DOCTOR OF PHILOSOPHY

Ecophysiology of ecotypes : An investigation in the ecophysiology of sand dune ecotypes

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Award date:
1983

Awarding institution:
Bangor University

ECOPHYSIOLOGY OF ECOTYPES:

AN INVESTIGATION IN THE ECOPHYSIOLOGY OF

SAND DUNE ECOTYPES

A Thesis

presented for the degree of

Philosophiae Doctor

in the University of Wales

by

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To my father...

ACKNOWLEDGEMENTS

Grateful thanks are due to Professor J.L. Harper for providing facilities in the School of Plant Biology to carry out this research.

I am greatly indebted to my supervisor, Dr John F. Farrar, for his interest, constant encouragement, enthusiasm and invaluable guidance during the course of this work, and for his help in writing and presenting the publication appended.

I would also like to thank the technical staff of Pen-y-Ffridd Field Station and the School of Plant Biology for their willing help, particularly Mr E. Williams and his expert son Mr G. Williams.

Thanks also go to Mr C. Whittaker for advice on statistical analysis of the data.

I wish to thank the British Council and the Government of Sudan for providing the scholarship. Many thanks go to Mrs E.M. Davies for her patience and skill in typing this thesis.

Lastly I am deeply indebted to my father, without whose encouragement throughout my life this would never have been.

ABSTRACT

Within populations of Trifolium repens L. and Festuca rubra L. growing on the sand dune system at Aberffraw, Anglesey, Trifolium repens shows ecotypes differentiated by their response to soil water status, but Festuca rubra does not. All populations of F. rubra grew best with moderate, and were adversely affected by both high and low, soil moisture status. Populations of T. repens showed a site-specific growth response; plants from 'dry' habitats were least affected by low soil moisture levels, whereas those from 'wet' habitats were quite adversely affected by low soil moisture status. Such differential response suggested the possible existence of these populations as ecotypes. More evidence was supplied by the reciprocal growth of plants from wet and dry sites on both sites in the field; each performed better when grown on its original site.

Physiological differences between ecotypes of T. repens at -1.0 MPa in the rooting medium, provided by solutions of polyethylene glycol (m.w.4000), were investigated. Plants from the wet site were not able to withstand such low water potential, and steadily their pressure potentials decreased, they lost turgor and wilted. Plants from the dry site showed their ability to keep their pressure potentials constant and thus maintain turgor, as their water potentials dropped. This ability of turgor maintenance was shown to be accomplished by osmotic adjustment through solute accumulation. Plants mainly accumulated K^+, Na^+, and the sugars sucrose, glucose, and fructose. However, low osmotic potentials in plants from the wet site were only due to tissue dehydration and consequent concentration of solutes in the cells.

Possibly as a consequence of turgor maintenance in plants from the

dry site, their stomatal resistance did not increase substantially as did that of plants from the wet site at low water potential, and therefore they were able to maintain relatively higher rates of net photosynthesis.

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INTRODUCTION

INTRODUCTION

Tolerance by a species of wide edaphic variability could be due either to individuals exhibiting extreme physiological tolerance or to the existence within a species of separate, genetically differentiated, physiological strains, each of which grows well on a limited range of soil conditions (Snaydon, 1962a). Such strains are termed ecotypes.

Sand dune systems contain a wide variety of soil types, especially in relation to moisture content (Salisbury, 1952; Onyekwelu, 1972; Pemadasa, 1973). The work undertaken in this study is designed to look for the existence of ecotypes; to investigate the differential responses of the plants growing in contrasting microhabitats of the sand dune system; and to see if it is possible to characterise ecotypes at the physiological level. This should provide insights into the mechanisms by which plants tolerate the environmental variables in response to which the ecotypes have been selected.

Ecotypes

Definition and mechanism of differentiation

Ecotypes are ecological or climatic races within a single species which have arisen by natural selection by distinct combinations of environmental factors (Hiesey and Millner, 1965). Valentine (1949) defined them as "groups forming genetically distinct components of species, adapted to special types of environment and capable of unlimited gene exchange". Hence they are interfertile and can exchange gentic material in crossing (Cooper, 1959). Variation, however, could be determined either by genetically controlled physiological differences (Snaydon, 1962a, 1970; Snaydon and Bradshaw, 1961) or by contrasting

environmental conditions prevailing in different habitats in which the species naturally occur. For example, the dwarfness of Plantago coronopus in dune habitats is a phenotypic effect caused by nutrient deficiency (Salisbury, 1952; Onyekwelu, 1966). Alternatively, the occurence of genetically controlled intraspecific variation can result in ecotypic differentiation. Cooper (1963) demonstrated a multiple-gene inheritance of most characters that distinguish ecotypes, for which Mather (1941) had developed the theoretical basis.

Ecological significance of ecotypes

The ecotypes most frequently described in the literature are those which occur along latitudinal and altitudinal gradients (Barber, 1955; Callaham and Liddicoet, 1961; Mooney and Billings, 1961). Bradshaw (1959) and Briggs (1962) had shown clearly recognizable races within a species occuring in strictly local areas characterised by differences in substrate, topography or microclimate. Snaydon (1962a,b) and Snaydon and Bradshaw (1961), showed the existence of edaphic ecotypes of Trifolium repens growing in single pastures in North Wales.

Ecotypes are best identified by their differential growth and development when grown under identical conditions (Hiesey, 1953; Irgens-Moller, 1957). They may, for example, be distinguished by their differential responses to water supply (Hiesey and Millner, 1965). Slatyer (1963) examined three species differing in their capacity to withstand drought and showed that they differed in the degree of turgidity their leaves displayed when all were grown at the same, low,water potentials. He showed that two of these species were only killed at water potentials much lower than those the third could withstand.

McCormick and Platt (1964) correlated the heat and drought resistance of Diamorpha cymosa ecotypes with differences in the seasonal rainfall patterns of their native habitats. Blum (1974) found that one of the two genotypes of sorghum he examined was able to maintain high rates of soil water extraction at lower soil moisture content than the other.

Ecotypes of Trifolium repens growing at different altitudes showed differential growth responses to light intensity (Mächler and Nösberger, 1977). They also showed different photosynthetic rates when grown at the same temperature (Mächler et al, 1977). The rate of photosynthesis is arguably the most crucial physiological process in a plant since it determines the ability of a plant to develop and grow (Hiesey and Millner, 1965). It is closely dependent on the supply of water to the plant as well as other factors such as light, temperature, CO_2 concentration and nutrient supply. Change in any of these factors would be expected to elicit differential response from ecotypes (Mooney and Billings, 1961).

The Sand Dune System

On coastal areas, sand dune systems are mainly from sand wind-blown from beaches, which then accumulates around obstacles such as plants. This accumulation is enhanced and established by more plant growth. Eventually, under effects of wind which can include the movement of sand dunes, a complex system of dune ridges will develop, separated by low areas or slacks on which can be superimposed low sandy hillocks. Plant cover is very important on sand dune systems, since it reduces the effect of wind on sand and therefore leads to more stable dunes. That is why grazing by rabbits and trampling by man and animals could lead to the removal of plant cover, exposing the sand to the effect of

wind. Plant cover also provides the soil with organic material. Hence slacks and old dunes are richer in organic material than young ones (Salisbury, 1952). Addition of organic material could also decrease the mobility of surface sand grains.

Water relations of sand dune systems

The main input of fresh water in the dune system is rainfall. However, Salisbury (1952) showed that surface soil, which in summer experiences high temperatures and conditions favouring rapid evaporation, could have a supplementary supply of water from internal dew formation, by distillation of water vapour carried by damp air moving up from deeper parts of the dune, or could be brought about by the humid air from above the sea passing into pores in the soil and condensing as it contacts cold sand grains inside the dune (Hill and Hanly, 1914). Salisbury (1952) had concluded that at times of long spells of drought, it is that part obtained by dew formation within the dune that plays an important part in sustaining plant life on the dunes, since roots cannot obtain water from the water table by capillary forces.
In the dune slacks and low dunes, moisture can be rendered available around plant roots by capillary forces. Water tables fluctuate with the tides (Hill and Hanly, 1914), and at times they could bring water to within reach of roots of plants in the slacks, and at times may cause flooding in the slacks (Onyekwelu, 1966). The fluctuations of the water tables also influence the rate of drainage of rainwater from the surface (Willis et al, 1959a). Therefore the water regime of dune soil is subject to frequent violent fluctuations.

The poor water retention by sand dune soil is improved by the addition of organic matter, which increases its water-holding capacity and decreases its liability to extreme dryness (Downs and Hellmers, 1975).

The sand dunes are considered as temperate deserts (Salisbury, 1952) whose drought conditions are not caused by climatic factors like true deserts, but due to the poor ability of sand to retain water. They have also been called "edaphic deserts". Moreover there is a large contrast between the slack and dune habitats in their soil water regimes (Jones, 1971).

The effect of the dune system environment on vegetation

Shortage of available water in dune soils is a primary factor in limiting the vegetation that can grow successfully on them (Ranwell, 1972). It had been reported that some dune plants undergo daily wilting in dry weather (Oosting, 1954). The contrast in moisture content between dune soil and that of the slacks is greatest during the summer months.

The particular importance of the soil moisture regime in determining the variation of vegetation within the dune habitat has been stressed by several workers (Willis *et al*, 1959a,b; Onyekwelu, 1966; Pemadasa, 1973). Some plant species such as *Juncus articulatus* L. are confined to sites subject to flooding whereas others such as *Ammophila arenaria* (L.) Link. and *Ononis repens* L. are almost entirely restricted to sites which are dry. Other species extend both into wet zones and some distance up the dunes; these include *Festuca rubra* L., *Trifolium repens* L., *Carex arenaria* L., and *Agrostis stolonifera* L.

Plants growing on sand dunes are exposed to a complex of adverse conditions, which they must be able to tolerate, or to avoid like annuals which complete their life cycle before the advent of the dry season and succulents that store water in order to use at time of shortage. Plants can tolerate drought by means of morphological and physiological modifications which could reduce water loss and increase water uptake, such as deep root system, thick cuticle, responsive stomata · or tolerance of some degree of cellular dehydration without injury (Kramer, 1980).

Water Relations

A consistent and widely applicable terminology describing the water status in plants and soil is that of water potential. Total water potential, Ψ, has been considered lately as the best measure of the water status in the plant (Kramer, 1974). The water potential describes the state of water by its chemical potential within the system relative to that of pure water. It gives a measure of the capacity of water at any point to do work (Slatyer and Taylor, 1960). The total water potential is composed of the following components:

$$\Psi = \Psi_s + \Psi_p + \Psi_m$$

Ψ_s is the osmotic potential resulting from dissolved solutes.
Ψ_p is the pressure or turgor potential arising from hydrostatic forces.
Ψ_m is the matric potential due to surface forces.

Leaves are often the part of the plant most sensitive to low water potentials, so they serve as good indicators of the water status of the plant, and often leaf water potential Ψ_l is used in the literature.

A good understanding of the water relations of a plant under water deficit can be made by measuring both the total water potential and the osmotic potential and then calculating the pressure potential (actually, pressure plus matric potential).

Soil water potential Ψ_{soil} also gives a good measure of the state of soil moisture conditions.

The unit used here is the SI unit of pressure, the Pascal; 1MPa (= 10 bars) is of a convenient magnitude.

Alternatively, the status of tissue water can be described in terms of the water content of the plant relative to the saturated water content, which is called the relative water content RWC :

$$\text{Relative water content} = \frac{\text{fresh weight - dry weight}}{\text{turgid weight - dry weight}} \times 100$$

(Weatherley, 1950).

Plants and low water potentials

Plants originally evolved in the medium of water; their life is totally dependent on it for function and survival. The roles of water in the plant include being a reactant, serving as a medium for ionization of metabolites, stabilization of membranes, and the maintenance of the turgidity of cells and hence the structure of the plant. It is also known that the removal of only 10-15% of the water held in the plant tissue at full turgor could clearly affect its metabolism (Hsiao *et al*, 1976). Thus reduced water availability which is frequently encountered by plants, could have great effects on all phases of plant growth, from the seedling to the mature plant (Slatyer, 1967). Low water status also has a profound influence on plant metabolism from the subcellular to the plant organ level (Hsiao, 1973).

Effect of low water potentials on plants

The many effects of low water potentials are reflected in the growth of the plant through decreased accumulation of dry matter, decreased extension growth and changes in morphology such as a decreased leaf area and increased root:shoot ratio (Hsiao, 1973; Begg and Turner, 1976).

The overall effect of low water status on plant growth is a reduction in total dry matter. This could be very severe as was reported by Kaul (1966) for wheat, oats and barley when grown in high concentrations of polyethylene glycol, and by Lawlor (1969) who found that total dry weight of ryegrass, maize, bean and cotton decreased with decreasing osmotic potential of the growth medium. Belesky et al (1982) found that four fescue cultivars showed reduced biomass at reduced water potentials. Extension growth is reduced by low water potential through its dependence on water flux into expanding cells in the zone of elongation (Wolf and Parrish, 1982).

Shields (1950) had suggested that a reduction in leaf growth as a response to drought conditions could reduce the total transpiring surfaces of the plant provided leaf number is not increased. Begg and Turner (1976) considered that in addition to some reduction of effective leaf area at low water potentials by reduction of leaf expansion, there is also accelerated senescence or rolling and flagging of leaves when wilted. Accelerated senescence at low water potentials will lead to a reduction in the number of green leaves per plant (Boyer and McPherson, 1975), which in turn will lead to a reduction in total production of dry matter (Slatyer, 1973). Begg and Turner (1976) concluded that reduction in leaf area under low water supply is the most important consequence of the sensitivity of cell enlargement to water deficits. Low leaf water potential influences leaf production

through its effect on leaf initiation in meristems and subsequent rates of cell division (Boyer and McPherson, 1975), therefore causing a decrease in the rate of production of new leaf area. However, leaf initiation could cease altogether at low water potentials (Husain and Aspinall, 1970). A slower rate of leaf enlargement was shown for maize and soybean by reduction in leaf water potential to values below -0.2MPa, and growth halted at a leaf water potential of -0.7MPa in maize (Acevedo et al, 1971) and -1.2MPa in soybean (Boyer, 1970a). It is interesting to note that in the two previous cases big reductions in leaf enlargement compared with the controls took place whilst photosynthesis was not affected, thus reflecting the sensitivity of cell expansion to a fall in water potential. Even in well watered plants, leaf water potential drops significantly at mid-day, and cell enlargement is stopped, but enlargement can continue during the night (Boyer, 1968). Lawlor (1969) examined the effect of low water potentials on specific leaf area (leaf area per unit leaf dry weight), which reflects the distribution of dry matter in the plant and found that it was usually decreased at low water potentials. Fereres et al (1978) showed a similar response in sorghum plants which were left unirrigated and then measured after 90 days. However, Watts (1974) showed little effect on leaf extension in maize at leaf water potentials down to -0.8 to -0.9MPa. Furthermore, a decline in leaf water potential down to -1.3MPa did not affect total leaf extension in sorghum (McCree and Davis, 1974; Chu and Kerr, 1977) but leaf extension was reduced at lower water potentials and stopped at -1.7MPa. McCree and Davis (1974) also indicated that cell division is as important as cell expansion in determining leaf extension. Therefore the exact water potential that results in a reduction in growth varies with the condition under which the plant is grown.

However, Hsiao and Acevedo (1974) suggested that the primary effect of low water supply on plant growth appears to be physical, through the loss of turgor. Turgor pressure sustains plant structure (Hsiao, 1973). Controls gas-exchange through the stomata (Meidner and Mansfield, 1968) and may regulate certain metabolic events in the plant (Hanson and Hitz, 1982). Lawlor (1969) showed that the growth of all species he used was affected more severely by a unit decrease of turgor potential than by one of osmotic or total water potentials, indicating that loss of turgor is particularly damaging. The same author (Lawlor, 1969) concluded that decreased turgor caused a reduction in cell expansion, which he considered to be the probable cause of leaf area reduction under water deficits. Hsiao and Acevedo (1974) noticed that changes in the water potential of the culture medium would cause nearly instant changes in leaf expansion, and they concluded that such changes were too rapid to be mediated metabolically and could only be explained in terms of turgor potential. In young maize leaves which had been exposed to mild water deficit, the very rapid resumption of elongation after rewatering indicates that only lack of turgor prevented expansion (Acevedo et al, 1971). Ordin (1960) found that decrease in turgor limited cellulose synthesis, thus causing reduced cell wall growth, an effect that paralleled reduction in cell elongation. Therefore, it is reduced turgor potential that affects cell enlargement during developing water deficits in plants.

Since at low turgor cell enlargement is reduced, meristematic cells would not expand to the size required before further division (Barlow et al, 1980). Therefore a reduction in rate of cell division can be a consequence of decreased cell expansion caused by low turgor (Gardner and Nieman, 1964; Hsiao, 1973). Munns et al (1979) showed that a decline in turgor potential from 0.45 to 0.15 MPa caused a

cessation of shoot apex elongation in wheat plants. However, McCree and Davis (1974) concluded that reduced cell division rate is more crucial. to plant growth than decreased cell enlargement under plant water deficits, since only the latter could be restored after rewatering and resumption of growth.

Turgor in the guard and subsidiary cells controls stomatal opening and closure. Therefore water deficits causing turgor potential difference changes would lead to loss of turgor in the guard cells and subsequent stomatal closure (Jarvis, 1980), thus resulting in a decrease in photosynthetic rates. Hanson and Hitz (1982) suggested that turgor potential reduction is the factor affecting chloroplast activity observed during developing water deficits in plants.

Low osmotic potentials induced by plant water deficits may produce changes in enzymatic activity, since they induce conformational changes in proteins (Crafts, 1968). Such an effect might be attributed to the concentration of solutes at low water potentials, but Plaut (1971) showed no inhibition of isolated enzymes of the photosynthetic carbon reduction cycle by -1.2MPa osmotic potential. However, he observed inhibition of these enzymes when assayed in isolated but intact chloroplasts at the same osmotic potential. Potter and Boyer, 1973, showed only 5% reduction of isolated chloroplast activity when the osmotic potential was lowered from -0.8 to -1.2MPa, whereas the same decrease in osmotic potential during desiccation <u>in vivo</u> was accompanied by an inhibition of chloroplast activity of 33%. Low osmotic potentials may be quite beneficial in some plants since they maintain turgor potential in the tissue as the water potential declines.

It was mentioned above that a reduction of leaf water potential to values below -0.4 to -0.6MPa brings about cessation of cell enlargement and growth in some species (Boyer, 1970; Acevedo et al, 1971). However, in other species some leaves may almost always have a water potential below -0.4 to -0.6MPa, and these plants must behave differently if cell enlargement and growth are not to be affected (Boyer, 1976). Meyer and Boyer (1972) suggested that solutes accumulate in enlarging cells, lowering solute potential and hence maintaining turgor, as a means of permitting cell enlargement under dry conditions. This phenomenon of turgor maintenance, through solute accumulation in cells resulting in lowering of osmotic potentials, is one of the most important features of plants growing in dry habitats. It is discussed below in more detail.

Effect of plant water deficits on root:shoot ratio

Root:shoot ratio tends to increase with decrease in soil moisture (Harris, 1914; Kaul, 1966; Pearson, 1966; El Nadi et al, 1969). Gwendolyn and Bray(1970) found that for plants growing on both dry and moist soils the root:shoot ratio tends to increase with increasing dryness of the soil. Sharp and Davies (1979) suggested that a high root:shoot ratio is a very important feature of plants growing in dry habitats. It increases plant access to soil water (Hoffman et al, 1971; Caldwell, 1976). Absolute increases in root growth caused by low water potentials, resulting in a higher root:shoot ratio, had been reported by Bennett and Doss (1960), Hsiao and Acevedo (1974), and Sharp and Davies (1979). The increased root growth under low water potentials may be due to their capacity to adjust osmotically (see below) under mild water deficits (Sharp and Davies, 1979). Stomata may remain open, and photosynthesis may continue whilst shoot growth is reduced due to

reduced turgor. Then the increased supply of assimilates made available by the reduced strength of the sink in the shoot may permit osmotic adjustments in the roots. Extra root growth will then follow (Hsiao and Acevedo, 1974), enabling the exploration of more soil such that more soil water will become available to the plant. Perennial grasses and shrubs of dry regions generally have root:shoot ratios above 1, higher than those of plants from humid areas (Oppenheimer, 1960).

It has been mentioned above that low water potentials induce an acceleration in leaf senescence (Boyer and McPherson, 1975), resulting in a decrease in the shoot dry weight. This shoot dry weight decrease, rather than an absolute increase in root growth, was suggested by Lawlor (1969) to be responsible for the increase in the root:shoot ratio of maize, cotton, bean and ryegrass.

Processes affected by low water potentials

New Leaf Area

Low soil water potentials reduces the number, rate of expansion, and final size of leaves (Zahner, 1968). As a result production of new leaf area will be greatly reduced.

Photosynthesis

Inhibition of photosynthesis under low water potentials was reported by Kozlowski (1949), Ashton (1956), and by more recent workers like El-Sharkawy and Hesketh (1964), Strain (1970), and Bazzaz (1974). If plants are to grow when exposed to low water potentials they must maintain reasonable rates of photosynthesis. Since the stomata control both water and CO_2 exchange, reduction of water loss will also reduce CO_2 uptake, but owing to the difference in lengths in the diffusion pathways of

CO_2 and water, water loss will be more affected by stomatal closure than CO_2 uptake (Meidner and Manfield, 1968). Thus photosynthesis may decline more slowly than transpiration, and this is considered to be of great importance for dry matter production, and hence competition and survival under drought conditions (Crafts, 1968). Studying the rate of photo-synthesis of leaves at low water potentials simultaneously with the rate of transpiration (or the diffusion resistance to water loss), Brix (1962), and Willis and Balasubramaniam (1968) found that the stomata are likely to exert the major control over photosynthesis at low water potentials. Brix (1962) found that the net rate of photosynthesis decreased at -0.4 and -0.7 MPa and ceased at -1.1 and -1.4 MPa for loblolly pine and tomato respectively; the pressure potentials were not shown. Data of Willis and Balasubramaniam (1968) showed significant changes in leaf diffusive resistance before changes in photosynthesis occurred during the early part of desiccation and recovery; relevant water and pressure potentials were not quoted. Barrs (1968) found that the stomata accounted for virtually all the effects of desiccation on photosynthesis.

However, Boyer (1976) argued that stomatal closure may not exert complete control over photosynthesis. Involvement of a non-stomatal factor in the reduction of photosynthesis at low soil moisture levels was also suggested by Shimshi (1963a,b). This factor, synonomous with the mesophyll resistance of Gaastra (1959), increases with developing water deficits. Boyer (1970b) and Hansen (1971) came to the same conclusion. Ackerson et al (1977a) showed maximum photosynthetic rates in cotton plants at -1.2 MPa; these decreased at lower water potentials reaching a minimum rate at about -2.8 MPa since stomatal aperture stayed virtually constant over that range of water potential, they attributed the effect on photosynthesis to decreased rates of the Hill reaction,

and of translocation. Graziani and Livne (1971) removed the epidermis from tobacco leaves and noticed then considerable loss in photosynthesis during severe desiccation. There is clearly a significant change of photosynthetic activity at low water potential, not attributable to stomatal closure. It is then expected that changes take place in the liquid phase pathway and at the site of CO_2 fixation at low water potentials. Boyer (1971) identified an inhibition of chloroplast activity in sunflower at low water potential as a cause of reduced photosynthesis, as reported by Heichel and Musgrave in maize (1970) and Redshaw and Meidner (1972) in tobacco leaves. Todd and Basler (1965) suggested that the Hill activity in isolated chloroplasts was affected by desiccation. Nir and Poljakoff-Mayber (1967) showed that both Hill reaction and cyclic photophosphorylation were inhibited when chloroplasts were isolated from leaves that had previously been severely desiccated. Leaf water potentials below -0.8 MPa limited photosynthesis in sunflower by an inhibition of chloroplast electron transport (Boyer, 1971; Boyer, 1976). Limitation on photosynthesis may shift from electron transport to photophosphorylation when sunflower leaves reach water potentials of -1.7 MPa and below (Keck and Boyer, 1974). Leaf desiccation also alters the activity of enzymes involved in the dark reactions of photosynthesis (Plaut, 1971).

At least in some species, when water is deficient enough to cause stomatal closure, the increase in stomatal resistance is commonly accompanied by an increase in mesophyll resistance (Redshaw and Meidner, 1972). It is sometimes argued that even if mesophyll resistance to CO_2 uptake is substantial then, at times of water deficits, the same increase in epidermal resistance for water and CO_2 would affect transpiration more than photosynthesis simply because epidermal resistance accounts for

a smaller portion of the total resistance to CO_2 than to water (Slatyer, 1970). However, higher photosynthetic rates coupled with low transpiration can only be achieved if the stomatal resistance is high and the mesophyll resistance is low; this is clearly a desirable feature in drought resistance (Slatyer, 1970; Gifford, 1974).

Variations in the effect of low water potentials on photosynthesis within the same species was noted by Heichel and Musgrave (1970) in different maize varieties, and by Blum (1974) in different sorghum genotypes.

Ways of maintaining metabolism at low soil water potentials

Stomatal closure

One mechanism for regulating water loss and reducing development of low leaf water potential is stomatal closure (Waggoner et al, 1964). Usually stomata remain unaffected until the leaf pressure, or water, potential drops to some critical threshold value (Hsiao, 1973). This threshold value usually differs between species and also depends on the growing conditions (Brown, 1974; Davies, 1977).

Stomatal closure by low water potentials is not simply due to an overall loss of turgor from the leaf. Rather it appears to involve a loss of solutes (mainly K^+) from the guard cells which then result in a selective reduction in guard cell turgor (Stålfelt, 1955; Hsiao, 1973). This mechanism ensures that stomata close before the onset of water deficits in the remainder of the leaf, thereby preventing deleterious deficits from developing (Ludlow, 1980). This may take place diurnally or in response to a drop in atmospheric humidity.

Osmotic adjustment or osmoregulation

This is defined as the regulation of osmotic potential within the cell by addition or removal of solutes from solution until the intracellular osmotic potential approximately equals that of the surrounding medium (Borowitska, 1981).

Considering the water potential components:

$$\Psi = \Psi_s + \Psi_p + \Psi_m$$

as cell water potential drops in response to lowered external water potential, the components Ψ_s and Ψ_m must be lowered if Ψ_p is to be kept constant. Since Ψ_m remains insignificant down to very low water potentials, alteration must occur in Ψ_s. This could take place either by dehydration of the cell due to water loss (Osonubi and Davies, 1978) or by positive accumulation of solutes by uptake or internal production of osmotically active substances (Hsiao et al, 1976).

When a plant has a high concentration of solutes, the removal of a small amount of water would cause a large decrease in solute potential Ψ_s, since

$$\Delta \Psi_s = -RTN_s/\Delta Vw$$

(R = gas constant; T = absolute temperature;

N_s = number of moles of solute; ΔVw = change in volume of water)

thus the more solutes the cell contains, the larger N_s, and the greater is the effect of water removal on the value of Ψ_s and on cell water potential (Hsiao and Acevedo, 1974).

Evidence for osmotic adjustment

The role played by solute accumulation in its maintenance of turgor and growth was first recognized by Pfeffer (1877) in his investigations

of osmotic potential. Osmoregulation has long been recognized in halophytes (Bernstein, 1961) but lately its role in mesophytes has also been appreciated (Hsiao, 1973; Begg and Turner, 1976). Seasonal drought-induced and diurnal osmotic adjustment in leaves takes place, helping to maintain turgor and thus sustain growth despite fluctuations in water potential (Fereres et al, 1978).

Seasonal osmotic adjustments

Osmotic potentials as low as -9.2 MPa are reported for Artemisia herba-alba leaves growing in the Negev desert, in the middle of summer (Kappen et al, 1972). Moore et al (1972) reported that the halophyte Atriplex confertifolia showed a seasonal osmotic adjustment with its osmotic potential decreasing from -5.0 MPa at the beginning of summer to -20.0 MPa in midsummer. Further, apple trees decrease their leaf osmotic potentials by 0.5 MPa when left unirrigated from July to September (Goode and Higgs, 1975). One of the most striking examples is that of sorghum (Fereres et al, 1978) which maintains its turgor pressure by dropping its osmotic potential from -1.4 to -2.0 MPa from the time of panicle initiation to maturity while the plants are unirrigated; this is due to solute accumulation. In a review, Walter and Stadlemann (1974) concluded that some plant species had up to a 3-fold decrease in their osmotic potential in the dry season as compared to the wet one.

Diurnal osmotic adjustment

Leaf water potential decreases during the day reaching minimum values at mid-day, and then starts to rise in the afternoon reaching

maximum values before dawn. Such oscillation of osmotic potential was shown by Acevedo et al (1979) in maize leaves, with the osmotic potential reaching a minimum value 2 hours after the leaf water potential minimum, thus giving higher values of pressure potential than would be caused by simultaneous changes in solute and water potential. They concluded that the drop in osmotic potential was mainly due to accumulation of soluble sugars. Ackerson et al (1977b) observed diurnal changes in leaf osmotic potential similar to those in water potential, such that turgor was maintained in field grown sorghum and cotton. Davies and Lakso (1979) showed the ability of apple trees to lower their leaf osmotic potentials by as much as 1.65 MPa during the day, partly due to dehydration and partly due to osmotic adjustment, with consequent maintenance of turgor.

Experimental evidence for osmotic adjustment

Many workers have shown evidence for osmotic adjustment in plants experimentally subjected to low water potentials (see Hsiao, 1973; Begg and Turner, 1976). Janes (1966) reported osmotic adjustment in leaves of pepper and beans grown in polyethylene glycol. Chu et al (1976) working on barley noticed osmoregulation with the water potential dropping to around -1.8 MPa and osmotic potential down to around -2.4 MPa, when plants were grown in NaCl at about -1.0 MPa. In experiments where the low water potential is developed by decreasing the osmotic potential of the growth medium, osmotic adjustment only takes place when low water potential is imposed gradually (Janes, 1961). Indeed, in nature drought often develops gradually. Meyer and Boyer (1972) showed that when low water potential was imposed over 24 hours, soybean hypocotyls lowered their osmotic potentials by 0.5 MPa and maintained turgor. However, when the same water potential was imposed by applying pressure to the hypocotyls, little

osmotic adjustment took place. When they removed the cotyledons, which were the site of solute synthesis, negligible osmotic adjustment took place. Morgan (1977a) showed evidence for osmotic adjustment in some wheat genotypes which were capable of maintaining constant turgor by dropping their osmotic potentials from initial values around -1.5 MPa down to -3.0 to -3.5 MPa, whilst the leaf water potential fell to about -1.5 MPa. Osmotic adjustment was also reported by Jones and Turner (1978), in leaves of sorghum which were left unwatered, and in expanding and fully expanded leaves of sunflower which were subjected to low water potentials (Jones and Turner, 1980).

Substances used in osmotic adjustment

Inorganic solutes

Osmotic adjustment could be accomplished by accumulation of inorganic solutes such as Na^+, K^+ and anions. In some marine algal cells osmoregulation is preferentially regulated by K^+ and Cl^-, the main cation and anion in the vacuole, whilst others have Na^+ instead of K^+ (Zimmermann, 1978). Also measures of K^+ showed its involvement in osmoregulation of bacteria growing in saline media (Measures, 1975). However, osmotic adjustment in higher plants in saline media is well documented. Many plants adjust at least partly by the uptake of solute from the media (Slatyer, 1961). The solutes accumulated will depend on the composition of the external solution. K^+ accumulation can be very marked; Na^+ and Cl^- are also responsible for lowering osmotic pressure, as concluded by Bernstein (1963) after finding that K^+ accounted for most of the diurnal change in osmotic potential in beans growing in saline media. He also found in another study (Bernstein, 1961) that an initial adjustment of

osmotic pressure under low water potentials was an accumulation of K^+ and change in organic acid content, followed by accumulation of NaCl. However, Gutknecht (1968) showed that net K^+ influx is greatly increased by the lowering of turgor, and reduced when turgor is maintained. Janes (1966) reported that a decrease in the osmotic potential of expressed juice of pepper and beans leaves was mainly due to an accumulation of inorganic solutes, namely K^+, Na^+, Ca^{2+}, and Cl^-. These accounted for 50 - 75% of the total decrease in Ψ_s.

Organic solutes

Some organisms osmoregulate by the accumulation of metabolites; for example, Chlorella pyrenoidosa increased its sucrose content when in a medium at -1.0 MPa (Hiller and Greenway, 1968). Iljin (1957) showed sugar accumulation in plants at low water potentials, and considered that low water potential causes polymers such as starch to be converted into more osmotically active substances such as sugars. Supporting this Henckel (1964) mentioned that dehydration of plants under drought conditions causes hydrolysis of starch. Prasad et al (1982) noticed that in barley plants on polyethylene glycol solutions, glucose concentration changed much more than sucrose when low water potentials were imposed. Soluble sugars also accounted for part of the osmotic adjustment of maize plants (Hsiao et al, 1976). Other osmotically active organic molecules which help in osmoregulation include polyols such as glycerol, and mannitol and nitrogen derivatives such as proline, glycine, and betaine (Zimmermann, 1978).

Localisation of solutes

It has been suggested that osmotic adjustment in cells is effected

by quite different solutes in the various cell compartments (Hanson and Hitz, 1982). This is suggested by the need to maintain ion concentration in the cytoplasm within narrow limits, and supported by the compartmentation of metabolites in plants (Wyn Jones et al, 1977; Hall et al, 1978). There is no direct evidence on compartmentation of the solutes affecting osmotic adjustment in plants during water deficits (Hanson and Hitz, 1982). However, Wyn Jones et al, (1977) have shown in three halophytes, concentrations of glycinebetaine develop high enough for the compound to be used as the major osmoticum in the cytoplasm provided it is largely but not exclusively localized there. The same authors (Wyn Jones et al, 1977) had hypothesised that the concentration of inorganic ions in the cytoplasm, with K^+ the dominant cation, remains fairly constant and does not normally exceed 200 - 250 mM, since metabolic reactions and enzyme activity are very sensitive to high concentrations of salts. They suggested that osmotic adjustment in the cytoplasm is achieved by the accumulation of non toxic organic molecules such as glycinebetaine and proline (Hall et al, 1978), which are known collectively as "compatible solutes" (Borowitzka, 1981). Support for this hypothesis came from investigations of vacuoles isolated from the red beet storage tissue (Wyn Jones et al, 1977). In most of the studies made on osmotic adjustment the analysis ignored the possibility of solute compartmentation, hence contributions of some organic molecules, for example proline, to the osmotic adjustment is underestimated. This is because calculations were made for whole tissue, but if the assumption was made that for instance proline was confined to the cytoplasm then its contribution to the osmotic potential change under water deficits would be substantial (Jones et al, 1981). On the other hand there is great flexibility in the compounds used in osmotic adjustment in the vacuole (Mott and Steward, 1972) including

inorganic ions such as K^+, Na^+, Ca^{2+} and Cl^- as well as organic compounds like soluble sugars, carboxylic acids.

Processes shown to be maintained by osmotic adjustment

Cell enlargement and growth

It has been said that a reduction in cell water potential of 0.3 - 0.4 MPa is enough to stop cell expansion (Boyer, 1970), but it is rather turgor pressure which really affects cell enlargement (Boyer, 1974; Hsiao, 1976). Plants growing under saline or drought conditions, as well as leaves in tree canopies, can overcome low water potentials by lowering their osmotic potentials and hence maintain turgor and therefore expansion growth (Morgan, 1977b). Under very low soil water potentials turgor pressure may fall to zero; in such a situation the plant can maintain some growth only through osmotic adjustment. Boyer (1970) reported that as a result of osmotic adjustment growth of soybean hypocotyls was less sensitive than growth of soybean leaves when tissue water potential was decreased, Sharp and Davies (1979) showed that at low water potentials reduction of the rate of leaf expansion correlated well with the reduction in leaf turgor. They also showed an increase in growth in root dry weight and length under low water potential, which maintained favourable root: shoot ratio. This they related to the accumulation of solute in root tips which maintained turgor and elongation growth. The other important role of osmotic adjustment is in the diurnal regulation of the leaf osmotic potential and thus turgor (Fereres et al, 1978).

Stomatal response

Lower stmatal resistance is maintained at lower leaf water potentials in plants that are capable of osmotic adjustment than in plants that are

not (Turner and Jones, 1980). Indications of such a feature were reported by McCree (1974) and Davies (1977). Brown et al (1976) had proposed that such stomatal adjustment in previously water-stressed plants may be expalined by solute accumulation and turgor maintenance as leaf water potential drops. The work of Osonubi and Davies (1978) supports this hypothesis, as does that of Turner et al (1978). Ludlow (1980) concluded that there is a strong correlative and mechanistic evidence that osmotic adjustment, particularly in the guard and subsidiary cells, is the main process responsible for stomatal opening at low water potentials. In sorghum plants that adjust osmotically by 0.2 MPa or 0.5 MPa, Jones and Rawson (1979) showed a decrease to -2.1 and-2.4 MPa respectively in the water potential at which the stomatal resistance reached 6 scm^{-1}. Differences in the water potential at which stomata close between plants growing in controlled environments and those in the field when subjected to water deficits may reflect differences in osmotic adjustment taking place in the plants. Plants in controlled environments are usually grown in small pots, thus when watering ceases it takes only days to reach the same water potentials that occur after weeks of drying in the field. In the field, the additional time allows plants to adjust osmotically and maintain turgor and stomatal opening at lower leaf water potentials (Begg and Turner, 1976; Ludlow, 1980).

Photosynthesis

When leaf water potential falls, there is loss of turgor and the stomata close, thereby reducing the amount of CO_2 entering the leaf. Therefore the maintenance of stomatal opening by osmotic adjustment is a necessary step in maintaining CO_2 fixation by leaves. Plants that can adjust osmotically would be expected to maintain higher rates of

photosynthesis at a particular leaf water potential. This was verified by Jones and Rawson (1979) who showed that sorghum plants which were allowed to adjust osmotically during slow drying maintained a higher rate of photosynthesis at low water potentials than those plants in which hardly any adjustment took place.

Drought tolerance

This refers to the ability of the plant to endure low water potentials in its tissues without injury, or to be able to withstand the physiological drought of the soil or of high evaporative demand of air whilst continuing metabolic activity (Gates, 1968). A drought tolerant plant can experience low water potential in its leaves and recover when drought ends.

Low osmotic potential

When plant water potential declines under the effect of drought, it can decrease the subsequent dehydration effect by lowering its osmotic potential by a net increase in solute content. Production of low water potentials tends to create a high soil-to-leaf water potential gradient and thus to more efficiency in extraction of soil water, Salisbury (1952) stated that plants with a preference for wetter soils may be unable to reduce their osmotic potentials to sufficiently low levels. Drought tolerant plants on dry soils could compete successfully, showing continued gas exchange as a result of low osmotic potentials and high turgor at low soil moisture levels. Ackerson and Krieg (1977) showed that sorghum suffers a smaller decrease in relative water content per unit change in leaf water potential than cotton. A lower than expected drop in the turgor pressure of beet leaves was shown to be due to dehydration avoidance by osmotic adjustment (Biscoe, 1972).

Low turgor potential

Plants could tolerate drought by change in the physical properties of their cell walls which involve an increase in the cell wall extensibility (Levitt, 1972). This would allow cell enlargement and growth at a lower cell turgor. Bunce (1977) suggested that leaves of soybeans growing in drier environments require less turgor for enlargement. The very big differences in water lost before plants wilt between xerophytes and shade plants also points to the difference in the physical properties of their cell walls (Maximov, 1929). In sorghum Sanchez-Diaz and Kramer (1973) showed a smaller reduction in water content per change in water potential than maize, which they supposed to be due to a lower cell wall elasticity. Therefore changes in the cell wall extensibility would lead to the resumption of plant growth at reduced turgor (Green, 1968). However, Levitt (1972) stated that cell wall changes could increase plant tolerance by only a small degree. It is also known that plants can lower their threshold turgor potentials at which cell expansion ceases, thus growth can continue at lower turgor potentials (Hsiao, 1973).

In this thesis, populations of Festuca rubra L. and Trifolium repens L. growing in different habitats of the sand dune system are examined for possible ecotypic differentiation, by testing whether they exhibit differential growth responses under different soil moisture regimes.

Under low water potentials, possible ecotypes will then be examined for differences at the physiological level, on photosynthesis, stomatal response, and with special emphasis on their water and ionic relations. Such differences might be the overriding factors in the differentiation of these ecotypes, and the means that allow them to survive and grow in such diverse habitats. This will elucidate the role played by soil water regime in the natural selection of these ecotypes.

CHAPTER TWO

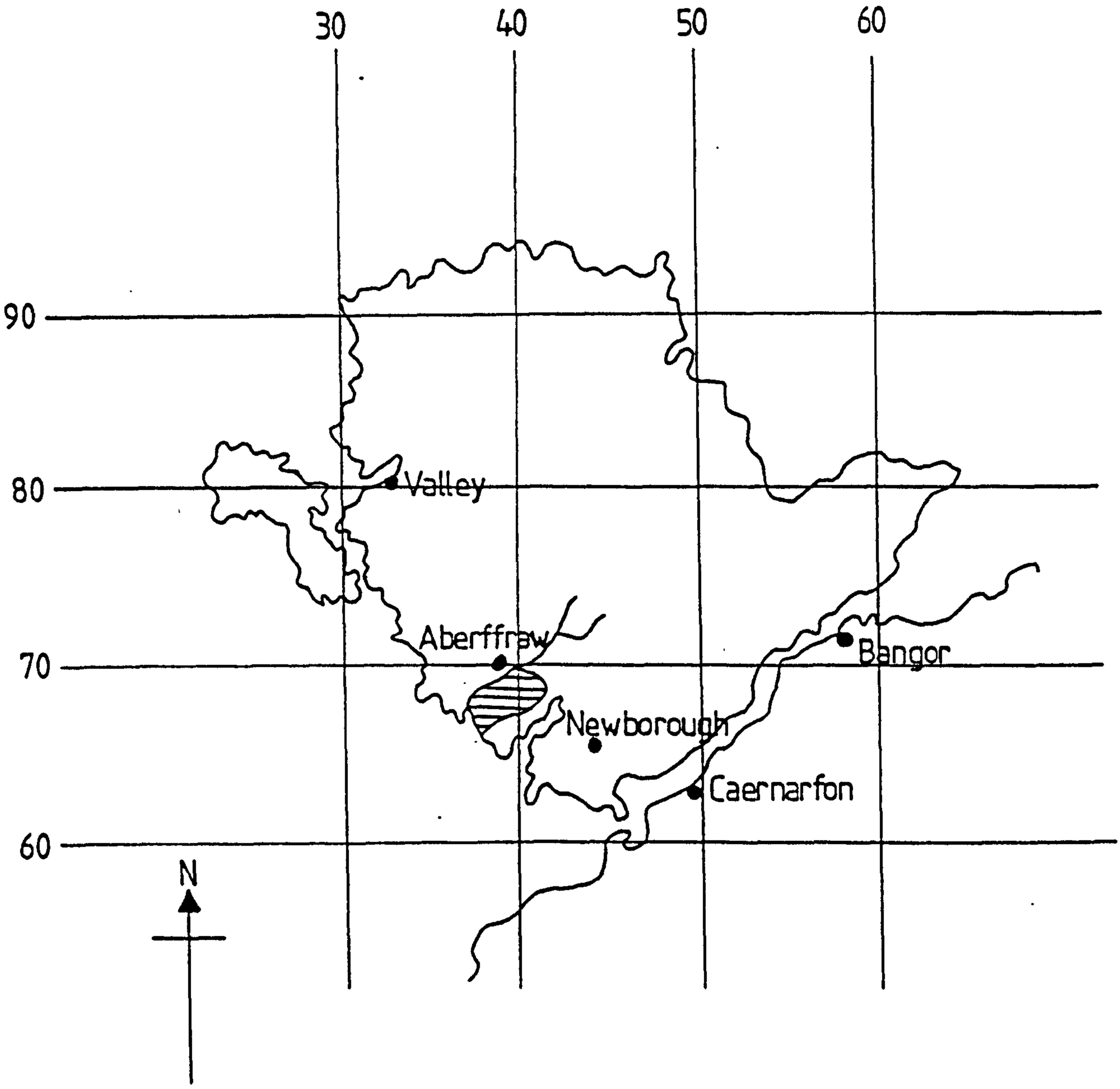
MATERIALS AND METHODS

MATERIALS AND METHODS

Field Site

The site chosen was the dune system at Aberffraw, Anglesey, (National Grid Reference SH3668, see also Map 2,1). The dunes cover an area of approximately 9km^2, in West Anglesey, about 5 kilometeres north west of Newborough Warren. The dune system is composed of three main dune ridges, two running approximately parallel to the coast and the third forming a connection between the two on one side (Map 2,2). Sandy hillocks and small low fixed blow-outs are especially common in the inland low-lying areas.

Throughout the dune system the soils are formed from wind blown sand with a fairly high content of shell fragments providing high amounts of calcium carbonates. In drier areas, the soil profiles are poorly developed, while in wet areas a thin band of humus is seen overlying yellow-brown sand. The growth of marram grass is the main factor leading to the building up and partial stabilization of the dunes. The inland dunes are more fixed, and hence more stable, than the coastal dunes. They also have a more complete plant cover especially on leeward slopes. The low fixed dunes and sand hillocks dispersed in the inland areas generally support a floristically rich vegetation. The low-lying areas provide two major types of habitat: dry slacks and wet slacks. In the dry slacks the soil is loose and dry, and vegetation is mainly dominated by grasses. On the other hand, the wet slacks show a wet compact and darker surface due to a surface layer of humus.



Map 2.1. Map of Anglesey showing the location of Aberffraw sand dunes. (striped area)

MAP 2.2

Map of the Aberffraw sand dune system, showing the sites from which plants were collected (shown by numbers, see also Table 2.2).

A.4080

Aberffraw

3

1

2

4

6

5

N

Aberffraw river

Aberffraw sands

sea

0 metres 150

main dune ridges

low fixed dunes and sandy hillocks

main road

The most interesting feature of this dune system is its relative dryness. The wet slacks are relatively dry and flooding is comparatively rare when compared to other dune systems, e.g. Braunton Burrows (Willis et al, 1959a,b) and Newborough Warren (Onyekwelu, 1972), in which the low-lying areas are subjected to varying degrees of lengthy seasonal flooding.

However, rainfall is fairly high during autumn and winter, while summers are usually warm and dry (Table 2.1a). Winter temperatures rarely fall below freezing point in this area (Table 2.1b).

Several workers have stressed the importance of soil moisture regimes in determining the vegetational variation within the dune habitat (Onyekwelu, 1972; Willis et al 1959a,b; Pemadasa, 1973).

Collection Sites

Plant material of white clover (Trifolium repens L.) and red fescue (Festuca rubra L.) was collected in the form of turves for subsequent cloning in the glasshouse, collected from 7 sites at Aberffraw (Map 2.2): F. rubra plants were taken from 7 sites and were used for growth experiments only; T. repens plants were collected from only 4 of the sites (Table 2.2).

Field Studies

Reciprocal Growth Experiment

Transplants of T. repens from sites 1 and 7 were left to establish in potted sand in the glasshouse before transfer to the field. Two sites were chosen, one on the wet slack (Site 1) and the other on the side of a dune in the middle ridge (Site 7), a dry habitat (See Map 2.2). Plots of 2x2m in each site were cleared of vegetation and dug over to a depth of 10cm. Each plot was then divided into 48 units, to which

Table 2.1 Monthly mean meteorological measurements for Valley, Anglesey.

(Courtesy of RAF Valley)

(a) Monthly rainfall (mm):

Month	1980	1981	1982
JAN	116.0	51.6	41.6
FEB	117.7	31.1	54.0
MAR	89.3	151.9	71.4
APR	7.0	65.9	22.2
MAY	29.8	55.0	35.9
JUN	67.3	51.4	56.9
JUL	29.9	40.6	30.6
AUG	99.1	26.0	66.9
SEP	71.4	101.6	102.8
OCT	109.2	172.2	99.8
NOV	95.3	76.8	169.2
DEC	77.6	77.6	84.5

(b) Monthly mean maximum and minimum temperatures (^{o}C)

	Temperature					
	Maximum			Minimum		
Month	1980	1981	1982	1980	1981	1982
JAN	6.7	8.1	7.1	2.3	4.8	2.7
FEB	8.8	7.2	8.8	5.0	2.3	3.9
MAR	8.3	10.0	9.5	3.4	5.8	4.2
APR	12.5	12.3	12.1	5.5	5.2	5.3
MAY	16.2	14.0	15.2	7.4	8.2	8.0
JUN	16.8	15.1	18.3	10.5	10.2	11.4
JUL	17.5	16.9	20.1	12.0	12.1	12.4
AUG	17.7	18.8	18.5	12.8	12.6	12.5
SEP	17.0	17.6	17.3	12.8	12.3	11.6
OCT	12.6	11.8	13.8	8.0	7.0	9.0
NOV	10.1	11.1	11.1	6.1	7.3	7.5
DEC	9.2	5.5	8.3	5.6	0.9	4.2

Table 2.2 Description of collection sites (see also Map 2.2)

Site No.	Location	Moisture status
* 1	Middle of wet slack	Wet
* 2	At dry end of wet slack	Fairly wet
* 3	Moist dry slack towards land side	Fairly wet
4	On an old fixed dune	Dry
5	Near the top of a very dry sea-facing mobile dune	Very dry
6	On a dry slack behind the dunes nearest the sea	Dry
* 7	On the side of a fixed dune on the middle ridge	Dry

*Site where T. repens plants were collected.

24 plants from either site were randomly allocated, forming a completely randomised design. Plants were watered for the first week to assist establishment. They were then left to grow under field conditions for six months (April-September 1981). After this they were harvested and the following measurements were taken for each plant:

1. Leaf number
2. Total leaf area
3. Leaf dry weight
4. Stolon and petiole dry weight
5. Total above-ground dry weight

Plant Water Status

Leaf tissue was collected from plants growing in the field, and placed in microcentrifuge tubes (1.5cm^3 Eppendorf vials) which were sealed tightly to ensure no water vapour loss. Collections were made at three different times when climatic conditions were as follows:

i. April 25 (1980), a mild dry day, temperature 15°C.

ii. June 18 (1980), a dry sunny day, temperature 18°C.

iii. July 27 (1980), a very warm dry day with temperature up to 24°C.

Leaf water potential measurements were taken immediately after samples were taken to the laboratory, using the psychrometric technique as described below. As for the osmotic potential measurement, plants were frozen and kept in a deep freeze (-20°C) for subsequent determination as described below.

Soil Sampling

Soil from the top 10cm layer was collected in air tight glass specimen tubes fitted with plastic tops. Care was taken to avoid plant material. The specimen tubes filled with soil were sealed *in situ* using 'Para-Film' wax paper to minimize loss of moisture. Soil sampling was done at the same time as that of plant material. Soil water potential was then determined in the laboratory using psychometry as described below.

Soil analysis for Exchangeable Sodium and Potassium

Soil samples were oven dried for 24 hours at 80°C and were then sieved through a mesh size of 2.032mm. 5g of dry soil was mixed with 50cm^3 of 0.1M acetic acid and the mixture shaken mechanically for three hours. The extract was then filtered and the filtrate made up to 100cm^3. Concentrations of sodium and potassium were then estimated using a flame photometer (Model A, Evans Electroselenium Ltd) and expressed as µg/g dry soil.

Glasshouse and Laboratory Studies

Plant Growth Conditions

Plants brought in from the field, were grown in 40x30x12cm wooden trays with John Innes No. 2 potting compost, three plants of *T. repens* per tray, and sixteen of *F. rubra* per tray. They were growing in a heated glasshouse with minimum temperature 18°C day, 17°C night. Supplementary lighting, when required, was provided by Philips 400W mercury vapour lamps (280 µmol quanta $m^{-2}s^{-1}$, at plant height over the wave band 400-700nm) giving a 16 hour day length.

Preparation of Plant Material for Water Culture

The terminal 3-4 internodes of T. repens stolons were cut and transplanted into 4.5-inch pots with sand. Full strength Long Ashton solution was added to provide nutrients (Table 2.3). Plants were left to establish for a week before transfer to Long Ashton culture solution in blackened 4-inch pots, two plants per pot. After that the plants were moved to a light bank in a controlled environment room with temperature 18°C and light 200 μmol quanta $m^{-2}s^{-1}$ from daylight fluorescent tubes over the wave band 400-700nm for 16 hours a day.

Known amounts of polyethylene glycol (average m.w. 4000) were dissolved in full strength Long Ashton solution to produce low water potentials (Lawlor 1970). The solution was measure psychometrically and adjusted until the required osmotic potential was reached.

Plant Growth in the Glasshouse:

i. Trifolium repens

Terminal 3-4 internodes of stolon were cut and transplanted into Plantpack half trays (Maldon, U.K.) with washed sand. Plants were left to establish for one week with daily watering and Long Ashton solution was added. At the start of the second week three watering regimes were begun as follows:

a. Daily watering.

b. Watering once every three days.

c. Watering once a week.

Table 2.3 The composition of Long Ashton solution as used in water culture experiments (Hewitt, 1966)

Element	Total conc. (ppm)
K	156
N	170
Ca	160
S	48
P	41
Mg	36
Na	34
Fe	2.8
Cl	3.5
Mn	0.55
B	0.54
Zn	0.065
Cu	0.064
Mo	0.048

Treatment (c) resulted in periodic drying out of the soil; the plants were near wilting by the time of re-watering. Long Ashton solution was added to all plants at weekly intervals to provide the required nutrients and thus reduce any effect due to soil nutrient deficiency. Four replicates (each of a different clone) from each collection site were allocated randomly to each of the three treatments. Trays were distributed on the glasshouse bench in a completely randomised design. Plants were left to grow for 8 weeks, at the end of which they were harvested and the following measurements were taken for each plant:

1. Leaf number
2. Total leaf area
3. Leaf dry weight
4. Stolon and petiole dry weight
5. Root dry weight
6. Total plant dry weight

Leaf area was measured using a Hayashi-Denko AAM-5 electronic planimeter, and dry weight following oven drying at 80°C.

ii. Festuca rubra

Young tillers were transplanted into 4.5-inch pots filled with washed sand. Care was taken to choose plants of uniform size and the same number of leaves. Long Ashton solution was added and they were watered daily for a week to assist establishment. Treatments were then started as described for T. repens. After ten weeks plants were harvested and the following measurements taken for each plant:

1. Leaf number
2. Tiller number
3. Total leaf length
4. Leaf dry weight
5. Stem dry weight
6. Root dry weight
7. Total plant dry weight

Water Relation Studies

Water Potential and Osmotic Potential Measurements

For both measurements dew-point thermocouple psychrometry was used (Begg and Turner, 1976). A Wescor model C-52 sample chamber connected to an HR-33T dew point microvoltmeter (Wescor Inc., Utah) was used, with readings taken on a Curken potentiometric recorder. The dew-point mode was used throughout. Solutions of NaCl of known molarity and solute potential (Weatherley, 1960; Robinson and Stokes, 1955) were used to calibrate the psychrometer. Apparatus was in a constant temperature room at 18°C to reduce error due to temperature fluctuations.

Leaf Water Potential (Ψ_l)

A leaf disc (diameter 0.5cm) was cut with a punch and placed quickly in the Wescor C-52 sample chamber and left to equilibrate for 2½ hours. This equilibration period was shown to be adequate by taking readings at intervals after putting up the disc into the sample chamber; constant readings were obtained after two hours.

Leaf Osmotic Potential (Ψ_s)

About 100mg fresh weight of leaf tissue was placed in 1.5cm^3 Eppendorf vials and immediately placed in a deep freeze at -20°C. For measurement, the frozen tissue was left to thaw in the vials and then centrifuged for 5 minutes at 14,000g in a microcentrifuge in order to expel the sap. A disc of Whatman No. 3 filter paper (0.5cm diameter) was dipped into the sap until fully saturated, then put into the psychrometer sample chamber and left to equilibrate. Here it was found that 30 minutes were quite sufficient for vapour equilibration. Measurements were then taken as described above.

Soil Water Potential (Ψ_{soil})

A soil sample was placed inside the sample chamber cup, filling it to 1mm from the top. Measurement was taken after an equilibration time of 30 minutes.

Relative Water Content (RWC)

This measurement which expresses tissue water content as a percentage of the turgid water content (Weatherley, 1950), is probably the most widely accepted way of expressing the quantity of water in plant tissue (Boyer 1969). A β-gauge technique was used for this purpose:

β-gauge technique

The theory behind the technique is that a stream of β-particles tends to be attenuated as the mass of material in its path increases (Mederski, 1961). Hence an increase in the leaf water content, which will result in an increase in its mass, can be detected by placing

the leaf between a source of β-particles and a detector, A C-14 source was used in this study, and counts were detected by a müller tube and a ratemeter (Panax Reigate Series). Counts were calibrated against leaf mass (ie. effective leaf thickness) where the log of the count rate was proportional to the leaf thickness (Fig. 2.1). This method permits measurements to be taken with minimal disturbance to the plant.

Turgid weight was taken after putting leaf tissue discs in water for 4 hours, then carefully wiped using blotting paper before being weighed (Barrs and Weatherley, 1962). Dry weight of discs was taken after oven drying at 85°C for 24 hours.

Relative water content was then calculated as follows:

$$RWC = \frac{Wf-Wd}{Wt-Wd} \times 100$$

RWC = Relative water content

Wf = fresh weight

Wt = turgid weight

Wd = dry weight

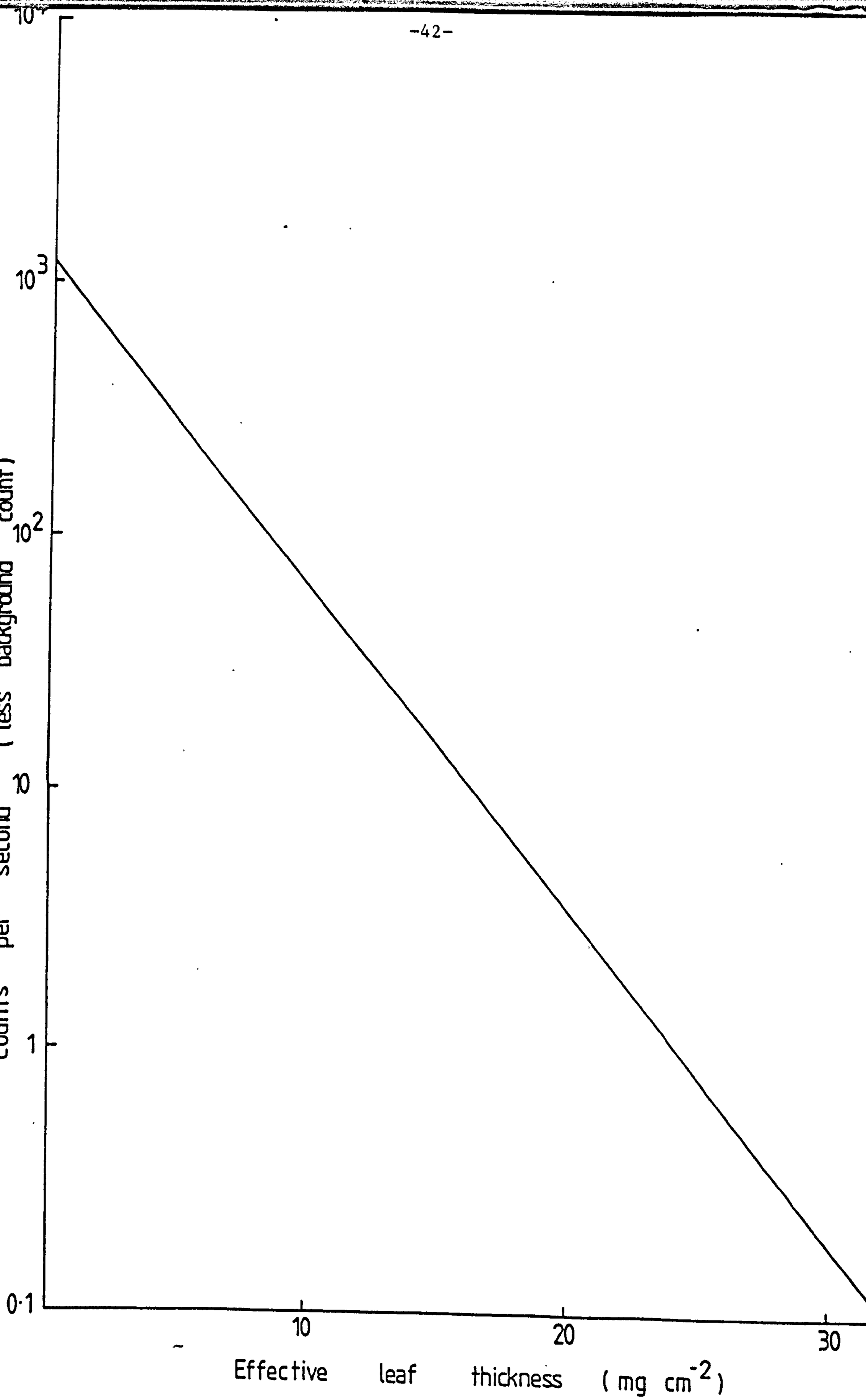
Paper Chromatographic Analysis of Soluble Sugars

Extraction

Analysis was carried out by extracting the leaf tissue with 95% (v/v) ethanol. 100mg fresh weight of leaf tissue was mixed with 50cm^3 of ethanol for 24 hours and then filtered. The filtrate was evaporated to dryness in a cold stream of air overnight and subsequently taken up in a known volume of the extraction medium for chromatographic analysis.

FIG. 2.1

β-gauge calibration of counts per second readings plotted against the effective leaf thickness.


10⁴
10³
10²
10
1
0·1
(less background count)
Counts per second
10
20
30
Effective leaf thickness (mg cm⁻²)

Chromatography

Development was carried out on Whatman No. 3 chromatography paper (Menzies, 1973). Five 20 µl spots were applied to each chromatogram, using Drummond microcaps, assigning samples to different chromatograms at random.

Solutions of different concentrations of standard compounds (Sugars) in 95% ethanol were used in order to locate the position of the sugar spots and to prepare the calibration curves. Chromatograms were developed in propanol/ethyl acetate/water (7:1:2 $^{v}/_{v}$) by descending chromatography for 24 hours (Cerbulis, 1955).

For detection of spots, freshly prepared 4-aminobenzoic acid reagent (0.7g 4-aminobenzoic acid in 100cm^3 methanol plus 0.4cm^3 of 88% ($^{w}/_{v}$) orthophosphoric acid) was used. Chromatograms were dipped through this solution and allowed to dry. They were then heated for exactly 4 minutes at 110^{o}C. A standard thermostatically controlled oven fitted with a fan to give uniform heating throughout was used for this purpose. Spot intensity was measured using a densitometer (vitatron modular photometer system), with a tungsten lamp and 616nm filter in conjunction with a potentiometric recorder. Standard sugar concentrations were plotted against peak height from the recorder trace to produce standard curves.

Analysis of Plant Material for Sodium and Potassium

Preparation of material for analysis by wet digestion

After harvest, leaves were dried in an oven at 80^{o}C. 50mg of the dried leaf tissue was placed in a Kjeldhal digestion tube (75cm^3 capacity) to which was added 5cm^3 of sulphuric acid digestion reagent. The reagent was prepared as follows: 14g of lithium sulphate was added to 420cm^3 of concentrated sulphuric acid, to which a solution of 0.42g of selenium in

350cm^3 of hydrogen peroxide was added slowly with stirring and cooling (Allen , 1974). Reagent was always prepared immediately prior to use. The digestion tubes were then heated in a block digestor (Tecam) at 180°C for one hour, and then at 350°C until the solution cleared (about 3 hours). The contents of the tubes were then transferred, with washing, to 50cm^3 volumetric flasks and made up to volume with distilled water. Aliquots of this solution were used to determine K^+ and Na^+ levels. A flame photometer (Model A, Evans Electroselenium Ltd) was used for this purpose.

Gaseous Exchange Measurement

Measurement of CO_2 Flux

An A.D.C. series 200 infra-red gas analyser was used in combination with an A.D.C. 6-channel automatic sampling unit operating in the differential mode (The Analytical Development Co. Ltd). The apparatus was kept at 18°C in a constant temperature room (see Fig. 2.2).

Ambient air was drawn from above the roof through a 230dm^3 drum and passed to a manifold serving six flow meters (Platon, 0.5-5.0dm^3 min^{-1}) each delivering 3dm^3 min^{-1} to air-sealed rectangular chambers made from clear acrylic perplex (16x12x1.3cm internal dimensions).

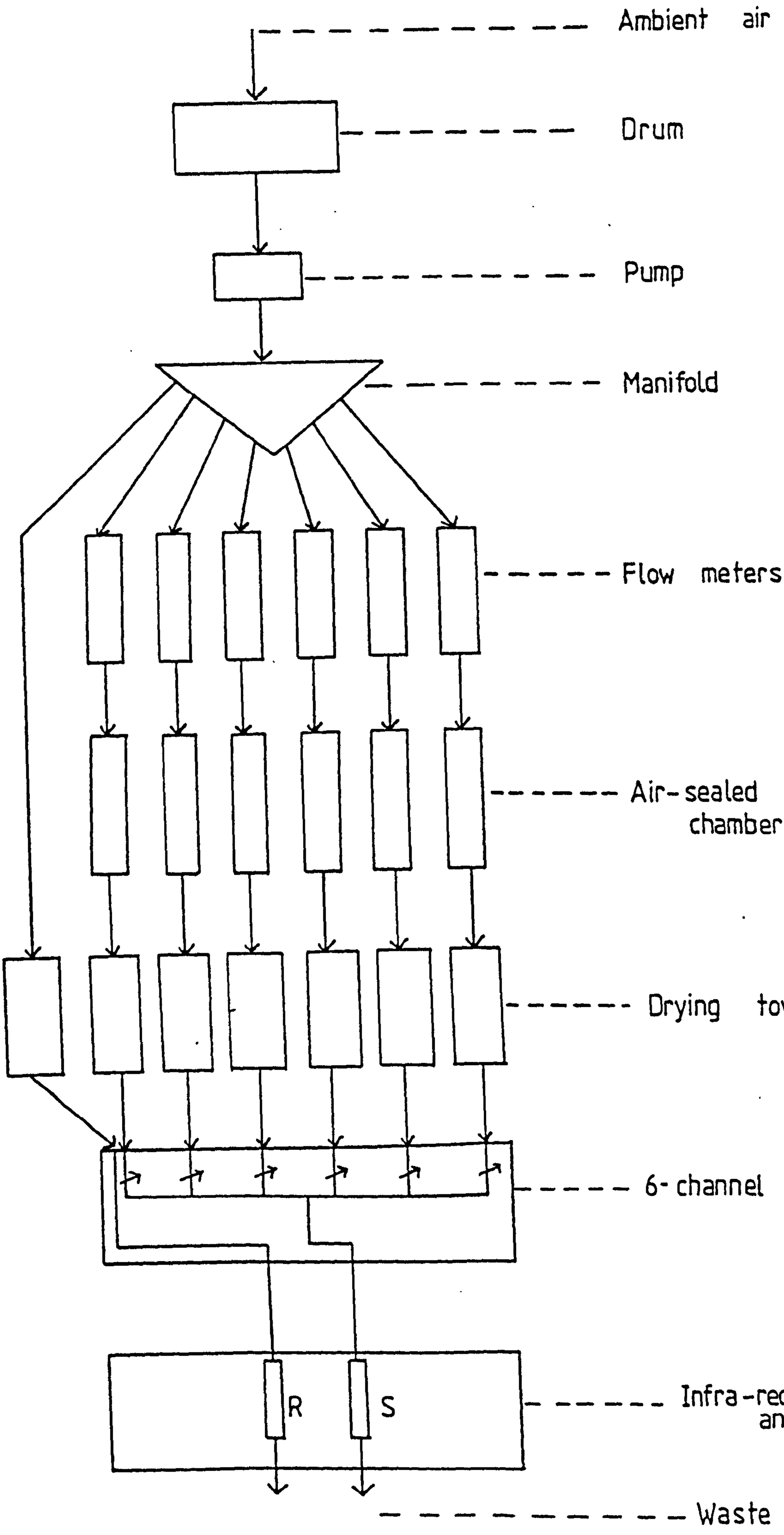
The integrity of the air seal was tested during design by supplying the chambers with CO_2 free air. No intake of ambient CO_2 was detected.

The infra-red gas analyser was calibrated using bottled CO_2 of known concentration (320 ppm CO_2) and the CO_2 concentration in the ambient air was measured. Air was drawn from the chambers at 1dm^{3}min^{-1} and dried by passing through a 500cm^3 column of self-indicating silica gel before passing to the gas sampling unit. Measurements were made at 18°C and 225 μmol quanta m^{-2}s^{-1} over the waveband 400-700nm from daylight fluorescent tubes (Measured by a quantum sensor, Macam SD101/Q).

Fig. 2.2

Flow diagram of gas circuits for the A.D.C. series 200 infra-red gas analyser in combination with an A.D.C. 6-channel automatic sampling unit, operating in the differential mode, as used for measurements of gaseous exchange. R : reference cell

S : Sample cell



Leaf area was measured using a Hayashi-Denko AAM-5 Electronic planimeter.

Measurement of Water Vapour Exchange

This was measured simultaneously with CO_2 exchange, using an A.D.C. 225-MKII gas analyser (Analytical Development Co. Ltd). Here the gas analyser was calibrated with a stream of air saturated with water vapour at controlled temperatures. Measurements of transpiration were thus obtained from which vapour phase resistance (r_v) was calculated as follows:

$$r_v = ([H_2O]\ \text{int.} - [H_2O]\ \text{ext}\ )/E$$

E = Evaporation rate (measured) g $m^{-2}s^{-1}$

$[H_2O]$ int.= water vapour concentration inside the leaf (estimated from leaf temperature and assuming saturation) g m^{-3}

$[H_2O]$ ext.= water vapour concentration outside the leaf (measured) g m^{-3}

Calculation of resistances to CO_2 uptake (r'):

i. Total resistance (r'_{total}):

This was calculated as follows:

$$r'_{total} + ([CO_2]_{ext} - [CO_2]_{int})/P$$

P = Rate of net photosynthesis (measured) mol $CO_2 m^{-2}s^{-1}$

$[CO_2]_{ext}$ = CO_2 concentration outside the leaf (measured at 13.6mmol m^{-3})

$[CO_2]_{int}$ = CO_2 concentration at the carboxilation site (assumed to be 0mol m^{-3})

ii. Gas phase resistance (r'_{CO_2}):

This was obtained from the water vapour resistance of the leaf by multiplying it by 1.37 (Hall and Loomis, 1972)

$$r'_{CO_2} = r_v \times 1.37$$

r_v = Vapour phase resistance (from above)

iii. Liquid phase resistance (mesophyll resistance) r'_m:

This was obtained by difference,

$$r'_m = r'_{total} - r'_{CO_2}$$

CHAPTER THREE

FIELD STUDIES

Field Sites: Vegetation, Soil Moisture Status and Plant Water Status

Vegetation

Vegetation on the sites was surveyed briefly. Marram grass (Ammophila arenaria (L.) Link.) was dominant on sites 5 and 6, while sites 4 and 7 showed colonization by species like Festuca rubra L., Ononis repens L. as well as Leontodon autumnalis L., L. hispidus L., Taraxacum officinale Weber., and Viola canina L. In site 7, however, other species also appear like Carex arenaria L., Trifolium repens L., and Sedum anglicum Huds. All these sites except site 6 represent the dune habitat, while site 6 is a dry slack. Site 1 was in the middle of a wet slack, with a wet and compact soil which is somewhat darker in colour probably due to the presence of humus. The site supports a vegetation composed of species like Carex flacca Shreb., Plantago coronopus L., Prunella vulgaris L., Trifolium repens L., and in the very moist areas Hydrocotyle vulgaris L. and Mentha aquatica L., Site 3 was on an extensive flat area beyond the dune ridges; the soil here is rich and peaty thus supporting a rich vegetation of Agrostis tenuis L., Carex arenaria L., Festuca rubra L., Trifolium repens L., and Salix repens L. However, site 2 which was on the dry end of a wet slack supports vegetation not very much different from Site 1.

Soil Moisture Status

Soil Moisture Status of the sites from which plants were collected was measured in samples from the top 10cm of soil. Soil samples were collected on three days when climatic conditions were different. The first sampling was on a mild day with temperature about 15°C (April 25, 1980), whilst the second was dry and sunny day with temperatures around 18°C (June 8, 1980). The third was a very warm sunny day with temperatures

reaching 24°C and conditions favouring very high evaporation (July 27, 1980).

There were clear differences between the sites in their soil water potential, especially on the hottest day sampled (Table 3.1). Sites 4,5,6 and 7 had low soil water potentials even on the mild day and these were even lower on the other two days. Site 1 had quite high soil water potentials, there being only slightly lower on July 27. Site 3 had soil water potentials not different from site 1 on any of the three days. However, site 2 showed soil water potentials intermediate between those of the dry and wet sites.

Plant Water Status

To see the effect of the soil moisture condition on plant water status, leaves of Trifolium repens were collected from plants growing on three sites of distinct water status : 1,2 and 7, these had moisture conditions which can be termed wet, intermediate and dry respectively for convenience. Leaves were collected on the same days as soil samples; the relevant soil water potentials are in Table 3.1. Subsequent measurements of leaf water potentials and osmotic potentials were conducted in the laboratory. Results are shown in Tables 3.2 to 3.4. The soil water status is mirrored by water and osmotic potentials of the plants. There were lower leaf water and osmotic potentials in plants from the dry and intermediate, as compared to the wet sites. Decreases in soil water potentials over the three days of sampling were paralleled by a drop in leaf water potentials. Thus plants from the dry site had a much lower leaf water potential on June 18 and July 27, whereas those from the wet site were not different on June 18 but their leaf water potential was slightly lower on July 27. However, plants from site 2, (intermediate in water status), showed different leaf water potentials on the three days, and had values intermediate between those of

Table 3.1 Soil water potential (MPa) of soil samples collected from the seven sites from which plants were collected. Samples were taken on three different days; each value is the mean of five replicates.

April 25, 1980 : Dry, temperature 15°C

	SITE NO.						
	1	2	3	4	5	6	7
SOIL WATER POTENTIAL	-0.03 ± 0.01	-0.09 ± 0.01	-0.04 ± 0.01	-0.16 ± 0.01	-0.19 ± 0.02	-0.17 ± 0.03	-0.15 ± 0.01

June 18, 1980 : Dry,sunny, temperature 18°C

	SITE NO.						
	1	2	3	4	5	6	7
SOIL WATER POTENTIAL	-0.05 ± 0.01	-0.12 ± 0.01	-0.06 ± 0.02	-0.29 ± 0.01	-0.32 ± 0.02	-0.30 ± 0.01	-0.29 ± 0.03

July 27, 1980 : Dry, sunny, temperature 24°C

	SITE NO.						
	1	2	3	4	5	6	7
SOIL WATER POTENTIAL	-0.15 ± 0.01	-0.28 ± 0.02	-0.10 ± 0.01	-0.64 ± 0.04	-0.70 ± 0.02	-0.70 ± 0.04	-0.68 ± 0.03

Table 3.2 Leaf Water, Osmotic, and Pressure Potentials (MPa) of Trifolium repens plants growing on wet, intermediate, and dry sites on the dune system. Samples collected on April 25, 1980, each value is the mean of five replicates.

	LEAF WATER POTENTIAL	LEAF OSMOTIC POTENTIAL	LEAF PRESSURE POTENTIAL
SITE 1 (WET)	-0.37 ± 0.01	-0.56 ± 0.01	0.19 ± 0.01
SITE 2 (INTERMEDIATE)	-0.41 ± 0.04	-0.60 ± 0.05	0.19 ± 0.02
SITE 7 (DRY)	-0.55 ± 0.01	-0.71 ± 0.02	0.16 ± 0.01

Table 3.3 Leaf Water, Osmotic and Pressure Potentials (MPa) of Trifolium repens plants growing on wet, intermediate, and dry sites on the dune system. Samples collected on June 18, 1980; each value is the mean of five replicates

	LEAF WATER POTENTIAL	LEAF OSMOTIC POTENTIAL	LEAF PRESSURE POTENTIAL
SITE 1 (WET)	-0.38 ± 0.02	-0.57 ± 0.02	0.19 ± 0.01
SITE 2 (INTERMEDIATE)	-0.52 ± 0.04	-0.70 ± 0.04	0.18 ± 0.03
SITE 7 (DRY)	-0.75 ± 0.02	-0.99 ± 0.02	0.24 ± 0.01

Table 3.4 Leaf Water, Osmotic and Pressure Potentials (MPa) of *Trifolium repens* plants growing on wet, intermediate, and dry sites on the dune system. Samples collected on July 27, 1980; each value is the mean of five replicates.

	LEAF WATER POTENTIAL	LEAF OSMOTIC POTENTIAL	LEAF PRESSURE POTENTIAL
SITE 1 (WET)	-0.58 ± 0.01	-0.79 ± 0.01	0.21 ± 0.04
SITE 2 (INTERMEDIATE)	-0.69 ± 0.04	-0.85 ± 0.03	0.16 ± 0.03
SITE 7 (DRY)	-1.00 ± 0.03	-1.29 ± 0.02	0.29 ± 0.01

the other two sites.

Osmotic potential measurements showed that this drop in leaf water potential was paralleled by a drop in the osmotic potential. This drop was highest in case of the plants on the dry site, which showed osmotic potentials lower by 0.5-0.6MPa on July 27, than April 25, whereas plants on the wet and intermediate sites were lower by about 0.2 and 0.25 MPa respectively. Plants from the intermediate sites did not show sufficient drop in their osmotic potentials on July 27 to equal the fall in water potentials. As a result their pressure potentials were lower on that day. On the same day osmotic potentials of plants from the dry site were much lower than on April 25. The major feature is the maintenance of large positive pressure potentials by the plants on all three days and on all sites.

Reciprocal Transplant Experiment in the Field

The information obtained from the soil and plant water status in the field indicated that there were large differences between sites, for both soil and plants. Such differences suggested possible specialization of the plants to their habitats, and possible ecotypic differentiation suiting range of conditions within each habitat. Therefore an experiment was designed to test the growth of T. repens plants from the two extreme sites - Site 1 which was very wet and Site 7 which was the driest - when grown reciprocally on both sites. Plants were left to grow under natural conditions in the dune system for six months following transplanting (April to September, 1981), and the results are shown in Tables 3.5 to 3.11.

The number of leaves produced per plant indicated that plants from the two sites performed much better in their original sites (Table 3.5). The plants from the wet site showed leaf production to be cut by 55% when grown on the dry site, while those from the dry site produced only half the number of leaves they had in the dry site when grown in the wet site.

That behaviour was mirrored by the total leaf area production by the plants (Table 3.6). There were marked differences in total leaf area per plant between the two sites. The plants from the wet site had, when grown on the dry site, only one third the total leaf area compared to the amount they produced in the wet site. Plants from the dry site, however, had a slightly larger total leaf area than those from the wet site when they were growing in their original sites, but the dry site plants had a 4-fold decrease when grown in the wet site. This improved performance of the plants on their original habitats is supported by the highly significant interaction between original site and growth site ($P < 0.001$) as shown by the analysis of variance.

There was a highly significant effect of both plant origin and the growing site on leaf dry weight per plant (Table 3.7). Here plants from the dry site had leaf dry weight almost double that of the wet site plants when each was growing on its original site. Plants from the wet site had their leaf dry weight cut to half when growing on the dry site, whereas that of the dry site plants was reduced to about 25% when grown in the wet slack.

The dry weight of stolon (including petioles) followed the same trend as leaf number and total leaf area. Plants from both sites had their highest production when grown on their original sites, and this was then cut down by 55% in case of wet site plants growing on the dry site, and 45% of those from the dry site growing on the wet one (Table 3.8).

Table 3.5 Number of Leaves Produced by Trifolium repens from Two Dune Sites, Grown Reciprocally on Both Sites

		SITE OF ORIGIN	
		WET (SITE 1)	DRY (SITE 7)
SITE OF GROWTH	WET	201.8 ± 10.4	103.3 ± 7.1
	DRY	89.1 ± 9.7	192.1 ±16.1

HSD(0.05) = 43.8

Analysis of Variance of Number of Leaves

SOURCE	MEAN SQUARE	P
Site of Origin	40.5	ns
Growing Site	1128.1	ns
Interaction	81204.5	0.001
Residual	1025.2	

Table 3.6 Total Leaf Area (cm^2) of Trifolium repens from Two Dune Sites, Grown Reciprocally on Both Sites.

		SITE OF ORIGIN	
		WET (SITE 1)	DRY (SITE 7)
SITE OF GROWTH	WET	138.95 ± 12.14	42.52 ± 3.53
	DRY	42.48 ± 4.67	162.24 ±16,31

$HSD_{(0.05)} = 40.91cm^2$

Analysis of Variance of Plant Leaf Area

SOURCE	MEAN SQUARE	P
Site of Origin	1088.46	ns
Growing Site	1081.24	ns
Interaction	93475.15	0.001
Residual	895.74	

Table 3.7 Leaf Dry Weight (g) of Trifolium repens from Two Dune Sites, Grown Reciprocally on Both Sites

		SITE OF ORIGIN	
		WET (SITE 1)	DRY (SITE 7)
SITE OF GROWTH	WET	0.51 ± 0.04	0.22 ± 0.02
	DRY	0.24 ± 0.03	0.96 ± 0.06

HSD(0.05) = 0.14g

Analysis of Variance of Leaf Dry Weight

SOURCE	MEAN SQUARE	P
Site of Origin	0.3648	0.001
Growing Site	0.4505	0.001
Interactin	2.0770	0.001
Residual	0.0112	

Table 3.8 Stolon Dry Weight (g) of Trifolium repens from Two Dune Sites, Grown Reciprocally on Both Sites.

		SITE OF ORIGIN	
		WET (SITE 1)	DRY (SITE 7)
SITE OF GROWTH	WET	0.99 ± 0.11	0.53 ± 0.05
	DRY	0.45 ± 0.07	0.97 ± 0.11

HSD(0.05) = 0.35g

Analysis of Variance of Stolon + Petiole Dry Weight

SOURCE	MEAN SQUARE	P
Site of Origin	0.0073	ns
Growing Site	0.0275	ns
Interaction	1.9614	0.001
Residual	0.065	

Total above-ground dry weights of the plants showed clearly that each of these plants grew better when grown in its own site. However, there was a significant difference between plants from the two sites, with those from the dry site always having higher above-ground dry weights than those from the wet site (Table 3.9).

Unlike leaf number, total leaf area, and stolon dry weight, the original site as well as the growing site had highly significant effect on the specific leaf area (Table 3.10). Plants from the wet site had higher specific leaf area in both sites, although this was markedly reduced when these plants were grown on the dry site. Plants from the dry site had much lower values, these being higher when grown on the wet site. This relates to the much larger leaf dry weight the plants from the dry site had compared to those from the wet site when each were growing in their original sites.

The effect of the site on individual leaf size was quite significant (Table 3.11). Plants from the wet site had their leaves reduced in size by 30% when grown on the dry site. However, the dry site plants had somewhat larger leaves which were halved in size when grown on the wet site.

The effect of growing the plants therefore on either of the dry or wet site depends mainly on the place of origin of the plants. Plants originally collected from the wet site showed better growth on the wet than the dry site, and similarly plants collected from the dry site grew better on the dry site. This is supported by the highly significant Original Site x Growth Site interaction seen from the analysis of variance for all the parameters measured.

Table 3.9 Above-Ground Dry Weight (g) of Trifolium repens from Two Dune Sites, Grown Reciprocally on Both Sites.

		SITE OF ORIGIN	
		WET (SITE 1)	DRY (SITE 7)
SITE OF GROWTH	WET	1.51 ± 0.14	0.75 ± 0.05
	DRY	0.69 ± 0.06	1.93 ± 0.13

HSD(0.05) = 0.40g

Analysis of Variance of Above Ground Dry Weight

SOURCE	MEAN SQUARE	P
Site of Origin	0.4757	0.05
Growing Site	0.2575	ns
Interaction	8.0697	0.001
Residual	0.0876	

Table 3.10 Specific Leaf Area ($cm^2 gdw^{-1}$) of Trifolium repens from Two Dune Sites, Grown on Both Sites

		SITE OF ORIGIN	
		WET (SITE 1)	DRY (SITE 7)
SITE OF GROWTH	WET	271.66 ± 14.61	196.44 ± 4.96
	DRY	175.09 ± 6.64	166.19 ± 7.42

HSD(0.05) = 29.21 $cm^2 gdw^{-1}$

Analysis of Variance of Specific Leaf Area

SOURCE	MEAN SQUARE	P
Site of Origin	10620.4	0.001
Growing Site	26599.1	0.001
Interaction	6012.9	0.001
Residual	456.5	

Table 3.11 Area per Leaf (cm^2) of Trifolium repens from Two Dune Sites, Grown Reciprocally on Both Sites

		SITE OF ORIGIN	
		WET (SITE 1)	DRY (SITE 7)
SITE OF GROWTH	WET	0.68 ± 0.03	0.41 ± 0.01
	DRY	0.48 ± 0.01	0.84 ± 0.02

HSD(0.05) = 0.07 cm^2

Analysis of Variance of Mean Area Per Leaf

SOURCE	MEAN SQUARE	P
Site of Origin	0.0156	0.05
Growing Site	0.0997	0.001
Interaction	0.7863	0.001
Residual	0.0027	

CHAPTER FOUR

WATER AVAILABILITY AND PLANT GROWTH

The Effect of Soil Moisture Regime on Plant Growth and Development

The reciprocal growth experiment in the field, described above, showed the preferential growth response of plants to their original sites. The field measurements (Table 3.1) showed variation in the soil water status between the sites. It was thought that soil moisture status could be the overriding factor controlling plant grown on the different sites. An experiment was, therefore, designed to test, under controlled conditions, the effect of soil moisture regime on growth and development of plants collected from all the sites. Plants were grown for 8 or 10 weeks in a heated glasshouse under three watering regimes : daily, every three days, and every seven days; details of methods are given in Chapter 2.

Effects of contrasting watering regimes on Festuca rubra

Festuca rubra was given three watering treatments for a 10 week period and then harvested.

The number of tillers produced by plants was significantly affected by the watering regime (Table 4.1). All plants invariably produced a larger number of tillers with moderate watering; the minimum number produced with daily watering. However, plants from the different sites of the dune system produced different numbers of tillers over the three watering regimes, with plants from the drier sites (Sites 4,6 and 7) producing significantly higher numbers of tillers than those from the wetter sites (Site 1,2 and 3).

Leaf production was also affected by the watering regime in the same way as tiller production, the highest number of leaves being produced by plants under the moderate watering regime (Table 4.2). Differences in leaf production between plants from different sites was notable but it did not follow the same trend as tiller production.

Table 4.1 Number of tillers per plant of Festuca rubra, from different sites, grown under three watering regimes for ten weeks in a heated glasshouse.

		WATERING REGIME		
		DAILY	EVERY THREE DAYS	EVERY SEVEN DAYS
SITE NO.	1	8.3 ± 1.3	24.3 ± 1.2	9.7 ± 2.9
	2	7.0 ± 2.6	35.0 ± 4.4	12.0 ± 2.5
	3	9.3 ± 1.3	40.0 ± 6.8	8.3 ± 2.6
	4	23.3 ± 0.9	51.7 ± 2.9	21.3 ± 1.8
	5	11.0 ± 1.2	29.7 ± 4.1	15.7 ± 1.2
	6	24.3 ± 2.3	52.0 ± 5.0	26.3 ± 4.2
	7	18.7 ± 0.7	43.0 ± 4.6	26.7 ± 7.1

HSD (0.05) FOR WATERING REGIME = 4.5

HSD (0.05) FOR SITE = 8.8

(HSD, obtained from Tables of Q, $\frac{Q.05}{\sqrt{2}} \cdot \sqrt{\frac{2S^2}{n}}$)

ANALYSIS OF VARIANCE

SOURCE	MEAN SQUARE	P
SITE	574.76	0.001
WATERING REGIME	3908.30	0.001
INTERACTION	48.56	ns
RESIDUAL	36.03	

Table 4.2 Number of leaves per plant of Festuca rubra, from different sites, grown under three watering regimes for ten weeks in a heated glasshouse.

		WATERING REGIME		
		DAILY	EVERY THREE DAYS	EVERY SEVEN DAYS
SITE NO.	1	57.0 ± 5.6	146.0 ± 4.5	75.3 ± 5.9
	2	64.7 ± 11.3	190.7 ± 14.7	76.0 ± 3.2
	3	77.0 ± 14.8	247.0 ± 5.7	81.0 ± 5.6
	4	141.3 ± 4.9	271.3 ± 21.8	162.0 ± 13.1
	5	63.3 ± 10.8	198.3 ± 8.8	65.7 ± 13.1
	6	130.7 ± 15.5	268.3 ± 27.4	148.7 ± 20.7
	7	87.7 ± 12.6	189.0 ± 9.3	158.7 ± 15.6

HSD (0.05) FOR WATERING REGIME = 24.9

HSD (0.05) FOR SITE = 48.5

ANALYSIS OF VARIANCE

SOURCE	MEAN SQUARE	P
SITE	32478.19	0.001
WATERING REGIME	3848.30	0.001
INTERACTION	1578.67	ns
RESIDUAL	1102.02	

Effect of watering regime on total leaf length was highly significant, and plants from different sites reacted differently to the treatment (Table 4.3). For example plants from site 1 (wet) did not show reduced total leaf length under daily watering, whereas those from the dry sites (Sites 5 and 7) showed reduced total leaf length under daily watering. However they maintained higher total leaf length when watered weekly. This was supported by the significant interaction between watering regime and collection site shown by the analysis of variance. Generally plants from the dry sites had shorter leaves and a more bushy appearance.

The watering regime had a profound effect on leaf dry weight; this was lower with daily watering and weekly watering than with the intermediate treatment (Table 4.4).

All plants produced maximum stem dry weight when watered every three days (Table 4.5); the weight was more than halved with daily watering. Plants from different sites showed differences in their stem dry weight production. The same was true for root dry weight (Table 4.6).

However plants from the different sites showed significantly different root:shoot ratios, and this was affected by the different watering regimes (Table 4.7). In general plants had a lower root:shoot ratio the more frequent the watering.

Dry weight of the whole plants was highly affected by the frequency of watering (Table 4.8). Dry matter production was reduced by up to 60% by daily watering,as compared to watering every three days. The latter treatment also gave the maximum dry matter production. Under weekly watering dry matter was lower, but not as low as with daily watering. However, analysis of variance did not show differences in total dry matter production of plants from the different sites to be

Table 4.3 Total leaf length (m) per plant of Festuca rubra, from different sites, grown under three watering regimes for ten weeks in a heated glasshouse.

		WATERING REGIME		
		DAILY	EVERY THREE DAYS	EVERY SEVEN DAYS
SITE NO.	1	5.01 ± 1.15	5.60 ± 0.24	5.40 ± 0.76
	2	3.32 ± 0.33	5.59 ± 1.12	5.05 ± 1.05
	3	3.57 ± 0.33	7.67 ± 0.45	7.25 ± 0.61
	4	5.55 ± 0.84	7.33 ± 0.87	6.94 ± 0.16
	5	3.74 ± 0.27	10.16 ± 0.97	8.79 ± 0.99
	6	3.50 ± 0.62	8.49 ± 1.13	5.64 ± 1.04
	7	5.27 ± 0.99	10.07 ± 1.58	5.55 ± 0.38

$HSD_{(0.05)}$ = 4.55m

ANALYSIS OF VARIANCE

SOURCE	MEAN SQUARE	P
SITE	8.7740	0.01
WATERING REGIME	67.3085	0.001
INTERACTION	4.9781	0.05
RESIDUAL	2.1468	

Table 4.4 Leaf dry weight (g) of Festuca rubra plants, from different sites, grown under three watering regimes for ten weeks in a heated glasshouse.

	WATERING REGIME		
	DAILY	EVERY THREE DAYS	EVERY SEVEN DAYS
SITE NO. 1	0.37 ± 0.02	1.09 ± 0.06	0.59 ± 0.03
2	0.57 ± 0.06	1.03 ± 0.06	0.57 ± 0.04
3	0.55 ± 0.07	1.03 ± 0.04	0.54 ± 0.04
4	0.48 ± 0.04	1.19 ± 0.08	0.59 ± 0.05
5	0.45 ± 0.01	0.96 ± 0.06	0.52 ± 0.02
6	0.45 ± 0.03	0.92 ± 0.08	0.53 ± 0.05
7	0.49 ± 0.04	1.14 ± 0.14	0.71 ± 0.10

HSD (0.05) FOR WATERING REGIME = 0.08g

HSD (0.05) FOR SITE = 0.15g

ANALYSIS OF VARIANCE

SOURCE	MEAN SQUARE	P
SITE	0.0276	0.05
WATERING REGIME	1.9788	0.001
INTERACTION	0.0131	ns
RESIDUAL	0.0106	

Table 4.5 Stem dry weight (g) per plant of Festuca rubra, from different sites, grown under three watering regimes for ten weeks in a heated glasshouse.

		WATERING REGIME		
		DAILY	EVERY THREE DAYS	EVERY SEVEN DAYS
SITE NO.	1	0.15 ± 0.004	0.52 ± 0.003	0.24 ± 0.02
	2	0.27 ± 0.04	0.68 ± 0.04	0.32 ± 0.02
	3	0.25 ± 0.02	0.57 ± 0.03	0.22 ± 0.01
	4	0.29 ± 0.03	0.73 ± 0.05	0.38 ± 0.08
	5	0.31 ± 0.03	0.52 ± 0.10	0.25 ± 0.02
	6	0.29 ± 0.06	0.42 ± 0.09	0.37 ± 0.06
	7	0.25 ± 0.03	0.65 ± 0.04	0.45 ± 0.13

HSD (0.05) FOR WATERING REGIME = 0.07g

HSD (0.05) FOR SITE = 0.14g

ANALYSIS OF VARIANCE

SOURCE	MEAN SQUARE	P
SITE	0.0314	0.01
WATERING REGIME	0.6398	0.001
INTERACTION	0.0172	ns
RESIDUAL	0.0091	

Table 4.6 Root dry weight (g) per plant of Festuca rubra, from different sites, grown under three watering regimes for ten weeks in a heated glasshouse.

		WATERING REGIME		
		DAILY	EVERY THREE DAYS	EVERY SEVEN DAYS
SITE NO.	1	0.73 ± 0.07	2.11 ± 0.14	1.13 ± 0.07
	2	1.17 ± 0.12	2.43 ± 0.27	1.07 ± 0.05
	3	1.38 ± 0.33	2.87 ± 0.07	1.48 ± 0.06
	4	1.03 ± 0.15	3.46 ± 0.48	1.67 ± 0.39
	5	1.08 ± 0.04	3.14 ± 0.31	1.64 ± 0.45
	6	1.08 ± 0.21	2.69 ± 0.47	1.39 ± 0.15
	7	1.00 ± 0.04	2.47 ± 0.56	1.45 ± 0.25

HSD (0.05) FOR WATERING REGIME = 0.36g

HSD (0.05) FOR SITE = 0.70g

ANALYSIS OF VARIANCE

SOURCE	MEAN SQUARE	P
SITE	0.5851	0.05
WATERING REGIME	16.4024	0.001
INTERACTION	0.1594	ns
RESIDUAL	0.2294	

Table 4.7 Root:Shoot ratio of Festuca rubra plants, from different sites, grown under three watering regimes for ten weeks in a heated glasshouse.

		WATERING REGIME		
		DAILY	EVERY THREE DAYS	EVERY SEVEN DAYS
	1	1.40 ± 0.08	1.31 ± 0.05	1.33 ± 0.02
	2	1.42 ± 0.09	1.42 ± 0.01	1.20 ± 0.12
SITE	3	1.69 ± 0.15	1.79 ± 0.15	1.96 ± 0.12
NO.	4	1.34 ± 0.09	1.78 ± 0.13	1.76 ± 0.08
	5	1.50 ± 0.05	2.13 ± 0.16	2.08 ± 0.48
	6	1.46 ± 0.11	1.98 ± 0.17	1.55 ± 0.08
	7	1.36 ± 0.18	1.35 ± 0.06	1.26 ± 0.09

HSD (0.05) FOR WATERING REGIME = 0.05

HSD (0.05) FOR SITE = 0.10

ANALYSIS OF VARIANCE

SOURCE	MEAN SQUARE	P
SITE	0.6440	0.001
WATERING REGIME	0.2686	0.05
INTERACTION	0.0344	ns
RESIDUAL	0.0695	

Table 4.8 Total plant dry weight (g) of Festuca rubra, from different sites, grown under three watering regimes for ten weeks in a heated glasshouse.

		WATERING REGIME		
		DAILY	EVERY THREE DAYS	EVERY SEVEN DAYS
SITE NO.	1	1.25 ± 0.08	3.72 ± 0.19	1.98 ± 0.11
	2	2.00 ± 0.21	4.15 ± 0.47	1.96 ± 0.11
	3	2.18 ± 0.42	4.47 ± 0.13	2.24 ± 0.09
	4	1.80 ± 0.60	5.38 ± 0.59	2.64 ± 0.51
	5	1.81 ± 0.08	4.62 ± 0.44	2.42 ± 0.48
	6	1.81 ± 0.30	4.04 ± 0.64	2.29 ± 0.25
	7	1.75 ± 0.11	4.26 ± 0.73	2.61 ± 0.45

HSD (0.05) FOR WATERING REGIME = 0.47g

HSD (0.05) FOR SITE = 0.92g

ANALYSIS OF VARIANCE

SOURCE	MEAN SQUARE	P
SITE	0.6982	ns
WATERING REGIME	39.1995	0.001
INTERACTION	0.3482	ns
RESIDUAL	0.3970	

significant, despite significant differences shown in leaf, stem and root dry weights when taken separately.

Effect of contrasting watering regimes on Trifolium repens.

Plants of T. repens from 4 different sites grown under uniform conditions in the glasshouse manifested clear, site-specific differences in their growth and morphological features such as leaf and stolon size, and petiole length (Plates 4.1a and b).

The effect of different watering treatments gave a response distinct from that for F. rubra. Plants produced the highest number of leaves under daily waterings, and those from the two wet sites (Site 1 and 3) showed this effect most strongly. Plants from Site 1 had a leaf number reduced by 60% when watered only weekly (Table 4.9). Plants from Site 2 (intermediate in its moisture status) showed no difference in the number of leaves they produced between the two most frequent watering treatments, but showed a 30% decrease with weekly watering. On the other hand plants from Site 7 (dry) were not adversely affected by the lowest frequency of watering, producing the same number of leaves in each treatment.

Total leaf area of plants from Site 2 was highest when watered every three days (Table 4.10). This was decreased by about 25% with daily watering, and by nearly 50% with weekly watering. Plants from wet sites (1 and 3) showed maximum total leaf area with daily watering with decreases of about 70% with watering weekly. However, watering only weekly did not reduce total leaf area of plants from the dry site. Plants from wet sites generally produced larger total leaf area than those from drier sites.

Plate 3.1 Plate showing the differential growth of Trifolium repens plants collected from different sites on the dune system when grown under the uniform conditions of the glasshouse. (a) From left to right plants are from sites 4, 1, 2 and 7.

(b) From left to right plants from sites 2, 7, 1 and 4.

Plate 4.1,a

Plate 4.1,b

Table 4.9 Number of leaves per plant of Trifolium repens, from 4 different sites, grown under three watering regimes for eight weeks in a heated glasshouse.

		WATERING REGIME		
		DAILY	EVERY THREE DAYS	EVERY SEVEN DAYS
SITE NO.	1	193.0 ± 4.0	105.8 ± 10.2	73.3 ± 17.0
	2	137.8 ± 4.6	138.0 ± 6.7	96.3 ± 4.1
	3	138.8 ± 12.8	72.0 ± 12.3	67.5 ± 6.7
	7	106.8 ± 3.9	106.5 ± 9.4	105.3 ± 6.5

HSD (0.05) = 45.15

ANALYSIS OF VARIANCE

SOURCE	MEAN SQUARE	P
SITE	2705.07	0.001
WATERING REGIME	13888.00	0.001
INTERACTION	3342.56	0.001
RESIDUAL	334.14	

Table 4,10 Total Leaf Area per plant (cm^2) of Trifolium repens from 4 different sites, grown under three watering regimes for eight weeks in a heated glasshouse.

		WATERING REGIME		
		DAILY	EVERY THREE DAYS	EVERY SEVEN DAYS
SITE NO.	1	135,04 ± 5,31	63,93 ± 15,41	43,92 ± 17,24
	2	53,70 ± 5,84	73,88 ± 9,55	38,96 ± 1,07
	3	336,08 ± 26,92	163,75 ± 27,09	86,45 ± 7,52
	7	49,44 ± 1,84	43,53 ± 4,12	34,62 ± 3,18

HSD (0.05) = 67,43cm^2

Analysis of Variance

SOURCE	MEAN SQUARE	P
SITE	23151.15	0.001
WATERING REGIME	87725.08	0.001
INTERACTION	13732.44	0.001
RESIDUAL	745.15	

The effect of frequency of watering on leaf dry weight per plant was highly significant, especially for plants from wet sites. Plants from Site 3 showed reduction in this character with watering weekly compared to daily (Table 4.11), whilst plants from Site 1 had their leaf dry weight halved. Plants from Site 2, however, performed best when they were watered every three days, with a decrease of about 15% under daily, and larger decrease with weekly, watering. Plants from the dry site (Site 7) showed a lower leaf dry matter production the less frequent the watering.

There was significant variation in stolon size between plants from differentsites. Those from the dry and intermediate sites (7 and 2 respectively) had much thinner stolons than those from the wet sites; plants from Site 3 had particularly thick stolons. The watering regime had a profound effect on stolon dry weight (which here includes petioles). Plants from the two wet sites (1 and 3) showed stolon dry weights decreased by 60-70% from daily down to weekly watering (Table 4.12). Plants from Site 2 had their highest stolon dry weights with daily watering, whilst stolons were about 13% lighter with intermediate, and 60% with weekly, watering. The intermediate frequency of watering increased the stolon dry weight in the case of plants from Site 7 (dry), whilst the weekly watering did not reduce it significantly, relative to daily watering.

Table 4.13 shows results for root dry weight measurement. Here again plants from the wet sites had maximum root production with frequent watering, and that decreased significantly with the other two treatments. Site 2 plants had a 30% decrease in their root production when watered only weekly. However plants from the dry site (Site 7) showed greater root dry matter production with decreasing frequency of watering.

Table 4.11 Leaf dry weight (g) per plant of Trifolium repens, from 4 different sites, grown under three watering regimes in a heated glasshouse for eight weeks.

		WATERING REGIME		
		DAILY	EVERY THREE DAYS	EVERY SEVEN DAYS
SITE NO.	1	0.56 ± 0.03	0.29 ± 0.06	0.23 ± 0.05
	2	0.28 ± 0.02	0.34 ± 0.02	0.20 ± 0.02
	3	1.34 ± 0.10	0.67 ± 0.13	0.46 ± 0.03
	7	0.25 ± 0.03	0.24 ± 0.03	0.22 ± 0.02

HSD (0.05) = 0.28g

ANALYSIS OF VARIANCE

SOURCE	MEAN SQUARE	P
SITE	0.8791	0.001
WATERING REGIME	0.4624	0.001
INTERACTION	0.1811	0.001
RESIDUAL	0.0131	

Table 4.12 Stolon dry weight (g) per plant of Trifolium repens from 4 different sites, grown under three watering regimes in a heated glasshouse for eight weeks.

		WATERING REGIME		
		DAILY	EVERY THREE DAYS	EVERY SEVEN DAYS
SITE NO.	1	1.16 ± 0.09	0.50 ± 0.12	0.43 ± 0.14
	2	0.80 ± 0.05	0.69 ± 0.05	0.30 ± 0.03
	3	2.61 ± 0.23	1.17 ± 0.29	0.86 ± 0.12
	7	0.61 ± 0.08	0.68 ± 0.13	0.54 ± 0.08

HSD (0.05) = 0.67g

ANALYSIS OF VARIANCE

SOURCE	MEAN SQUARE	P
SITE	2.6930	0.001
WATERING REGIME	2.6983	0.001
INTERACTION	0.5789	0.001
RESIDUAL	0.0730	

Table 4.13 Root dry weight (g) per plant of Trifolium repens from 4 different sites, grown under three watering regimes for eight weeks in a heated glasshouse.

		WATERING REGIME		
		DAILY	EVERY THREE DAYS	EVERY SEVEN DAYS
SITE NO.	1	0.74 ± 0.02	0.41 ± 0.13	0.22 ± 0.02
	2	0.32 ± 0.08	0.22 ± 0.02	0.20 ± 0.05
	3	1.08 ± 0.12	0.54 ± 0.11	0.38 ± 0.05
	7	0.21 ± 0.06	0.24 ± 0.05	0.33 ± 0.08

HSD (0.05) = 0.41g

ANALYSIS OF VARIANCE

SOURCE	MEAN SQUARE	P
SITE	0.4908	0.001
WATERING REGIME	0.4949	0.001
INTERACTION	0.1151	0.01
RESIDUAL	0.0276	

Table 4.13 shows results for root dry weight measurement. Here again plants from the wet sites had maximum root production with frequent watering, and that decreased significantly with the other two treatments. Site 2 plants had a 30% decrease in their root production when watered every three days compared to those watered daily, with only small decreases when watered only weekly. However, plants from the dry site (Site 7) showed greater root dry matter production with decreasing frequency of watering.

Total plant dry weight showed that T. repens plants behaved quite differently from those of F. rubra under these experimental treatments. As can be seen from the analysis of variance (Table 4.14) there was a highly significant interaction between watering regime and site for this species. Also shown by Table 4.14 was the site-specific response of plants to the moisture status of the soil. Plants from the two wet sites, and those from the intermediate one, had their dry weights more than halved when watered only weekly compared to daily watering, but in the case of plants from the dry site (7) there was no significant difference between the three treatments.

When root:shoot ratio was calculated (Table 4.15), highly significant differences were seen between plants from the different sites. Plants showed an increased ratio with weekly watering, those from the dry site (7) having the highest ratio, Site 2 (intermediate) being next and plants from the wet sites having the lowest ratio. Plants from drier sites thus seemed to have greater ability to increase their root:shoot ratio under dry soil conditions than those from wet sites.

Specific leaf area was substantially affected by the watering regime (Table 4.16). All plants with the exception of those from Site 2 greatly reduced their specific leaf area under infrequent watering, with those from the dry site having the lowest specific leaf area.

Table 4.14 Total plant dry weight (g) of Trifolium repens,from 4 different sites,grown under three watering regimes for eight weeks in a heated glasshouse.

		WATERING REGIME		
		DAILY	EVERY THREE DAYS	EVERY SEVEN DAYS
SITE NO.	1	2,46 ± 0,20	1,20 ± 0,17	0,87 ± 0,15
	2	1,40 ± 0,12	1,25 ± 0,13	0,71 ± 0,07
	3	5,03 ± 0,45	2,37 ± 0,51	1,71 ± 0,20
	7	1,07 ± 0,16	1,16 ± 0,14	1,09 ± 0,18

HSD (0,05) = 1,22g

ANALYSIS OF VARIANCE

SOURCE	MEAN SQUARE	P
SITE	9,9609	0,001
WATERING REGIME	8,5298	0,001
INTERACTION	2,2603	0,001
RESIDUAL	0,2459	

Table 4.15 Root:Shoot Ratio of Trifolium repens, from 4 different sites, grown under three watering regimes for eight weeks in a heated glasshouse.

		WATERING REGIME		
		DAILY	EVERY THREE DAYS	EVERY SEVEN DAYS
SITE NO.	1	0.44 ± 0.02	0.47 ± 0.08	0.34 ± 0.04
	2	0.29 ± 0.05	0.22 ± 0.01	0.40 ± 0.05
	3	0.27 ± 0.01	0.30 ± 0.04	0.29 ± 0.01
	7	0.23 ± 0.04	0.26 ± 0.03	0.43 ± 0.06

HSD (0.05) = 0.20

ANALYSIS OF VARIANCE

SOURCE	MEAN SQUARE	P
SITE	0.0473	0.001
WATERING REGIME	0.0241	0.05
INTERACTION	0.0193	0.05
RESIDUAL	0.0068	

Table 4.16 Specific Leaf Area ($cm^2 gdw^{-1}$) of Trifolium repens from 4 different sites, grown under three watering regimes for eight weeks in a heated glasshouse.

		WATERING REGIME		
		DAILY	EVERY THREE DAYS	EVERY SEVEN DAYS
SITE NO.	1	241.66 ± 4.84	216.75 ± 15.79	172.84 ± 27.37
	2	192.49 ± 6.60	216.37 ± 12.78	204.75 ± 21.15
	3	250.07 ± 6.46	253.47 ± 15.21	189.76 ± 6.73
	7	195.52 ± 15.66	183.66 ± 12.28	158.48 ± 2.95

HSD (0.05) FOR WATERING REGIME = 24.57 $cm^2 gdw^{-1}$

HSD (0.05) FOR SITE = 31.37 $cm^2 gdw^{-1}$

ANALYSIS OF VARIANCE

SOURCE	MEAN SQUARE	P
SITE	5412.73	0.001
WATERING REGIME	7485.66	0.001
INTERACTION	1522.24	ns
RESIDUAL	806.59	

Plants from different sites showed marked differences in their mean area per leaf (Table 4.17). Plants from the dry and intermediate sites had smaller leaves than those from the wet sites, especially plants from Site 3 which had large, broad leaves. However, the watering frequency greatly affected the mean area per leaf ; it was substantially reduced by infrequent watering. Again plants from the dry site had the smallest area per leaf.

Table 4.17 Area per leaf (cm^2) of Trifolium repens, from 4 different sites, grown under three watering regimes, for eight weeks in a heated glasshouse.

		WATERING REGIME		
		DAILY	EVERY THREE DAYS	EVERY SEVEN DAYS
SITE NO.	1	0.70 ± 0.01	0.58 ± 0.09	0.55 ± 0.08
	2	0.39 ± 0.03	0.53 ± 0.05	0.41 ± 0.01
	3	2.43 ± 0.05	2.29 ± 0.07	1.30 ± 0.09
	7	0.45 ± 0.01	0.41 ± 0.01	0.33 ± 0.01

HSD (0.05) = $0.27cm^2$

ANALYSIS OF VARIANCE

SOURCE	MEAN SQUARE	P
SITE	7.0650	0.001
WATERING REGIME	0.5810	0.001
INTERACTION	0.3373	0.001
RESIDUAL	0.01176	

CHAPTER FIVE

PHYSIOLOGICAL INVESTIGATIONS

Plant Water Relations

Water potentials of plants growing on the dune system were given above. They indicated that as soil water potential drops, plant leaf water and osmotic potentials drop as well, and turgor is maintained. However, plants growing on the wet site had not experienced really low soil water potentials. The glasshouse growth experiment also showed that when plants were grown on dry soil, growth of the plants from the wet sites was more adversely affected than those from the dry sites. An experiment was therefore conducted to see the effect of low soil water potential on plant water relations, and to see if there is any differential response to low water potentials between plants from the wet and dry sites.

In order to apply low water potentials consistently, polyethylene glycol (m.w. 4000) was dissolved in Long Ashton solution to give an osmotic potential of -1.0MPa. T. repens plants from Site 1 (wet) and Site 7 (dry) were used for the experiment. After the polyethylene glycol solution was added subsequent measurements of leaf relative water content, water and osmotic potentials were taken, and leaf pressure potential was obtained by difference, assuming the matric potential to be negligible

Effect of low water potential in the growth medium on the relative water content, water, osmotic, and pressure potentials of Trifolium repens leaves

Measurements were taken daily for the first seven days after polyethylene glycol was added, then every other day for the next fourteen days. Leaf water potential dropped sharply in the first five days after the start of the treatment for plants from either site (Figure 5.1 and 5.2). Plants from the wet site, however, started

showing signs of wilting on the first day although the pressure potential remained positive. Leaf water potential of plants from the dry site carried on falling rapidly reaching -2.1 MPa three weeks after polyethylene glycol was added. Plants from the wet site did not show as much decrease after the first five days, and they had leaf water potentials around -1.7 MPa three weeks after the treatment was started (Figure 5.1). Differences in the osmotic potentials between plants from the two sites were quite striking. Those from the dry site had a big drop in their osmotic potentials, of about 0.7 MPa, in the first five days, whilst at the same time plants from the wet site decreased their osmotic potentials by only about 0.25 MPa. Osmotic potentials of plants from the wet site continued decreasing slowly reaching -1.7 MPa three weeks from the start of the treatment. Further decreases in plants from the dry site were very small, and the osmotic potential measured about -2.1 MPa after twenty one days. These changes were reflected in the pressure potential of the plants which dropped steadily from about 0.65 MPa on the first day, for plants from the wet site, reaching zero values after 10-12 days (Figure 5.1), whilst those from the dry site showed pressure potentials which were virtually unchanged for the first four days, but then started dropping gradually and reaching zero after 16 days from the start of the experiment (Figure 5.2). Plants from the wet site showed a steady decline in their relative water content from about 95% at the beginning reaching around 50% 21 days later. However, plants from the dry site showed a decline from 94% to 80% over the first 12 days but after that their relative water content fell sharply to about 55% after 21 days (Figure 5.2).

Figure 5.1 Change, with time after transfer to polyethylene glycol (m.w. 4000), in leaf relative water content (□), water potential (O), osmotic potential (●), and pressure potential (X) of Trifolium repens from Site 1 (Wet).

Fig 5.1.

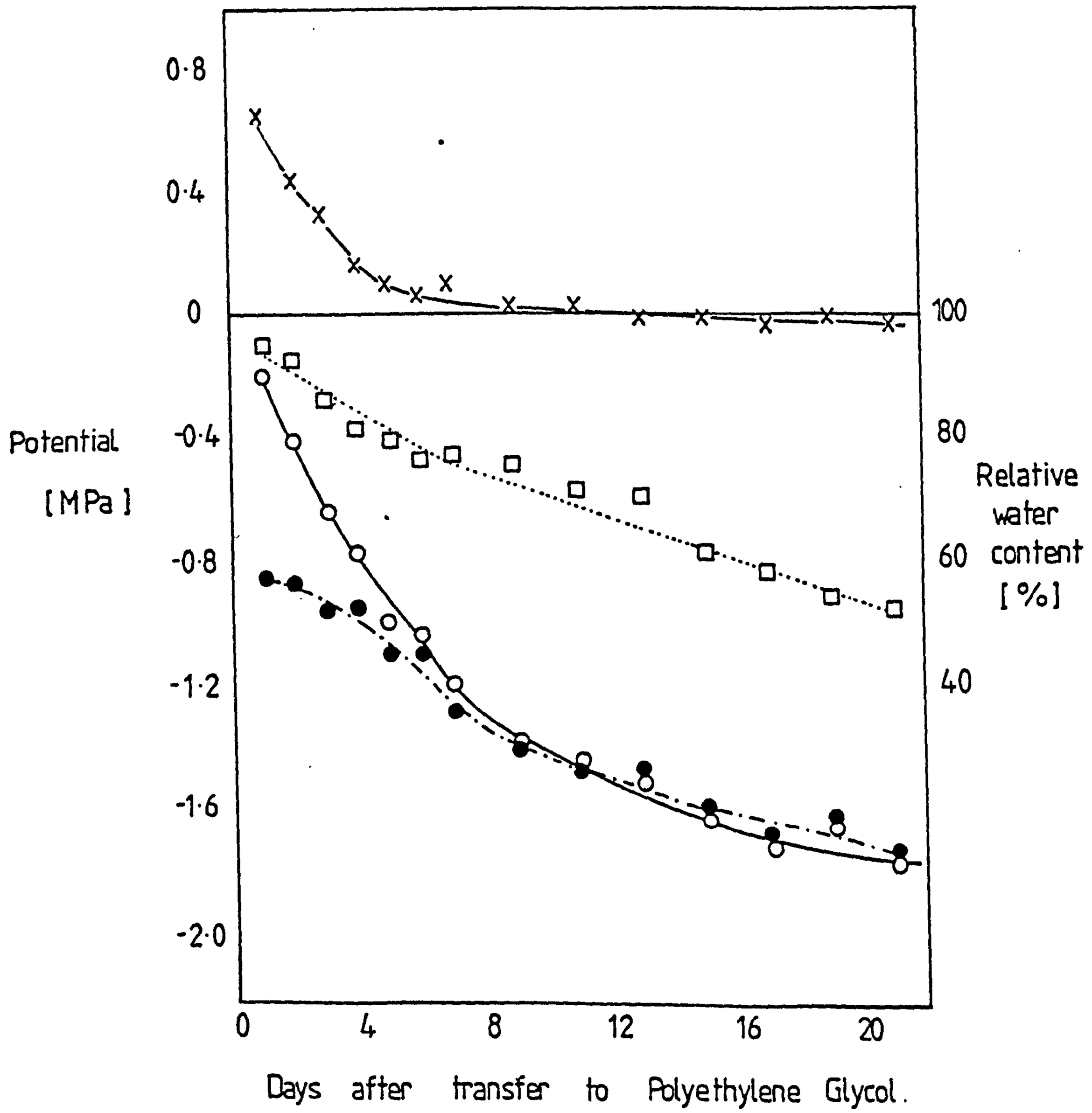
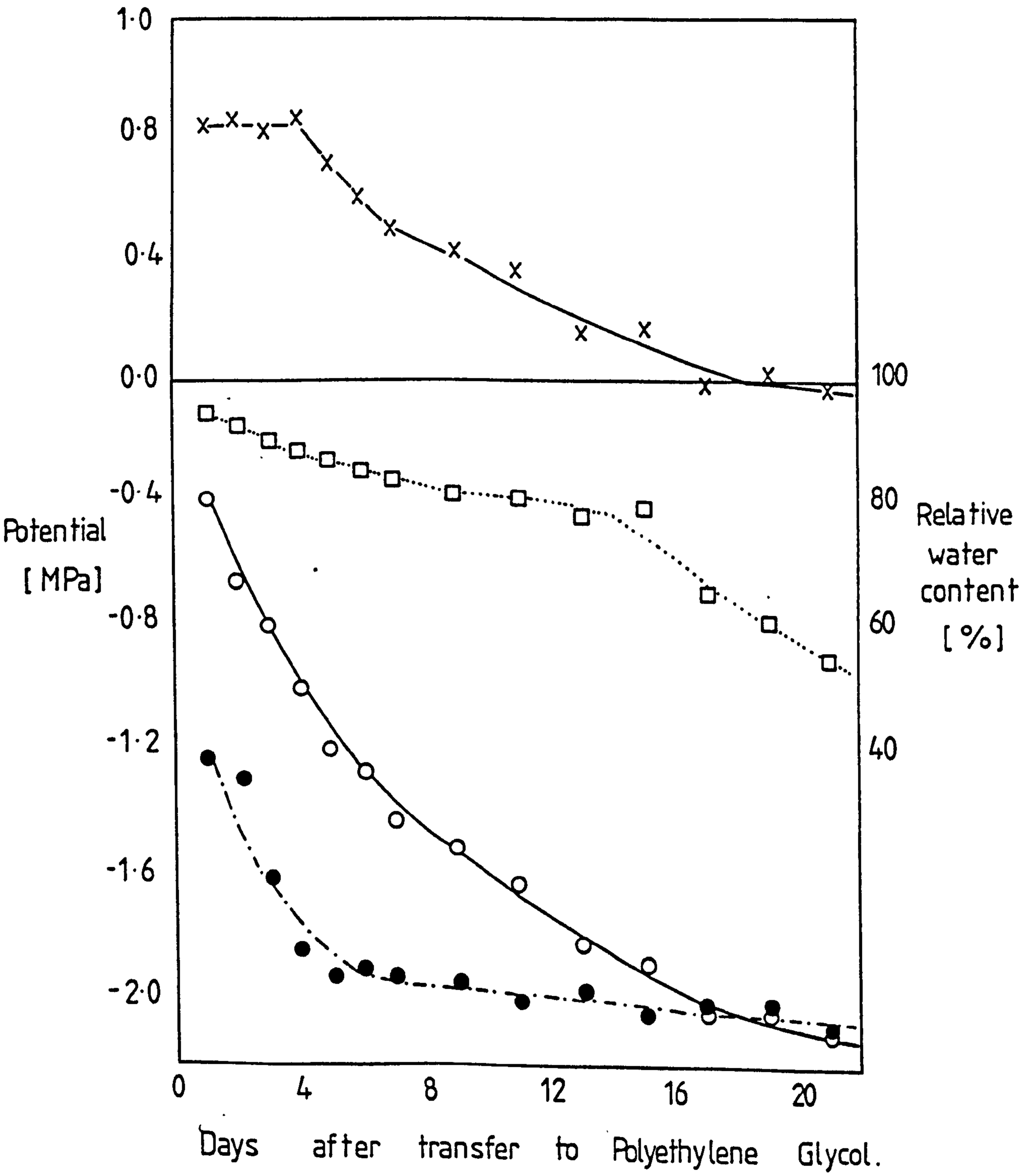

0·8
0·4
0
-0·4
-0·8
-1·2
-1·6
-2·0
Potential
[MPa]
100
80
60
40
Relative
water
content
[%]
0
4
8
12
16
20
Days after transfer to Polyethylene Glycol.

Figure 5.2 Change with time after transfer to polyethylene glycol (m.w. 4000), in leaf relative water content (□), water potential (O), osmotic potential (●), and pressure potential (X) of Trifolium repens from Site 7 (dry).

Fig 5.2.


1·0
0·8
0·4
0·0
-0·4
-0·8
-1·2
-1·6
-2·0
Potential [MPa]
100
80
60
40
Relative water content [%]
0
4
8
12
16
20
Days after transfer to Polyethylene Glycol.

Relationship between leaf water potential and osmotic potential

In Figure 5.3 leaf water potential is plotted against the corresponding osmotic potential, the dashed line representing equality of osmotic and water potentials, that is zero pressure potential. In the case of plants from the dry site the relationship was linear down to leaf water potentials of about -1.0 MPa and osmotic potentials of about -1.9 MPa therefore within this range changes in osmotic potential equalled those in water potential, resulting in the maintenance of the initial turgor. Beyond this range the relationship reflected a slower rate of decline in osmotic potential relative to that in water potential resulting in the steady drop in pressure potential seen as the experimental curve approach the dashed line. This part resembles the response of plants from the wet site where there was a slower rate of osmotic potential than of water potential decrease with a steady decline in the pressure potentials.

Relationship between leaf pressure potential and relative water content

The relationship for plants from the dry site could be represented by three straight lines (Figure 5.4) and for those from the wet site by two straight lines (Figure 5.5). In the case of plants from the dry site, pressure potentials did not change as the relative content fell from 94% to about 88%; they also had higher pressure potentials at 100% RWC than those from the wet site, (0.85 and 0.68 MPa respectively). It could also be seen that at any relative water content above 70% plants from the dry site had higher leaf pressure potentials than those from the wet site. However, there was no obvious difference in the relative water content at which plants reached zero pressure potential, which was around 75%.

Figure 5.3 Leaf water potential plotted against leaf osmotic potential of Trifolium repens growing in culture solution maintained at -1.0 MPa by the addition of polyethylene glycol (m.w. 4000). Plants are from Site 1 (wet) ●——●, and Site 7 (dry) O——O. Measurements were taken for 21 days after the start of the treatment, and each point represents a single measurement of Ψ_l and Ψ_s.

Fig 5 3.

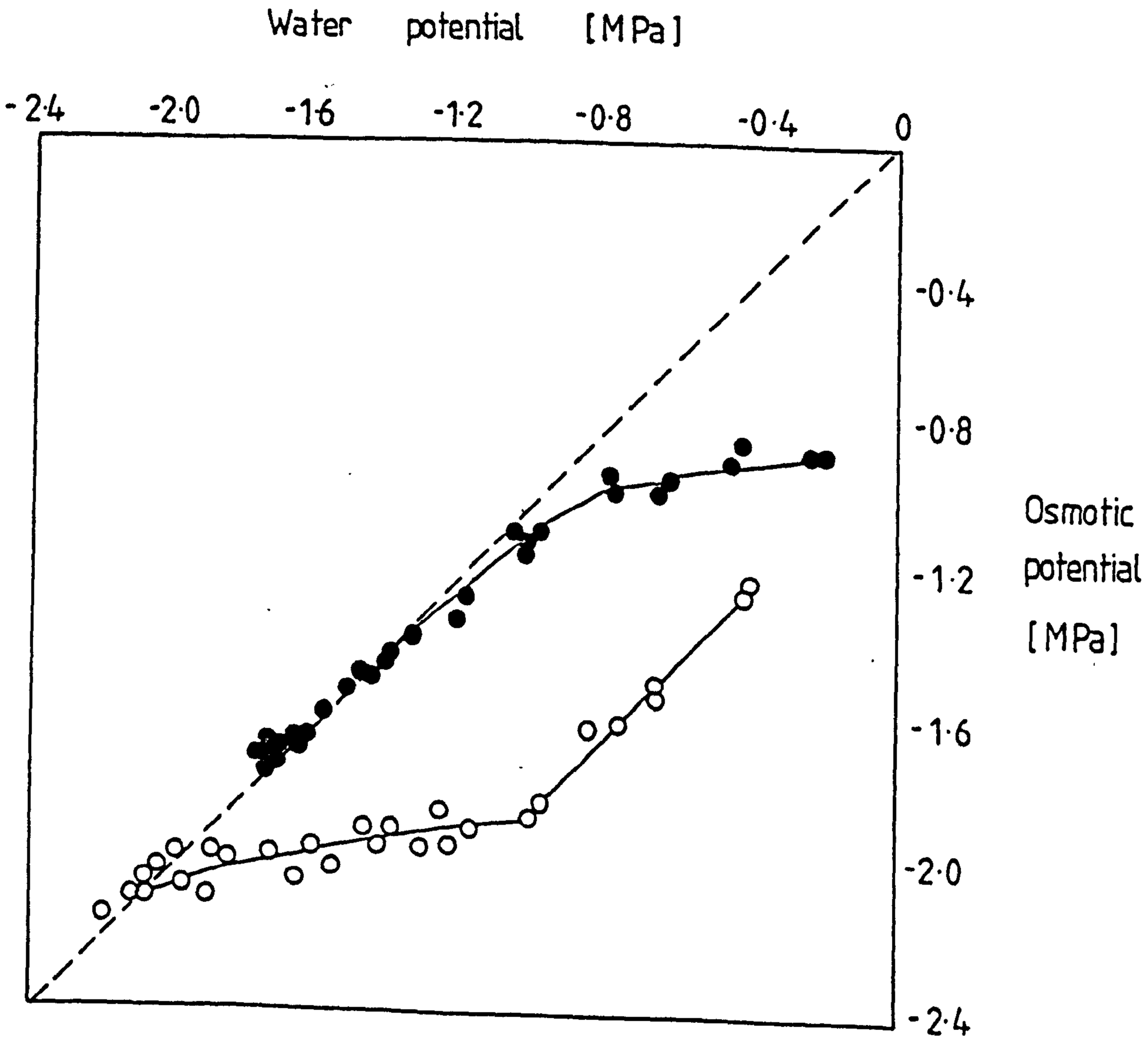

Water potential [MPa]
-2·4
-2·0
-1·6
-1·2
-0·8
-0·4
0
-0·4
-0·8
-1·2
-1·6
-2·0
-2·4
Osmotic potential [MPa]

Figure 5.4 Relationship between leaf pressure potential and relative water content of Trifolium repens from Site 7 (dry) growing in culture solution maintained at -1.0 MPa by the addition of polyethylene glycol (m.w. 4000). Each point represents a single estimate of Ψ_p and RWC, and lines are fitted linear regressions:

Line 1 : $\Psi_p = 0.0085\ RWC + 0.76\ ;\ R^2 = 0.004$

Line 2 : $\Psi_p = 0.059\ RWC - 4.44\ ;\ R^2 = 0.93$

Line 3 $\Psi_p = 0.0011\ RWC - 0.08\ ;\ R^2 = 0.05$

Fig 5.4.

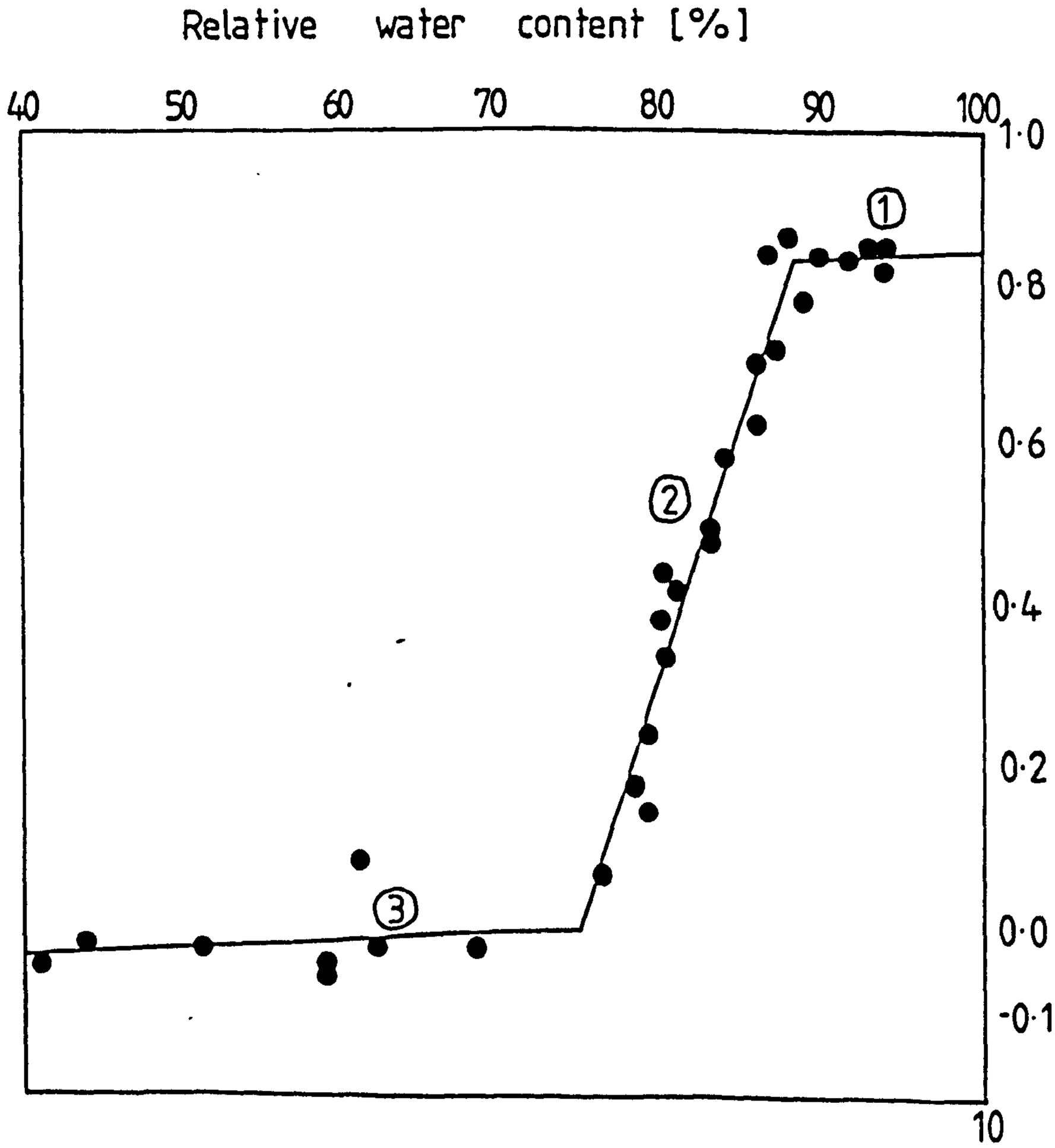
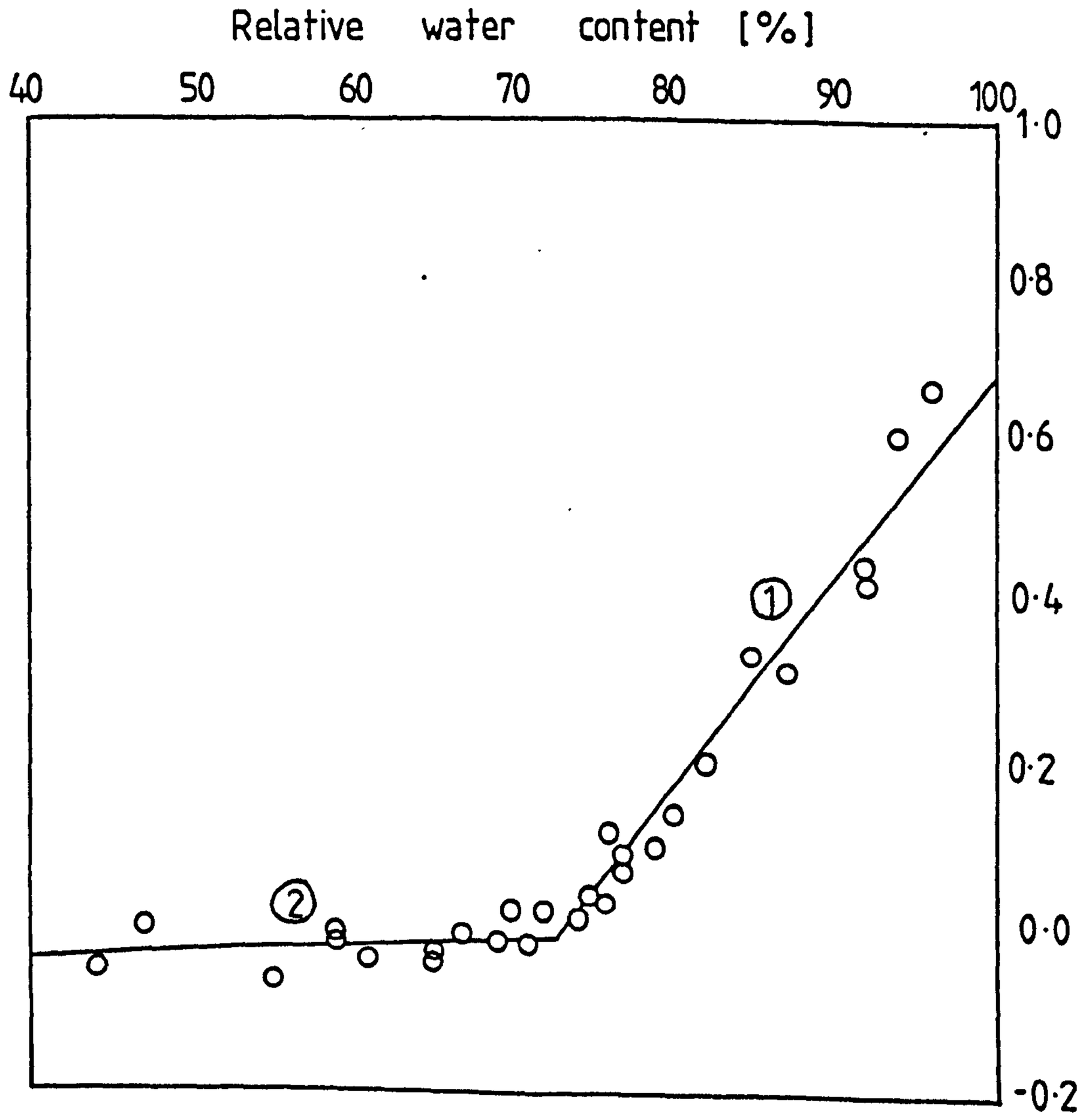

Relative water content [%]
40
50
60
70
80
90
100
1·0
0·8
0·6
0·4
0·2
0·0
-0·1
Pressure potential [MPa]
①
②
③
10

Figure 5.5 Relationship between leaf pressure potential and relative water content of Trifolium repens from Site 1 (wet) growing in culture solution maintained at -1.0 MPa by the addition of polyethylene glycol (m.w. 4000). Each point represents a single estimate of Ψ_p and RWC and lines are fitted linear regression:

Line 1 : $\Psi_p = 0.025\ RWC - 1.85;\ R^2 = 0.94$

Line 2 : $\Psi_p = 0.00075\ RWC - 0.07;\ R^2 = 0.04$

Fig 5.5.


Relative water content [%]
40
50
60
70
80
90
100
1·0
0·8
0·6
0·4
0·2
0·0
-0·2
Pressure potential [M Pa]
①
②

Relationship between leaf water potential and pressure potential

Plants from the dry site were capable of maintaining positive pressure potential at lower leaf water potentials than those from the wet site (Figure 5.6). They reached zero turgor at leaf water potential 0.6 MPa lower than those from the wet site. It is also clear from the figure that the plant from the dry site had always higher pressure potentials than those from the wet site at any leaf water potential.

Relationship between leaf water potential and relative water content

Figure 5.7 shows the relationship between leaf water potential and relative water content, which is known as the moisture release (or retention) curve. The relationship could be represented by two straight lines. Plants from the wet site showed a steady decline to about 70% relative water content below which a second linear relationship was seen. However, plants from the dry site had a more steep drop in leaf water potential with the decreasing relative water content down to about 75%, where the second linear relationship was found. Difference in the slope of the first line between the two sites indicate that this part of the relationship is site-dependent, where the second part is not, since the slope was not different between the two sites. However, the plants from the dry site had much lower water potential than those from the wet site at the same relative water content.

Figure 5.6 Relationship between leaf pressure potential and water potential of Trifolium repens growing in culture solution maintained at -1.0 MPa by the addition of polyethylene glycol (m.w. 4000). Plants are from Site 1 (wet) O——O, and Site 7 (dry) ●——●. Measurements were taken for 21 days after the start of treatment, and each point represent a single estimate of Ψ_l and Ψ_p.

Fig 5.6.

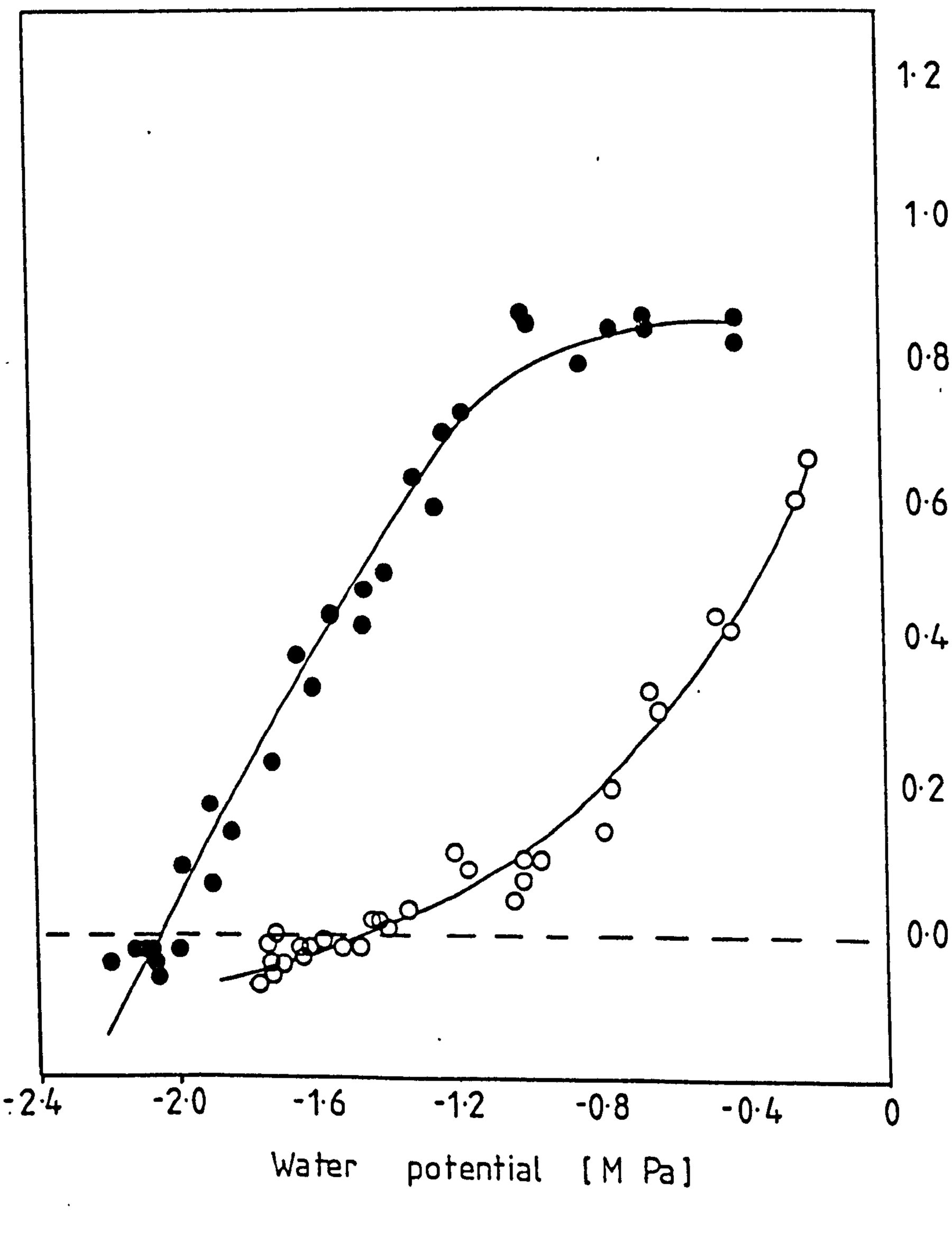

1·2
1·0
0·8
0·6
0·4
0·2
0·0
Pressure potential [M Pa]
-2·4
-2·0
-1·6
-1·2
-0·8
-0·4
0
Water potential [M Pa]

Figure 5.7 Relationship between leaf water potential and relative water content (moisture release curve) of Trifolium repens growing in culture solution maintained at -1.0 MPa by the addition of polyethylene glycol (m.w. 4000). Plants are from Site 1 (Wet) ●——●, and Site 7 (dry) O——O. Measurements were taken for 21 days after the start of treatment, and each point represents a single measurement of Ψ_1 and RWC. Lines are fitted linear regressions:

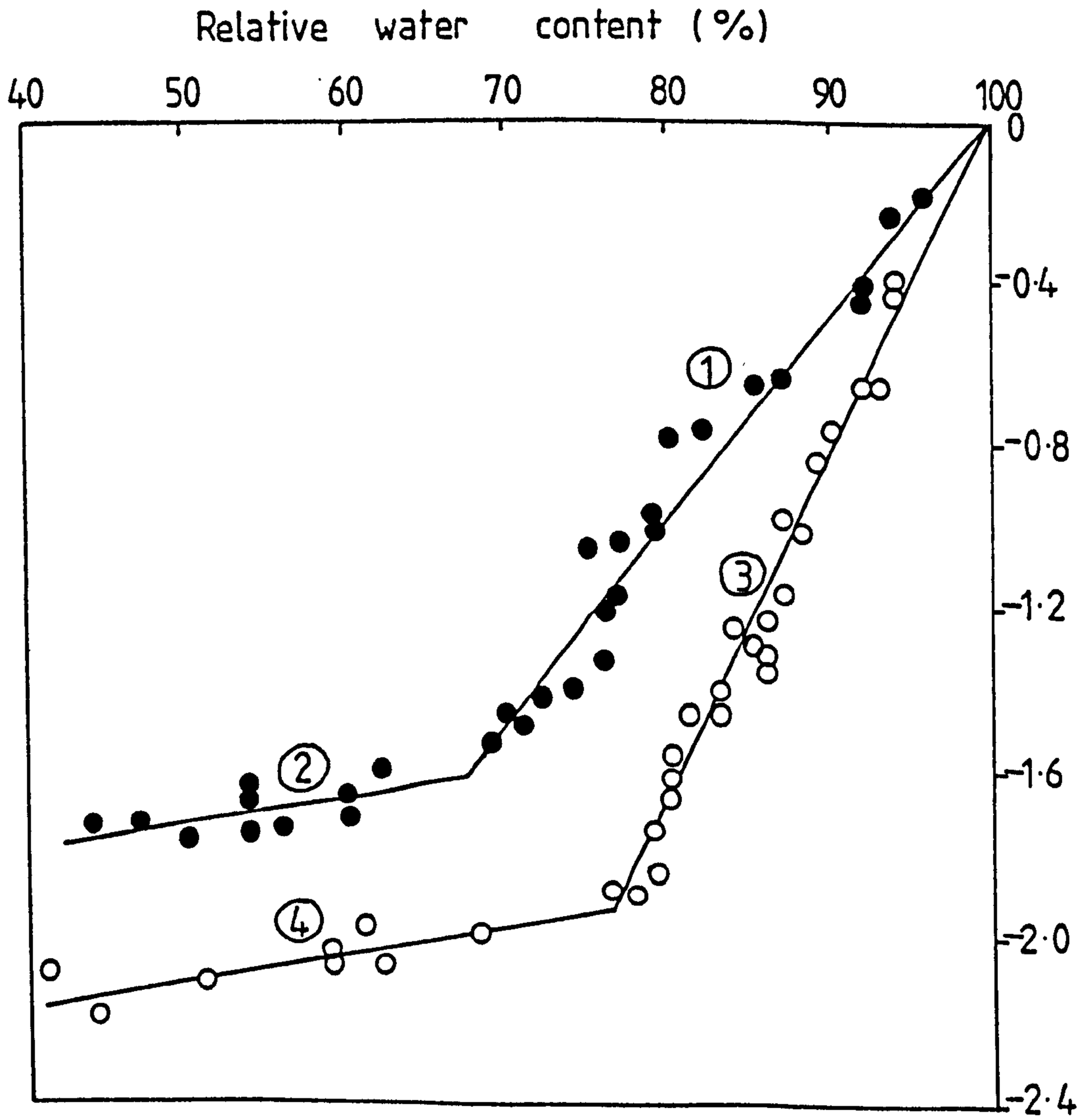
Line 1 : $\Psi_1 = 0.05$ RWC - 5.01 ; $R^2 = 0.95$

Line 2 : $\Psi_1 = 0.006$ RWC - 2.04 ; $R^2 = 0.39$

Line 3 : $\Psi_1 = 0.08$ RWC - 8.38 ; $R^2 = 0.97$

Line 4 : $\Psi_1 = 0.006$ RWC - 2.42 ; $R^2 = 0.70$

Fig. 5.7.


Relative water content (%)
40
50
60
70
80
90
100
0
-0·4
-0·8
-1·2
-1·6
-2·0
-2·4
Water Potential [M Pa]
①
②
③
④

Effect of Low Water Potential on Solutes, their Concentration and Contribution Towards the Osmotic Potential in Leaves of Trifolium repens

It was shown above that when plants in the field experienced low soil water potentials, their leaf water potential fell but they also showed lower osmotic potentials, thus maintaining turgor. When plants in the laboratory were subjected to low water potential by the addition of polyethylene glycol to the culture medium, only those from the dry site were able to maintain a high positive pressure potential at leaf water potentials as low as -1.0 MPa. This ability to maintain turgor by dropping the osmotic potential could be due to accumulation of solutes in cells, that is osmotic adjustment. This possibility has been investigated in plants growing in the field and in the laboratory.

A - Field grown plants

i - Concentration of solutes

T. repens leaf samples were collected from the dune system, from plants grown on Site 1 (wet) and Site 7 (dry) on the following dates: April 25, June 18, and July 27 (1980). They were analysed for some major constituents of low molecular weight, those found in concentrations high enough to influence the osmotic adjustments of the plants being K^+, Na^+ and the sugars sucrose, fructose and glucose. Concentrations of these solutes in tissue water of plant leaves collected from the two sites on the three days, together with their leaf water, osmotic, and pressure potentials are given in Table 5.1.

Here the osmotic potential did not change in plants on the wet site, between samples taken on April 25 and June 18, but it was 0.2 MPa lower on July 27 when the leaf water potential was also 0.2 MPa lower; thus the pressure potential was maintained.

Table 5.1 Leaf Water, Osmotic and Pressure Potentials (MPa), and the concentration of some of the major solutes (mol m^{-3} of tissue water) in leaves of Trifolium repens growing on two sites on the dune system. Samples were collected on: April 25, June 18, and July 27 (1980). Conditions and leaf water status data are the same as in Chapter 3. Each value is a mean of five samples.

APRIL 25:

	SITE 1 (WET)	SITE 7 (DRY)
LEAF WATER POTENTIAL	-0.37 ± 0.01	-0.55 ± 0.02
LEAF OSMOTIC POTENTIAL	-0.57 ± 0.01	-0.71 ± 0.02
LEAF PRESSURE POTENTIAL	0.20 ± 0.02	0.15 ± 0.01
Na^+	36 ± 2	50 ± 2
K^+	43 ± 2	61 ± 2
SUCROSE	15 ± 1	11 ± 1
FRUCTOSE	29 ± 2	10 ± 1
GLUCOSE	30 ± 2	27 ± 2
TOTAL	153 ± 6	159 ± 8

JUNE 18:

LEAF WATER POTENTIAL	-0.38 ± 0.02	-0.75 ± 0.02
LEAF OSMOTIC POTENTIAL	-0.57 ± 0.02	-1.00 ± 0.02
LEAF PRESSURE POTENTIAL	0.19 ± 0.01	0.24 ± 0.02
Na^+	35 ± 2	50 ± 2
K^+	49 ± 1	131 ± 1
SUCROSE	21 ± 1	17 ± 1
FRUCTOSE	24 ± 2	11 ± 1
GLUCOSE	32 ± 2	22 ± 2
TOTAL	161 ± 5	232 ± 12

JULY 27:

LEAF WATER POTENTIAL	-0.58 ± 0.01	-1.00 ± 0.03
LEAF OSMOTIC POTENTIAL	-0.79 ± 0.01	-1.29 ± 0.02
LEAF PRESSURE POTENTIAL	0.21 ± 0.01	0.29 ± 0.02
Na^+	51 ± 1	72 ± 3
K^+	72 ± 2	171 ± 3
SUCROSE	17 ± 2	11 ± 2
FRUCTOSE	26 ± 2	15 ± 1
GLUCOSE	58 ± 3	29 ± 2
TOTAL	224 ± 10	298 ± 8

In plants on the wet site concentrations of the inorganic cations K^+ and Na^+ did not differ significantly in the first two days of sampling but they were significantly higher on July 27, by around 40% for Na^+ and 35% for K^+. Within the sugars glucose had the highest concentration in all the leaves sampled. Sucrose concentration was the lowest of the sugars (unexpectedly), and was slightly higher on June 18 than on April 25, but was not much different on July 27. Fructose concentration was lower on June 18 than on the other two days. Glucose concentration on July 27 was almost double its concentration on either of the other days.

Plants growing on the dry site had leaf osmotic potentials about 0.3 MPa lower on June 18 than on April 25, and were lower by about 0.5 MPa on July 27. This accompanied differences in leaf water potential of the same magnitude, thus resulting in turgor maintenance despite the very low leaf water potential on July 27.

In plants on the dry site concentrations of Na^+ and K^+ were higher than in those on the wet site. However, in leaf samples collected from plants on the dry site there *were* no differences in Na^+ concentration between April 25 and June 18, but it was about 40% higher on July 27. K^+ had a concentration on June 18 double that measured on April 25, and it was higher still on July 27, by about 30%.

The sugar concentrations measured were lower in leaves of plants on the dry site compared to those on the wet site. In plants on the dry site sucrose concentration was only slightly higher in leaves collected on June 18, but there was no difference between those collected on the other two days. Fructose concentrations did not show any differences between April 25 and June 18 but was slightly higher on July 27. Glucose concentration was lower on June 18, than on either of the other days, between which there was no significant difference.

The striking thing to be noticed from these results is the low concentration of K^+ and the unexpectedly high concentration of glucose and fructose.

ii - The contribution of individual solutes to the leaf osmotic potential

In order to assess the relative contribution of the different solutes measured, the osmotic potential due to these solutes was calculated according to Slavik (1974) and results are shown on Table 5.2. In case of the inorganic cations the values shown in the table are double the calculated values to account for anions involved in charge balance.

Most of the osmotic potential of leaves is accounted for by the two inorganic cations K^+ and Na^+, provided their associated anions are taken into account and assumed to be monovalent. There was no difference in the osmotic potential due to Na^+ (+ anions) between leaves collected from the wet site on April 25 and June 18, but there was a decrease of 50% on July 27. K^+ and its balancing anions gave slightly lower osmotic potential than Na^+ in these plants but showed a similar trend to Na^+ between the three days of sampling. The osmotic potential due to sucrose and fructose did not vary much between leaves collected on April 25 and June 18, but that due to glucose was almost double on July 27.

The osmotic potential due to the inorganic cations K^+ and Na^+ (with their anions) was much lower in leaves of plants growing on the dry site than those on the wet site. Here again Na^+ contribution did not show any differences between samples collected on April 25 and June 18 but was 40% lower on July 27. However the osmotic potential due to K^+ (and its anions) was much lower than that of Na^+,

Table 5.2 The osmotic potential of Trifolium repens leaves (MPa) from two sites on the dune system, and the osmotic potentials due to Na^+, K^+, sucrose, fructose and glucose. Figures in parentheses indicate the percentage contribution of the solutes to the measured osmotic potential. Values for Na^+ and K^+ are double the calculated ones to take account of their associated anions. Osmotic coefficients of 0.93, 0.92, 1.01, 1 and 1 were used in calculations for Na^+, K^+, sucrose, fructose and glucose respectively. Samples were collected on: April 25, June 18 and July 27 (1980). Each value is a mean of five samples.

APRIL 25:

	SITE 1 (WET)		SITE 7 (DRY)	
LEAF OSMOTIC POTENTIAL	-0.57		-0.71	
OSMOTIC POTENTIAL DUE TO:				
Na^+	-0.16	(28)	-0.23	(32)
K^+	-0.19	(33)	-0.27	(38)
SUCROSE	-0.04		-0.03	
FRUCTOSE	-0.07	(32)	-0.02	(17)
GLUCOSE	-0.07		-0.07	
TOTAL	-0.53	(93)	-0.62	(87)

JUNE 18:

LEAF OSMOTIC POTENTIAL	-0.57		-1.00	
OSMOTIC POTENTIAL DUE TO:				
Na^+	-0.16	(28)	-0.23	(23)
K^+	-0.22	(39)	-0.58	(58)
SUCROSE	-0.05		-0.04	
FRUCTOSE	-0.06	(33)	-0.03	(12)
GLUCOSE	-0.08		-0.05	
TOTAL	-0.57	(100)	-0.93	(93)

JULY 27:

LEAF OSMOTIC POTENTIAL	-0.79		-1.29	
OSMOTIC POTENTIAL DUE TO:				
Na^+	-0.23	(29)	-0.32	(25)
K^+	-0.32	(41)	-0.76	(59)
SUCROSE	-0.04		-0.03	
FRUCTOSE	-0.06	(30)	-0.04	(11)
GLUCOSE	-0.14		-0.07	
TOTAL	-0.79	(100)	-1.22	(95)

and on June 18 it was twice as negative as thaton April 25. On July 27, K^+ (together with its balancing anions) contributed about 59% of the total osmotic potential.

The osmotic potential due to the three sugars, in plants on the dry site, did not vary much between the samples collected on the three days and altogether accounted for about -0.12 to -0.14 MPa.

B - Plants growing under low water potential in the laboratory

i - Concentration of solutes

To test the effect of low water potentials on the concentration of solutes in leaf tissue, Trifolium repens from Site 1 (wet) and Site 7 (dry) was grown in Long Ashton culture solution in a controlled environment room. The addition of polyethylene glycol (m.w. 4000) to the culture solution gave an osmotic potential of -1.0 MPa. Measurements of solute concentration, together with leaf water, pressure, and osmotic potentials, were made before and one, seven and twenty one days after the transfer to polyethylene glycol. Results are shown in Table 5.3. Control values did not change significantly during the experiment.

Before plants were transferred to polyethyleneglycol, those from the dry site had higher concentration of Na^+ and K^+ than plants from the wet site. However, the sucrose concentration in plants from the wet site was much higher than in those from the dry site.

Plants from the wet site showed a drop in osmotic potential of about 0.28 MPa one day after transfer to polyethylene glycol, but this was insufficient to lower the leaf solute potential below that of the culture solution, and plants showed signs of wilting. After seven days their osmotic potential fell to -1.31 MPa with the water potential measuring -1.20 MPa and reached values of -1.70 MPa and -1.73 MPa

Table 5.3 Leaf water, osmotic and pressure potentials (MPa) and the concentration of some of the major solutes (mol m^{-3}) in leaves of Trifolium repens, from two sites, after transfer to polyethylene glycol (m.w. 4000). Each value is a mean of three replicates.

DAY 0:

	SITE 1 (WET)	SITE 7 (DRY)
LEAF WATER POTENTIAL	-0.12 ± 0.02	-0.24 ± 0.03
LEAF OSMOTIC POTENTIAL	-0.58 ± 0.03	-0.72 ± 0.04
LEAF PRESSURE POTENTIAL	0.46 ± 0.03	0.48 ± 0.03
Na^+	27 ± 1	34 ± 2
K^+	52 ± 2	75 ± 2
SUCROSE	28 ± 3	12 ± 1
FRUCTOSE	15 ± 2	15 ± 2
GLUCOSE	32 ± 3	30 ± 2
TOTAL	154 ± 7	166 ± 4

DAY 1:

LEAF WATER POTENTIAL	-0.22 ± 0.01	-0.39 ± 0.01
LEAF OSMOTIC POTENTIAL	-0.86 ± 0.02	-1.23 ± 0.02
LEAF PRESSURE POTENTIAL	0.64 ± 0.02	0.84 ± 0.02
Na^+	32 ± 3	41 ± 3
K^+	81 ± 5	160 ± 7
SUCROSE	44 ± 2	16 ± 1
FRUCTOSE	58 ± 1	28 ± 1
GLUCOSE	80 ± 4	55 ± 2
TOTAL	295 ± 8	300 ± 11

Table 5.3 cont'd

DAY 7:

	SITE 1 (WET)	SITE 7 (DRY)
LEAF WATER POTENTIAL	-1.20 ± 0.01	-1.26 ± 0.03
LEAF OSMOTIC POTENTIAL	-1.31 ± 0.02	-1.92 ± 0.04
LEAF PRESSURE POTENTIAL	0.11 ± 0.02	0.66 ± 0.03
Na^+	47 ± 4	55 ± 3
K^+	131 ± 4	252 ± 6
SUCROSE	30 ± 3	33 ± 2
FRUCTOSE	71 ± 4	50 ± 4
GLUCOSE	93 ± 3	88 ± 2
TOTAL	372 ± 6	477 ± 6

DAY 21:

LEAF WATER POTENTIAL	-1.73 ± 0.01	-2.13 ± 0.03
LEAF OSMOTIC POTENTIAL	-1.70 ± 0.01	-2.13 ± 0.02
LEAF PRESSURE POTENTIAL	-0.03 ± 0.01	0.00 ± 0.01
Na^+	51 ± 3	62 ± 2
K^+	209 ± 4	290 ± 5
SUCROSE	15 ± 1	20 ± 2
FRUCTOSE	68 ± 3	14 ± 2
GLUCOSE	90 ± 5	62 ± 2
TOTAL	433 ± 13	448 ± 9

respectively on the twenty first day. They were then quite wilted.

In these plants from the wet site there was a significant increase in all solutes except Na^+ by one day after transfer to polyethylene glycol. Sodium showed a significant increase only after seven days, with no further change after 21 days. K^+ had the highest concentration of the solutes measured, showing a steady increase to reach values just over 200 mol m^{-3} 21 days after the treatment began. Sucrose concentration increased significantly one day after transfer to polyethylene glycol, but dropped again to values like the controls after seven days, and was even lower after 21 days. Fructose and glucose concentrations had increased dramatically from the first day reaching maximum values on day seven, but did not change significantly when measured 21 days after the start of the treatment.

Plants from the dry site showed a drop in their osmotic potential one day after the transfer to polyethylene glycol. This decrease was greater after seven days, reaching values of about -1.92 MPa. The pressure potential was therefore maintained at near full turgor, indicating that osmotic adjustment had taken place. When measured 21 days after the treatment began the osmotic potential was about -2.13 MPa, about equal to the leaf water potential, and plants were wilting.

Plants from the dry site, measured one day after the start of the treatment, showed a 20% increase in Na^+ concentration but K^+ concentration more than doubled. After seven days, further increase in Na^+ concentration was small whilst K^+ increased signficantly. By the twenty first day K^+ concentration had reached about four times control concentrations.

In these plants from the dry site, concentrations of the three sugars did not increase as much as those from the wet site when measured one day after the start of the treatment, but after seven days there was

a 3-fold increase in their concentration compared to the control. However there was a significant decrease in the concentration of sucrose and fructose by day 21, with glucose concentration maintained at about double that of the control.

ii - <u>The contribution of individual solutes to the leaf osmotic potential</u>

The inorganic cations, together with their associated anions, contributed a large part of the leaf osmotic potential (Table 5.4). In the control plants the osmotic potential due to Na^+ and its associated anions was 21%, where that due to K^+ (+ anions) was about 40-45%, of the leaf osmotic potential. However, the osmotic potential due to the sugars in control plants from the wet site was almost double that in those from the dry site.

The contribution of Na^+ to the osmotic potential in plants from the wet site remained constant during the experiment. The osmotic potential due to K^+ (+ anions) increased steadily contributing about 42% and 55% to the leaf osmotic potential one and twenty days after the treatment started, respectively. The contribution of sugars increased significantly on the first day, but dropped to below control levels on day 21.

In plants from the dry site K^+ contribution to the osmotic potential increased after transfer to polyethylene glycol, reaching about 61% on day 21. Na^+ and its associated anions contribution to the measured osmotic potential fell from 21% before the start of the treatment to 13% twenty one days after.

In plants from the dry site osmotic potential due to three sugars decreased after the treatment began, but its contribution to the osmotic potential stayed the same on day one and day seven, and fell on day twenty one.

Table 5.4 The osmotic potential of Trifolium repens leaves (MPa) from two sites, and the osmotic potentials due to Na^+, K^+, sucrose, fructose and glucose, after transfer to polyethylene glycol (m.w. 4000). Figures in parentheses indicate the percentage contribution of the solutes to the measured osmotic potential. Values for Na^+ and K^+ are double the calculated ones to take account of their associated anions. Osmotic coefficients of 0.93, 0.92, 1.01, 1 and 1 were used in the calculations for Na^+, K^+, sucrose, fructose and glucose respectively. Each value is a mean of three replicates.

DAY 0:

	SITE 1 (WET)		SITE 7 (DRY)	
LEAF OSMOTIC POTENTIAL	-0.58		-0.72	
OSMOTIC POTENTIAL DUE TO:				
Na^+	-0.12	(21)	-0.15	(21)
K^+	-0.23	(40)	-0.33	(46)
SUCROSE	-0.07	(33)	-0.03	(19)
FRUCTOSE	-0.04		-0.04	
GLUCOSE	-0.08		-0.07	
TOTAL	-0.54	(93)	-0.62	(86)

DAY 1

LEAF OSMOTIC POTENTIAL	-0.86		-1.23	
OSMOTIC POTENTIAL DUE TO;				
Na^+	-0.14	(16)	-0.18	(15)
K^+	-0.36	(42)	-0.71	(58)
SUCROSE	-0.11	(51)	-0.04	(20)
FRUCTOSE	-0.14		-0.07	
GLUCOSE	-0.19		-0.13	
TOTAL	-0.94	(110)	-1.13	(92)

Table 5.4 cont'd

DAY 7:

	SITE 1 (WET)		SITE 7 (DRY)	
LEAF OSMOTIC POTENTIAL	-1.31		-1.92	
OSMOTIC POTENTIAL DUE TO:				
Na^+	-0.21	(16)	-0.25	(13)
K^+	-0.58	(44)	-1.12	(58)
SUCROSE	-0.07	(36)	-0.08	(21)
FRUCTOSE	-0.17		-0.12	
GLUCOSE	-0.23		-0.21	
TOTAL	-1.26	(96)	-1.78	(93)

DAY 21:

LEAF OSMOTIC POTENTIAL	-1.70		-2.13	
OSMOTIC POTENTIAL DUE TO:				
Na^+	-0.23	(14)	-0.28	(13)
K^+	-0.93	(55)	-1.29	(61)
SUCROSE	-0.04	(24)	-0.05	(11)
FRUCTOSE	-0.16		-0.04	
GLUCOSE	-0.21		-0.15	
TOTAL	-1.57	(92)	-1.81	(85)

It should be emphasized here that the changes in solute concentrations in plants from the wet site were consistent with what had been observed in plants growing in the field. Firstly, in the laboratory and in the field K^+ and Na^+ concentrations were higher when the leaf water potential was lower. Sugar concentration increased in the field with low water potential, and in the laboratory it increased but only at small negative water potentials; there was no further increase with further decline in the leaf water potential. Percentage contributions of the solutes, in plants from the wet site, showed the same trend in the laboratory as in the field.

In plants from the dry site there was also broad agreement between field and laboratory findings. K^+ concentration change, as well as its contribution to the leaf osmotic potential, with changes in leaf water potential was similar in the two cases. Change in Na^+ concentration in the laboratory was more sensitive to low leaf water potential than in the field, even though its concentration was not as high as in the field. Despite the large increase in sugar concentration in plants from the dry site in the laboratory, which kept a constant percentage contribution to the osmotic potential, they did not increase as much with low leaf water potentials in the field, and therefore their contribution to the leaf osmotic potential was lower under these conditions.

The osmotic adjustment seen in this experiment could be due simply to water loss concentrating the remaining solutes, or represents the net import of ions, and net accumulation of organic molecules, in leaves. Some of these data will now be re-expressed on a leaf area basis to investigate these possibilities.

When solute concentrations were expressed on a leaf area basis (Table 5.5), they showed clearly that for plants from the wet site there was virtually no increase in K^+ concentration per unit leaf area, along the first seven days, and only a small increase was measured twenty one days from the start of the treatment. Sodium concentration showed a steady decrease. However, the concentration of sugars per unit leaf area increased by about 50% on the first day after exposure to polyethylene glycol, but fell back to equal control plants seven days later, and to below the control after 21 days. Therefore these plants from the wet site did not accumulate the two inorganic cations, but significantly accumulated sugars in the first day after exposure to polyethylene glycol. The rate of change in the concentration of these sugars (Table 5.6) as calculated from measurement on the seventh and twenty first days reflected an export from the leaves. Calculation of the assimilation rates taken as sucrose produced by photosynthesis (Table 5.7, from data shown below) showed rates that could possibly account for the high increase in sugar concentrations shown on the first day. Plants were severely dehydrated by the treatment as could be seen from the very big reduction in their leaf water content (Table 5.5).

Plants from the dry site behaved very differently. When concentrations were expressed on a leaf area basis, they showed an almost doubling of K^+ concentration on the first day after the treatment began (Table 5.5), and increased again by nearly 50% seven days later, with a slight decrease when measured 21 days from the start of the treatment. Na^+ concentration showed small increases both on day one and day seven but decreased again on day twenty one. The sugars concentration, per unit leaf area, increased by about 50% after one day from the start of the

Table 5.5 Water Content Per Unit Leaf Area ($m^3 m^{-2}$) and Solute Concentration Per Unit Leaf Area ($mol\,m^{-2}$) In Leaves of Trifolium repens From Site 1 (Wet) and Site 7 (Dry) After Transfer to Polyethylene glycol (m.w. 4000) At -1.0 MPa

DAYS	WATER CONTENT	CONCENTRATION K^+	CONCENTRATION Na^+	CONCENTRATION SUGARS
		PLANTS FROM SITE 1 (WET)		
0	20.2×10^{-4}	0.105	0.055	0.152
1	12.9×10^{-4}	0.104	0.041	0.235
7	8.1×10^{-4}	0.106	0.038	0.157
21	4.3×10^{-4}	0.134	0.033	0.111
		PLANTS FROM SITE 7 (DRY)		
0	17.9×10^{-4}	0.134	0.061	0.104
1	16.2×10^{-4}	0.259	0.066	0.160
7	15.0×10^{-4}	0.378	0.083	0.257
21	12.4×10^{-4}	0.360	0.077	0.119

Table 5.6 Rate of change in amount of solutes per unit leaf area ($mol\ m^{-2} d^{-1}$) in leaves of Trifolium repens from Site 1 (Wet) and Site 7 (Dry) after transfer to Polyethylene Glycol (m.w. 4000) At -1.0 MPa

DAYS	RATE OF CHANGE K^+	Na^+	SUGARS
	PLANTS FROM SITE 1 (WET)		
0-1	-0.001	-0.014	0.083
1-7	0	0	-0.013
7-21	0.002	0	-0.003
	PLANTS FROM SITE 7 (DRY)		
0-1	0.105	0.005	0.056
1-7	0.020	0.003	0.016
7-21	-0.001	0	-0.010

Table 5.7 Assimilation Rate (mol sucrose $16h^{-1}$) of *Trifolium repens* from Site 1 (Wet) and Site 7 (Dry) after transfer to polyethylene glycol (m.w. 4000) -1.0 MPa, calculated from net photosynthetic rates. (See Tables 5.11 for details of Photosynthesis)

DAYS	PLANT FROM SITE 1	PLANT FROM SITE 7
0	0.073	0.068
1	0.032	0.053
2	0.015	0.043

treatment and by a further 60% after seven days, but decreased to almost control levels after twenty one days. The rate of increase in solute concentration (Table 5.6) reflects the ability of the plants from the dry site to accumulate large amounts of K^+ and sugars very rapidly after exposure to low water potentials. Calculated assimilation rates (Table 5.7) expressed as sucrose (from data shown below) shows that these plants (from the dry site) were capable of assimilating enough carbon to account for the increases in sugar concentration and possibly still export some outside the leaf even during the very rapid accumulation in the first day (Table 5.6). The increased K^+ per unit leaf area must have been imported in the xylem. The increase in K^+ concentration in xylem sap needed to account for this K^+ transport was calculated by dividing the rate of increase of K^+ per unit leaf area by the transpiration rate; there was a large increase in K^+ concentration in the xylem, especially on the first day (Table 5.8). However, the increase for Na^+ was not as high.

The results shown above indicate a big difference between the plants from the wet site and those from the dry one under low water potentials. Those from the wet site can accumulate sugars quite effectively as a rapid response to low water potential in the rooting medium, but the concentration of sugar falls with the continuation of the water deficit, and therefore much of the noticed increase in their solute concentrations was due to dehydration of the leaves. The dry site plants, on the other hand, can positively accumulate K^+, sugars, and to a less extent Na^+. They can do so through transport of the inorganic ions from the roots, and the assimilation of sugars within the leaves.

Table 5.8 Transpiration Rate ($m^3 m^{-2} 16h^{-1}$) and excess concentration of K^+ and Na^+ in the xylem sap ($molm^{-3}$) of Trifolium repens from the dry site after transfer to Polyethylene glycol (m.w. 4000) At -1.0 MPa. See Table 5.9 for details of transpiration. The excess concentration in xylem sap was calculated by dividing the rate of accumulation of the ion per unit leaf area by the transpiration rate.

DAYS	TRANSPIRATION RATE	EXCESS CONCENTRATION K^+	Na^+
0-1	0.86×10^{-3}	122	5.8
1-7	0.57×10^{-3}	28	4.3

Effect of low water potential on stomatal response and photosynthesis

The effect of low water potential on stomatal response and photosynthesis was examined in Trifolium repens. Plants from Site 1 (Wet) and Site 7 (Dry) growing in culture solutions were subjected to low water potential by bringing the osmotic potential of the culture down to -1.0 MPa by the addition of polyethylene glycol (m.w. 4000). Photosynthesis and water vapour loss from the leaves were measured simultaneously using an infra-red gas analyser. Measurements were made before, one and two days after transfer to polyethylene glycol. Control plants showed no significant change in gas exchange rates during the experiment.

Water vapour loss

Water vapour loss measurements (Table 5.9) show that the low osmotic potential of the culture medium caused a very significant drop in transpiration indicating an almost immediate closure of the stomata. This was also shown by the big increase in the vapour phase resistance of the leaves, over 6-fold, after two days from the start of the treatment. (Table 5.10).

However transpiration by plants from the dry site only decreased by about 30% on the first day, and 45% on the second day, after the treatment began (Table 5.9). The vapour phase resistance of their leaves did not increase as much as did that of plants from the wet site, it only increased by 45% on the first day, and was 80% higher on the second day compared to the control.

Table 5.9 Water vapour loss (µmol H_2O $m^{-2}s^{-1}$) from Trifolium repens leaves after transfer to polyethylene glycol (-1.0 MPa) , as measured by an infra-red gas analyser at 18°C and 200 µmol quanta $m^{-2}s^{-1}$ each value is a mean of three measurements.

DAYS	SITE (1) WET	SITE 7 (DRY)
0	1264 ± 29	987 ± 35
1	584 ± 26	676 ± 17
2	195 ± 8	550 ± 19

Table 5.10 Diffusive Resistance to Water Vapour ($5cm^{-1}$) of *Trifolium repens* leaves after transfer to Polyethylene glycol (-1.0 MPa). Values calculated from measurements made at 18°C and 200µmol quanta $m^{-2}s^{-1}$. Each value is a mean of three measurements.

DAYS	SITE 1 (WET)	SITE 7 (DRY)
0	3.5 ± 0.1	4.5 ± 0.2
1	7.6 ± 0.3	6.6 ± 0.2
2	22.8 ± 0.9	8.1 ± 0.3

Rate of net photosyntheses

Measurements of net photosynthetic rate of the plants before transfer to polyethylene glycol did not show much difference between plants from either site (Table 5.11). However, after imposition of low water potentials, plants from the different sites were affected differently.

One day from the start of the treatment, plants from the dry site had rates reduced by only 20%, while those from the wet site had up to a 60% reduction. Plants from the dry site continued to photosynthesise at a rate around 60% of the control after 2 days, but those from the wet site only showed 20% of the control rate.

Tables 5.12 and 5.13 show the total resistance, and mesophyll resistance to CO_2 uptake by the leaves. The control plants did not show significant difference in the total resistance to CO_2 uptake between plants from the two sites, but it increased quite dramatically one day after the start of the treatment in plants from the wet site, and showed a 5-fold increase on the second day. However, total resistance to CO_2 uptake by the plants from the dry site only increased by about 30% after one day and 55% after two days from the start of the treatment. Table 5.13 shows that plants from the dry site had a lower mesophyll resistance, which was hardly affected by the treatment, while that of plants from the wet site had increased by 3-fold after two days, thus accouting for a large part of total resistance to CO_2 uptake in those plants.

Table 5.14 shows the transpiration:photosynthesis ratio for plants from the two sites after transfer to polyethylene glycol.

Table 5.11 Net Photosynthetic Rate ($\mu mol\ CO_2 m^{-2} s^{-1}$) of *Trifolium repens* after transfer to polyethylene glycol (-1.0 MPa), as measured by an infra-red gas analyser at 18°C and 200 μmol quanta $m^{-2} s^{-1}$. Each value is a mean of three meesurements.

DAYS	SITE 1 (WET)	SITE 7 (DRY)
0	15.34 ± 0.40	14.10 ± 0.25
1	6.59 ± 0.19	10.97 ± 0.52
2	3.07 ± 0.23	8.99 ± 0.39

Table 5.12 Total resistance to CO_2 uptake (scm^{-1}) of *Trifolium repens* leaves after transfer to Polyethylene glycol (-1.0 MPa). Values calculated from net photosynthetic rate measurements made at 18°C and 200 µmol quanta $m^{-2} s^{-1}$. Three replicates per data point.

DAYS	SITE 1 (WET)	SITE 7 (DRY)
0	8.90 ± 0.23	9.68 ± 0.28
1	20.72 ± 0.62	12.48 ± 0.58
2	44.88 ± 3.14	15.22 ± 0.69

Table 5.13 Mesophyll Resistance to CO_2 Uptake (scm^{-1}) of *Trifolium repens* leaves after transfer to Polyethylene glycol (-1.0 MPa). Values calculated from measurements made at 18°C and 200μmol quanta $m^{-2}s^{-1}$. Three replicates per data point.

DAYS	SITE 1 (WET)	SITE 7 (DRY)
0	4.06 ± 0.35	3.47 ± 0.33
1	10.26 ± 0.31	3.48 ± 0.64
2	13.60 ± 1.87	4.17 ± 0.67

Table 5.14 Transpiration:Photosynthesis Ratio of Trifolium repens from Site 1 (Wet) and Site 7 (Dry) after Transfer to Polyethylene Glycol (m.w. 4000) at -1.0 MPa.

DAYS	SITE 1 (WET)	SITE 7 (DRY)
0	82.7	70.1
1	88.6	61.9
2	64.5	61.5

CHAPTER SIX

DISCUSSION

The soil water potential measurements showed clear differences between the sites on the dune system with respect to their soil moisture status. Such differences would be expected to affect the distribution of plants on the dune system (Harper and Sagar, 1953). The brief vegetation survey showed some restricted distribution patterns, some species being confined to distinct habitats on the dune system. The spread of other species over a wide range of habitats shows a variability that could be either due to genetic specialisation or to plasticity of a single genotype.

Varying the soil moisture regime clearly affected the growth of Festuca rubra, but since plants from all the sites responded similarly, showing the best growth under moderate soil moisture, this showed the plasticity of this species and was not consistent with ecotypic differentiation between these populations strongly related to water status. The vulnerability of F. rubra plants to high soil moisture was shown. Pemadasa (1973) showed that some dune annual species which grow mainly on the drier parts of the dune system also grow best under moderate soil moisture regimes in the glasshouse.

However, performance of Trifolium repens under the different soil moisture regimes showed that these plants are site specific. Further, growth of the plants reciprocally on the dune system gave more evidence to this. Unlike the slack plants, the dune plants growth was not much affected by low soil moisture, but when grown in the wet slack their growth was reduced possibly due to the anaerobic conditions due to the high moisture levels (Kramer, 1949), which could also be affecting the growth of the slack plants on the dune system. Tolerance to low soil

moisture is thus shown by the dune plants, whereas the slack plants were vulnerable to low soil moisture levels in the soils. This differential response suggests the existence of these T. repens populations as ecotypes in the dune system.

The way in which these plants, whether ecotypes or not, survive low soil water potentials in their growth is varied. Plant growth, especially that of plants from wet sites, was affected by low soil moisture status. Leaf growth is reduced by low moisture levels which is probably due to the decreased cell expansion, which is sensitive to water deficits (Boyer, 1970; Begg and Turner, 1978), at times of drought this reduction of the transpiring surfaces of the plant might reduce whole-plant transpiration (Shield , 1950). Decreased specific leaf area under water deficit shows that plants accumulate more dry matter in the leaves rather than producing extra leaf area. It had been suggested (Fischer and Turner, 1978), that this decrease in the specific leaf area is due to the plants investing heavily in such non-photosynthetic components as fibres, vessels, cell walls and sclerenchyma. Such production of mechanical tissue could be beneficial as low turgor may be caused by water deficits, and hence mechanical support for the tissue would preserve rigidity. Pearce et al (1969) showed that clones of alfalfa with higher specific leaf weight (ie. lower specific leaf area) have higher net photosynthetic rates. Bunce (1982) showed an increase in specific leaf area of sunflower and soybean with water deficits. Stocker (1960) considered the production of smaller denser leaves by herbaceous plants as a response to water deficits to be important in reducing water loss as well as improving photosynthetic rates.

Festuca rubra produced fewer leaves with lower soil moisture status which could reduce water loss, but this in effect will reduce the photosynthetic tissue of the plant and hence growth. However, production of shorter and bushier types of leaves could create higher relative humidity in the immediate vicinity of the leaves and thus lead to a cut in the transpiration and reduce the water loss by the plants. In this work, the dune populations of F. rubra tended to produce more tillers than the slack plants, a feature that helps the plant to spread by rhizomes and hence reach more of the available water in a larger volume of soil.

Under the long drying cycle in the glasshouse, plants of both species showed signs of wilting by the time of rewatering, meaning that they would be experiencing large water deficits and zero turgor. Complete cessation of growth can take place if turgor is reduced by a small magnitude (Hsiao, 1973). Plants could overcome this effect by keeping their turgor unaffected. Growth of the dune plants of T. repens in the glasshouse, expressed as total plant dry weight, was virtually unaffected by the drying cycle. This suggests that probably these plants sustained growth by turgor maintenance during developing water deficits. These same plants had also shown an increase in root growth, that increased the root:shoot ratio, which could also be a result of osmotic adjustment and turgor maintenance in the roots (Sharp and Davies, 1979). Dune populations of T. repens, therefore, show features consistent with drought resistance, while the slack ones did not show an ability to grow well in relatively dry soil; they only performed best under high soil moisture levels which could possibly resemble those encountered in their natural habitat.

Physiological difference between ecotypes

Variation in the response of plants to water deficits, due to genetic factors, undoubtedly exists. The differential growth response of plants must be an indication of inherent physiological differences betweeen these ecotypes. Knowledge of the physiological variability within a species could give an understanding of the autoecology of the species and the factors limiting its distribution. In the forthcoming discussion an attempt is made to reveal the role played by one of these factors, water availability, and to show how ecotypes of Trifolium repens differ at the physiological level.

Photosynthesis and transpiration

The influence of leaf water status on photosynthesis is well known. The effect on photosynthesis at low soil moisture levels could be due to an effect on the stomata or in the mesophyll that probably increases during plant desiccation. Plants growing in habitats subject to frequent droughts would be at an advantage were they able to maintain a reasonable photosynthetic rate under low water potentials.

Exposure of T. repens plants to low water potentials in the growth medium, resulted in a reduction of their net photosynthetic rates. The dune slack plants were more affected than the dune plants, with net photosynthetic rates 20% and 65% of control rates, respectively, at low water potentials. This indicates a clear difference in the photosynthetic ability between these ecotypes at low water potentials. This is similar to findings of Blum and Sullivan (1972) for different sorghum genotypes, where they showed that just two varieties maintained relatively high net photosynthetic rates at low leaf water potential,

and they explained that by their ability to maintain low stomatal resistance in spite of decreasing water potentials. However, they did not investigate possible osmotic adjustment in those two varieties, which might allow for the low water potentials without much water loss from the tissue, and hence the low stomatal resistance. T. repens from the dry site showed an increase in the vapour phase resistance (from 4.5 to 8.1 scm^{-1}), but plants from the wet site showed an even greater increase (from 3.5 to 22.8 scm^{-1}), which could indicate complete stomatal closure. This could reflect the high sensitivity of the stomata of the plants from the wet site to low water potentials which would result in deleterious effects on photosynthesis. On the other hand the plants from the dry site responded by partial stomatal closure at low water potentials and therefore lowering their transpiration whilst maintaining reasonable net photosynthetic rates. Indications that some plants can maintain a low stomatal resistance at low water potentials was reported by McCree (1974) and Davies (1977). This could be attributed to turgor maintenace by osmotic adjustment as leaf water potential falls (Brown et al, 1976; Osonubi and Davies, 1978). Moreover there is more efficient gain of CO_2 per unit of water lost by plants from the dry site, as shown by the transpiration:photosynthesis ratio, and thus an increase in their efficiency, with the development of water deficits. Plants from the wet site showed lower efficiency of water use, reflected by the increase in their transpiration:photosynthesis ratio, 24 hours after exposure to low water potentials, with recovery after 48 hours. These changes are part of a consequence of an increase in stomatal resistance affecting CO_2 entering the leaf proportionately less than water, due to the presence of the mesophyll resistance in the

pathway of CO_2 into the leaf, which accounts for a large part of the total resistance to CO_2 uptake (Meidner and Mansfield, 1968). It has been shown that artificially induced partial closure of the stomata can improve water use efficiency (Shimshi, 1963a,b; Slatyer and Bierhuizen, 1964; Mansfield, 1976).

It has long been argued whether reduced photosynthesis at low water potentials, is due to stomatal closure alone or whether factors in the mesophyll are also involved. An increase in the mesophyll resistance with developing water deficits was shown by Fischer (1968), and Jarvis and Slatyer (1970); others who have reported non-stomatal reduction of photosynthesis include Redshaw and Meidner (1972) in tobacco, Boyer (1971), and Doley and Trivett (1974) in Mitchell grass. The calculated mesophyll resistance in T. repens was substantial. However, for plants from the wet site it increased quite significantly at low water potential (3-fold) and thus accounted for a large part of the total resistance to CO_2 uptake. Such a large increase did not allow the decreases in transpiration:photosynthesis ratio to take place as the stomatal resistance increased. Bunce (1982) found that a substantial part of the reduction in net photosynthesis at low water potential in sunflower and soybean was due to mesophyll resistance, which he suggested to be due to changes in the chloroplast structure and enzyme activity, probably due to the effect of solute concentration on enzymes (Boyer, 1976).

In the plants from the dry site, mesophyll resistance accounted for a large part of the total resistance to CO_2 uptake, and it did not change significantly under low water potential. Therefore moderate increase in their gas phase resistance will bring about more effect on water loss than on CO_2 uptake, and hence increase their water use efficiency.

The relatively lower diffusive resistance of plants from the dry site under low water potentials also means that continued import of nutrients into leaves, via the transpiration stream in the xylem, is possible.

It may be that some of the controversy in the literature over the importance of changes in the mesophyll resistance is due to intra-specific variability being underestimated.

Water relations

One of the problems of studying plant water relations under water deficits was to have a constant and prolonged low soil water potential. To overcome this problem workers commonly use dissolved solutes in culture media. The most widely used solute is polyethylene glycol of high molecular weight. Its effect on decreasing the potential of the rooting medium will render water less available to the plant. Polyethylene glycol of high molecular weight is also preferred because it has been shown not to enter the plant in concentrations that could affect metabolism (Lagerwerff *et al*, 1961; Jarvis and Jarvis, 1965; Janes, 1966; Kaul, 1966; Lawlor, 1970). Leaf water potential and its components, together with relative water content, measured after the addition of polyethylene glycol to the culture medium showed its effect on creating water deficits in *T. repens* plants.

Clear differences in response of plants from the wet and dry sites, after transfer to polyethylene glycol was shown. For plants to continue growth under low water potentials, they should be able to maintain high pressure potentials. The dry site plants were able to keep their pressure potential unaffected even at leaf water potentials at which plants from the wet site wilted. This response shows the tolerance of

the plants from the dry site to low water potentials. Turgor maintenance by osmotic adjustment, at low water potentials, is considered to be one of the most important mechanisms for survival of water deficits and it has been correlated with drought tolerance. Plants from the dry site were shown to drop their osmotic potentials and hence maintain their leaf turgor. They had lower osmotic potentials than plants from the wet site at all the leaf water potentials studied. Furthermore the leaf water potential at which they reached zero leaf turgor (which is of important ecological significance) was about 0.6MPa lower than in the plants from the wet site. Therefore, plants from the dry site can, at times of drought, achieve water potentials low enough to maintain a positive water potential gradient from soil to leaf and extract soil moisture. Thus at any given relative water content plants from the dry site had lower leaf water potentials, as well as higher pressure potentials, than those from the wet site, and this difference was greatest at low relative water contents. The same response was shown by Jones and Turner (1980) for sunflower plants which were stress-hardened, as compared to controls, and by the same authors (Jones and Turner, 1978) for sorghum plants.

The relationship between leaf water potential and osmotic potential for plants from the dry site is similar to that obtained by Morgan (1977a) for some genotypes of wheat which are considered to be drought resistant and showed osmotic adjustment by solute accumulation. That for the wet site plants was typical of plant species which do not show osmotic adjustment (Kassam, 1973; Morgan, 1977a,b).

Data obtained from T. repens growing on the field showed values of osmotic potential always lower than the water potential with a result that turgor was always maintained at high positive values, for plants from both the dry and the wet sites. However, under the low water potentials imposed by the addition of polyethylene glycol in the laboratory only plants from the dry site showed turgor maintenance, whilst plants from wet site showed a steady fall in turgor. Leaf water potentials down to which plants from the dry site kept their pressure potentials constant (-1.0MPa) are comparable to those measured for these plants while growing on the dunes on July 27 (1981), when soil moisture and climatic conditions were extreme, and plants still maintained their leaf turgor potentials. Therefore these plants are capable of maintaining turgor under the moisture conditions prevailing in their natural habitat, and extreme conditions encountered in their habitat are within their physiological tolerance. On the other hand, the steady drop in pressure potentials in plants from the wet site under polyethylene glycol treatment, shows that these plants cannot withstand such low water potentials. Growing in the field, these plants are only capable of limited osmotic adjustment under very small reduction in the soil water potential. However, under much lower potentials, like the one created by polyethylene glycol addition in the laboratory, these plants fail to adjust, lose turgor and wilt. There are, therefore, clear differences in the drought tolerance of the plants from the dry site and those from the wet site.

Solute accumulation

Osmotic adjustment is due to the accumulation of osmotically active solutes. A wide range of solutes accumulate when different plant species experience water deficits. Data presented above show that Trifolium repens plants concentrate K^+, Na^+ and the sugars, sucrose, glucose and fructose. More than two thirds of the measured osmotic potential is contributed by K^+ and Na^+ together with their associated anions. These balancing anions could be monovalent like Cl^- which was shown by Ford and Wilson (1981) to increase substantially in green panic and buffel grass thus largely balancing the increased cation concentration at low water potential, or di- or multi-valent anions which are relatively immobile and slowly accumulated (Jones et al, 1980). However, balancing excess cation concentration by di- or multivalent anions would result in smaller osmotic potential values than in the case of monovalent anions. Concentration of solutes measured in plants growing in the dry site suggest that probably only K^+ is used effectively in the osmotic adjustment of these plants in the field, since sugar concentrations did not vary significantly, and Na^+ only slightly with low osmotic potentials. Plants growing in the wet site probably use sugars in osmotic adjustment, and the relative differences in K^+ and Na^+ concentrations, when the plants osmotic potentials were lower, suggest their possible equal contribution to the osmotic adjustment. The more detailed study of ionic relations in the laboratory under low water potentials show clearly how different these ecotypes are in their response. The increase in sugar concentration shown by plants

from the wet site one day after they were subjected to low water potentials suggests the limited ability of these plants to adjust osmotically by solute accumulation. These plants showed turgor maintenance in the field when their soil water potential was about 0.1MPa lower. Sugar accumulation in the laboratory was only observed one day after the treatment started but not to high enough concentrations to maintain turgor. This reflects the inability of these plants to cope with prolonged low soil water potentials and could possibly explain the poor growth performance of these plants when grown on the dry site, where low soil water potentials are not uncommon.

Plants from the dry site, on the other hand, showed an ability to adjust osmotically by the accumulation of K^+ and sugars (and to a lesser extent Na^+), unlike in the field where the involvement of sugars in the osmotic adjustment was not clear. Na^+ concentration in the laboratory did not reach that measured when the plants were under large water deficits in the field. The dune soil contains relatively high levels of exchangeable sodium (295±12 and 262±14μg g^{-1} dry soil in the wet and dry sites respectively, compared with 128±4 and 103±5μg g^{-1} dry soil K^+). Sugars accumulated in leaves appear to be produced locally either by assimilation or by degradation of starch with a possible cessation of phloem transport.

However, that osmotic potentials due to the solutes measured were sometimes higher than the measured osmotic potential could be due to the overestimation of the anions associated with K^+ and Na^+, which were taken into account, since some of them could be di- or multi-valent. Another reason could be that the osmotic potential of tissue sap measured

is underestimated, since the sap could be diluted by the apoplastic water (Borowitzka, 1981).

The significant contribution of K^+ towards osmotic adjustment has been shown by many workers (Munns et al, 1979; Jones et al, 1980; Ford and Wilson, 1981). Janes (1966) reported an accumulation of K^+, Na^+, Ca^{2+} and Cl^- in pea plants grown in polyethylene glycol that acounted for 50-75% of the osmotic adjustment. Ford and Wilson (1981), in their study of some grass species under water deficits, showed that most of the contribution towards the osmotic adjustment came from K^+, Na^+, Cl^- and the sugars, sucrose, glucose and fructose. Jones et al (1980) showed that increases in sucrose, glucose, K^+ and Cl^- fully accounted for the osmotic adjustment in fully expanded sorghum leaves at low water potentials, but in sunflower leaves the sugar did not show any contribution.

Stomatal closure, causing reduced water transport through the leaf, will bring about changes in the concentration and fluxes of various solutes in the xylem (Hanson and Hitz, 1982). Thus the major part in the osmotic adjustment in plants from the dry site is the maintenance of transport, as shown by the high accumulation of the inorganic cation that must be transported into the leaves via the xylem, which is allowed for by the relatively low diffusive resistance these plants had under low water potentials.

Solute accumulation allows the plant to have low leaf water potentials, without large decreases in their water contents and hence maintain a water potential gradient between soil and leaf, and thus to extract water from the soil. It also maintain turgor which is vital for the growth of the plant.

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