shoot elongation, TNC concentration and water potential in the field was the possibility of differences between the two. No significant differences were found. However, Freeman *et al.* (1976) found significant differences between the number of males and females of *Atriplex confertifolia*, and several other dioecious species, on dry and moist sites in Northern Utah, with more males occupying drier sites. Given that such a distribution occurs on the spatially heterogeneous sites occupied by

A. vesicaria it is possible that selection of greater drought hardiness has occurred in male populations. Experiments designed specifically to test this may establish such differences.

These aspects of gas exchange and growth all relate to the survival and productivity of *A. vesicaria* to some extent and are of considerable interest in themselves but the details of mechanisms behind some of the observed responses are unlikely to add much to the management of shrub populations. One of the main issues in this thesis is the role of stored carbohydrate in growth either after defoliation or a period of water stress. Most of the discussion of this subject is based on the assumption that fluctuations in TNC imply their direct use at the growing point. This may be a reasonable conclusion from the correlations between changes in TNC concentration, vigour or individual growth events but it is nevertheless an assumption. Perhaps one of the most important areas of research, apart from the more practical aspects of physiological responses to grazing mentioned earlier, is the study of patterns of movement and reallocation of stored photosynthate and its incorporation into structural or metabolic components of new growth. It is likely that such studies on *A. vesicaria* will show that TNC is used at the growing point, as shown for alfalfa by Smith and Marten (1970).

On this basis the ability of *A. vesicaria* to photosynthesize at low water potential is important in that as well as maintaining the plant during dry periods it also results in the accumulation of TNC which can be used to support growth after summer rains when photosynthetic rates are initially low. The consequence of TNC depletion during summer growth is that any removal of existing or new growth by grazing animals will reduce the shrub's capacity to replace TNC stores. Since shrubs apparently use stored carbohydrate throughout the winter growth period, if stores of TNC have not been replaced before the end of the dry season regrowth in winter will be slow and shrub vigour may decline.

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