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**THE EFFECT OF WATER STRESS ON
WATER RELATIONS, CARBON ISOTOPE
DISCRIMINATION, AND SHOOT AND ROOT
GROWTH OF SAINFOIN (*Onobrychis viciifolia* Scop.)
AND LUCERNE (*Medicago sativa* L.)**

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in partial fulfilment of the requirements
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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ
لِحَمْدِ اللَّهِ رَبِّ الْعَالَمِينَ
وَالصَّلَاةِ وَالسَّلَامِ عَلَى خَاتَمِ الْأَنْبِيَاءِ وَالرَّسُلِ

**In the name of Allah,
the Compassionate, the Merciful,
Praise be to Allah, Lord of the Universe,
And Peace and Prayers be upon
His final Prophet and Messenger.**

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ABSTRACT

Sainfoin (*Onobrychis viciifolia* Scop.) is a useful forage legume regarded as having drought resistant attributes. Also, it does not cause bloat in ruminants and is not sensitive to alfalfa weevil (*Hypera postica* L.). Although the physiological and morphological responses to water stress of lucerne (*Medicago sativa* L.) are well known the responses of sainfoin to water stress have not been fully studied. In this study the physiological and morphological responses of sainfoin to water stress were investigated, with lucerne used as a reference plant.

The results of the indoor and outdoor studies showed sainfoin had useful characteristics for forage production in dry conditions. Relative to lucerne it had a lower yield, due to lower leaf area, lower stem number and poor regrowth. However, sainfoin responded to water stress at least as well as lucerne. Sainfoin had a higher root:shoot ratio and a lower specific leaf area ratio than lucerne, indicating a higher allocation of carbohydrate to the roots, and a lower leaf surface area for transpiration in sainfoin than for lucerne. Water stress decreased the yield of lucerne proportionally more than sainfoin mostly due to the greater reduction in the above ground dry weight of lucerne.

The indoor study of root characteristics of sainfoin and lucerne in 1m tall tubes showed that in terms of root development sainfoin responded to water stress better than lucerne. Although sainfoin had equal root mass and root length to lucerne, the root distribution of sainfoin at below 0.6 m depths was greater than for lucerne. As water stress developed sainfoin roots grew below 0.6 m earlier than lucerne roots. Sainfoin had a higher root osmotic adjustment than lucerne and also maintained higher (less negative) leaf water potential than lucerne.

The stomatal resistances (R_s) of sainfoin and lucerne were equal, but R_s was not distributed equally between adaxial and abaxial leaf surfaces. The R_s of the adaxial leaf surface of sainfoin was lower and more sensitive to water stress than the R_s of the abaxial leaf surface. The different R_s of the adaxial and abaxial leaf surfaces of sainfoin was partly due to the different stomatal frequencies of the respective surfaces.

Comparison of sainfoin cultivars in a climate room showed that the water use efficiencies (WUE) of Remont, Fakir, Cotswold-Common, and Eski, were similar. Remont was more sensitive to water stress than the other three cultivars, and Eski produced a greater root length and mass than other cultivars. The growth of Eski was initially slower than that of the Remont in both the indoor and the outdoor studies. However, lucerne grew faster than all the sainfoin cultivars. Over three harvests in the field the yields of Eski and Remont were similar but lucerne out yielded both sainfoin cultivars. Sainfoin produced a greater proportion of its yield earlier than lucerne, whereas lucerne distributed its yield throughout the whole season, indicating that sainfoin is adapted to regions with precipitation in only winter and spring.

The results of the carbon isotope discrimination (Δ) analysis for the indoor and outdoor studies showed Δ had a negative correlation with WUE, leaf water potential, osmotic potential, and stomatal resistance, but had a positive correlation with relative water content, turgor potential, transpiration rate, and photosynthetic rate. These correlations demonstrated the usefulness of this technique for evaluating the responses of plants to water stress. The stressed plants always had lower Δ than the control plants showing the higher WUE of stressed plants. The Δ of roots was higher than the Δ of the leaves suggesting that the growth of leaves occurred in conditions that were an average drier than for the growth of roots. This was supported by the lower (more negative) water potential of leaves than roots. The Δ of the roots below 0.6 m depth was higher than the Δ of roots above 0.1 m depth suggesting the roots above 0.1m grew under higher water stress than the roots below 0.6m depth. Over three harvests in the field the Δ of Eski and lucerne were similar and the Δ of Remont was higher than for Eski and lucerne.

In conclusion, sainfoin was found to have several useful attributes for growth and survival in dry regions. Of the sainfoin cultivars examined Eski was the best adapted to water stress. Relative to lucerne, sainfoin yielded less, but had a similar water use efficiency, a shorter season of growth, a greater root: shoot ratio, deeper roots and better maintenance of leaf water potential under water stress.

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LIST OF ABBREVIATIONS:

- A= Assimilation rate ($\mu\text{mol CO}_2/\text{m}^2/\text{s}$)
ABA= Abscisic Acid
ANOVA= Analysis of variance
 C_a = CO_2 concentration of the air (ppm)
 C_i = CO_2 concentration inside of the leaf (ppm)
 C_s = Stomatal conductance (cm/s)
D= drainage
DAP= Days after planting
DS= Days of stress
DW= Dry weight
E= Transpiration rate ($\text{mol H}_2\text{O}/\text{m}^2/\text{s}$)
 e_a = Vapour pressure of the air
 e_i = Vapour pressure of the leaves
FR= Fine roots (<0.3 mm diam.)
GSWC= Gravimetric soil water content (%)
I= Interception of rainfall by crop canopies
LA= Leaf area (cm^2)
LAI= Leaf area index
LDW= Leaf dry weight (g)
P= Turgor potential (MPa)
 P_a = Partial pressure of CO_2 concentration of the air (MPa)
 P_i = Partial pressure of CO_2 concentration of the leaf (MPa)
 P_n = Net photosynthetic rate ($\mu\text{mol CO}_2/\text{m}^2/\text{s}$)
R= run-off
 R_s = Stomatal resistance (s/cm)
RH= Relative humidity (%)
RSE= Relative stem elongation (mm/mm/day)
RWC= Relative water content of the leaf (%)
RWD= Root weight density (g/m^3)

SDW= Stem dry weight (g)

SLA= Specific leaf area (cm^2/g)

SEM= Standard error of the mean

S/R= shoot:root ratio

TAC= Total available carbohydrate

TDR= Time domain reflectometer

TR= Thick roots (>0.3 mm diam.)

Tr= Transpiration rate ($\text{ml H}_2\text{O}$)

VPD= Vapour pressure deficit

VSWC= Volumetric soil water content (%)

W= Transpiration efficiency $\{(\mu\text{mol CO}_2/\text{m}^2/\text{s})/(\text{mol H}_2\text{O}/\text{m}^2/\text{s})\}$

WUE= Water use efficiency

Y_{ec} = Economic yield

Δ = Carbon isotope discrimination

π = Osmotic potential (MPa)

π_{100} = Osmotic potential at full turgor (MPa)

v = Water vapour pressure difference between the intercellular spaces and the atmosphere

ϕ = Loss of carbon or water not through stomata

Ψ = Leaf water potential (MPa)

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Chapter 1

Introduction and objectives

1. Introduction and objectives

Despite an increasing world population world land area is fixed, and the effective area for agriculture is further reduced by drought. By the year 2000 when the global population is projected to be 6-7 billion, only 0.2 ha per person will be available and producing food will be even more difficult (Simpson 1981).

Wise development and management of the semi-arid and arid zones of the world for greater crop production demands better knowledge about the nature of water stress and of ways of mitigating its harmful effects on crop production.

In most regions of the globe, water is usually a significant rate-limiting factor in maintaining high productivity of crop plants throughout the potential growing season. In most circumstances natural rainfall limits the amount of growth or number of crops that can be grown in a year. Clearly, the study of water stress provides information that can help to optimize plant growth under agricultural conditions.

Iran with a total area of 1,600,000 km² is the second largest country in the Middle East. It is located between 44° and 66° east longitude, and between 25° and 40° north latitude. The climate is of Mediterranean type with rain in the cool season and a long summer drought. Approximately 17% of the land surface of Iran has 250-500 mm of annual precipitation. The monthly rainfall and mean temperature suggest that most of the precipitation is snow in winter and the rain mainly occurs in spring (Choudhary 1992).

Drought in Iran has two characteristics: a) limitation from low rainfall b) distribution of precipitation mostly in winter and early spring. In consideration of these two points, research on drought resistant plants and their use potential for use in the country is justified.

Sainfoin (*Onobrychis viciifolia* Scop.) is a perennial forage legume capable of out-yielding lucerne (*Medicago Sativa* L.) in extremely dry regions (Cooper and Roath 1965, Murray and Slinkard 1968). Sainfoin appears to have some morphological adaptations to water stress such as a deep rooting system, and a low specific leaf area (Koch *et al.* 1972, Sheehy and Popple 1981). Nevertheless, the

little study (Bolger 1988). The high water use efficiency of sainfoin in spring (Bolger and Matches 1990) is another attribute suitable for plants in regions with enough rainfall in spring but with dry summer. Sainfoin has several attributes which make it a desirable forage species. It has a high feed quality (Smoliak and Hanna 1975, Carleton *et al.* 1968, Sheely 1977), is non-bloating due to the presence of tannin (Hanna *et al.* 1972, McGraw and Marten 1986, Fortune 1985), yet is palatable to sheep (Osborne *et al.* 1966), fixes nitrogen (Hume 1981, Bolger 1988), grows on low fertility soil (Roath and Graham 1968), and is resistant to some lucerne insects pests such as the alfalfa weevil (*Hypera postica* L.) (Eslick 1968, Hanna *et al.* 1977), and spotted alfalfa aphid (*Therioaphid maculta*) (Lance 1980).

The objectives of this study were:

- i) To examine physiological and morphological response of sainfoin cultivars to water stress, using lucerne as a reference plant
- ii) To determine the important physiological or morphological characters of sainfoin,
- and iii) To determine the potential of sainfoin as an alternative plant to lucerne for forage production in dry conditions.

Chapter 2

Review of literature

2 Review of literature

2.1 Plant adaptation to water stress

2.1.1 Definition and concepts

The ability of a crop to grow satisfactorily in areas subjected to water deficit has been termed "drought resistance" (Turner 1986a). Modifications of the structure and function that increase the probability that a plant will survive and reproduce in a particular environment are termed "adaptation" (Kramer 1980). "Acclimation" is the ability to slowly adapt to new environmental condition (Turner 1986a).

Bielorai (1992b) pointed out plants have to contend with three kinds of situations in which they are subjected to moisture stress:

- a) Transient drought: the occasional transient stress periods at various stages of plant development, a major characteristic of semi-arid regions
- b) Terminal drought: causing moisture stress towards the end of the growing period, which prevents normal formation and ripening of grain. This is the most common form encountered in a Mediterranean climate
- c) Seasonal drought: where insufficient precipitation for economic crop production occurs, generally in cycles, in the fringe areas of semi-arid regions and occasionally in semi-arid regions.

2.1.2 Categories of drought resistance

Drought resistance has been attributed to a number of adaptive mechanisms, but there is as yet no consensus on the most useful breakdown of categories of drought resistance (Turner 1986a). In many cases, the distinctions are more semantic than substantive. Three principal ways in which plants can adapt to drought are acknowledged (Levitt 1972).

- Stress escape: the ability of a plant to complete its life cycle before serious soil and plant water deficits develop.
- Stress avoidance: Maintaining a favourable internal water balance, thereby postponing the negative effects of drought.
- Stress survival: Surviving relatively long periods of drought (drought survival).

2.1.2.1 Drought escape

A: Ephemeral

Many desert plants germinate at the beginning of the rainy season and can have as short as five to six weeks growing period (Polunin 1960; Kassas 1966) .

B: Early maturity

Early maturing cultivars usually encounter fewer moisture stress periods, and avoid terminal stress; these do not, however, escape drought completely, and usually have additional drought resistance attributes. For example, in a study of drought stress on wheat cultivars during the period of ear growth and development Blum and Pnuel (1990) found that early cultivars tended to also have more osmotic adjustment.

In areas with alternate rainy and dry seasons, the ability to achieve maturity before the soil dries out is the main adaptation to drought of cultivated crops, and matching crop phenology to available moisture supply has always been a major breeding objective (Bielorai 1992b).

C): Developmental plasticity

Developmental plasticity is where the duration of growth varies according to the extent and timing of water deficit (Ludlow and Muchow 1990), and frequently occurs in indeterminate cultivars. Early maturity may be induced by water stress, which is an advantage in dry years; in more favourable years, maturity is delayed and the plant can benefit from a longer growing period. Phenological plasticity will be more beneficial than earliness, where soil moisture supply is less predictable.

Sinclair *et al.* (1987) state that cowpeas have an advantage over other grain legumes through their ability to delay development under moisture stress so that flowering and pod formation resume when a favourable moisture regime is re-established.

D) Seed dormancy

The seeds of many species that grow in regions with a hot, dry season are not

able to germinate at high temperatures, they are thereby protected from destruction (Evenari 1962). In desert plants, the process of germination can be stopped and re-started at different stages between imbibition and the appearance of the radicle and shoot, without loss of germination ability (Evenari 1962).

2.1.2.2 Avoiding stress

A favourable water balance under conditions of limited water supply can be achieved (a) by improving water uptake sufficiency, so as to replenish lost water (so-called water spenders), (b) by conserving water, i.e. restricting transpiration before or as soon as stress is experienced (water savers), or (c) postponing dehydration and thereby enabling plants to avoid the effects of stress by maintaining turgor and cell volume.

A: Maintaining water uptake

In annual plants of the dry savannah, roots may account for 30–40% of total dry matter, whilst the proportion may rise to 90% in perennial desert species, with the roots growing to great depths. By contrast, annual ephemeral species, that grow during short moist periods as and when they occur, or in depressions in which water has accumulated, have poorly developed root systems (Fitter and Hay 1987). The role of root systems in the maintenance of water uptake will be discussed in Section 2.6

B) Reducing water loss

The most common way plants regulate water balance and maintain turgidity is to reduce water loss. Several mechanisms are possible, and can be grouped as a) reducing absorption of radiation, or b) increasing stomatal, cuticular and root resistance.

a) Reduced absorption of radiation.

Leaf area reduction is a common trait following the imposition of water stress because it decreases the probability of the crop depleting water before reaching maturity (Ludlow and Muchow 1990). Leaf shedding increases the root/leaf ratio and

modifies the carbon balance of plants. Generally, nitrogen is recovered before abscission (Schulze 1988). Many desert species, such as *Artemisia herba-alba* have two leaf types, large winter leaves that are shed at the end of the wet season, and replaced by very small summer leaves (Zohary 1961). Krieg (1983) pointed out that plasticity of leaf area development is a useful trait allowing plants to conserve water during the vegetative stage that can be used during seed formation. Excessive reduction in leaf area, will impose an irreversible limitation on yield due to the close association between leaf area and seed number in most crop plants.

Another way water loss from plants is reduced is through leaf movements such as leaf rolling, folding and wilting of leaves at the time of water stress. These movements help reduce the heat load and water loss.

Active movement of leaves is also an adaptation to both low and high light intensities. A special case of leaf orientation is the active leaf movement commonplace in Cucurbitacea, Leguminosae, and others. When water supply is adequate, the leaves are oriented perpendicular to incoming radiation. Thus the maximum photosynthetic rate is assured, but water loss is high. When stress occurs, leaves orient parallel to incoming radiation, thereby reducing heat load and transpiration. Indirect light is still sufficient for photosynthesis (Schulze 1988).

Leaf reflectance caused by the presence of epicuticular wax (Ludlow and Muchow 1990) is another factor which reduces water loss. Besides increasing reflectance, the wax also lowers epidermal conductance (Bielorai 1992a) and transpiration, thereby increasing water use efficiency (WUE).

b) Increased resistance to water flow

Leaf structure can also influence water loss. Leaves can have characteristics such as waxy surfaces, thick cuticles, sunken stomata, the presence of spines, and hairiness that help to reduce the transpiration rate under dry conditions.

Stomatal resistance, stomatal number and location are also involved in the control of water loss. This will be discussed in Section 2.5.4.

Reduced cuticular conductance, is important since it is the main pathway of water loss when stomata are closed (Bielorai 1992a). Low epidermal conductance

delays leaf dehydration and, therefore, promotes leaf survival (Sinclair and Ludlow 1986). Since water loss through the cuticle is significant only when the stomata are closed, low cuticular conductance has no adverse effect on photosynthesis and does not reduce yield potential. It should therefore enhance plant survival in intermittent stress environments without any cost in performance (Ludlow and Muchow 1990).

C) Mitigating stress

Osmotic adjustment, high root/shoot ratio, and transpiration efficiency are adaptations to drought that permit plants to maintain a high internal water potential in spite of the stress. They are thereby able to maintain cell turgor and growth, avoid secondary drought-induced stress, as well as direct and indirect metabolic injury due to dehydration (Levitt 1972).

Maintenance of turgor in spite of leaf water deficits is a major factor in mitigating stress since many biochemical, physiological, and morphological processes in the plant are sensitive to leaf turgor (Turner and Burch 1983). Osmotic adjustment is an adaptive process which enables a plant to maintain turgor despite lower internal water potential. This factor is explained in detail in Section 2.4.2.

For short-term water stress (e.g. at midday) a balance between water uptake and loss can be achieved through stomatal closure. For longer-term stress, however, adaptation by increased ratio between effective root surface and leaf area (Loomis 1983) is necessary. Begg and Turner (1976) pointed out that a high root:shoot ratio is a very effective means of plant adaptation to water stress.

Transpiration efficiency (W) is defined as mass or moles of C or CO_2 fixed per unit of water lost from a leaf. This contrasts with WUE of a plant which is dry matter produced per unit of water lost (Ludlow and Muchow 1990).

In principle, there should be no cost for higher W and it should contribute to yield potential and stability. Ludlow and Muchow (1990) concluded that "this trait has great promise and potential for increasing yield of crops in the semi-arid and arid tropics" (see Section 2.2).

2.1.2.3 Dehydration tolerance (low lethal water status)

The degree to which plant parts withstand desiccation can be expressed as the relative water content (RWC) or water potential (ψ) at which leaves die, these are called lethal values (Ludlow and Muchow 1990).

There are large differences between different plant organs in their ability to withstand desiccation. In many perennial species, the above-ground parts die off with the onset of the hot, dry season and the underground parts, such as rhizomes, bulbs, corms, and tubers, remain alive but dormant (Vegis 1963).

The ability of plants to store water in their tissue is another useful character in dry conditions. Succulents are a special group, which tend to be preponderant in arid regions which have short annual rainy seasons (Walter 1962). They are able to survive periods of absolute drought during which they are almost completely unaffected by their environment, both aerial and edaphic, through use of the reserves of water that they accumulate in their storage organs during periods when water is available in the soil.

2.2 Water use efficiency

2.2.1 Introduction

Plant WUE was a topic of early scientific study (Woodward 1699, Lawes 1850; Lawes (1980); Briggs and Shantz 1914). The determination of factors influencing WUE has been the objective of many studies of water relationships. Wittwer (1975) identified water as the second-most limiting factor, behind land area, to increasing food production. He argued that a high research priority should be an improvement in the efficiency of water use by plants.

2.2.2 Definitions

Water use efficiency is an ambiguous term, with a wide range of meanings and usages, although the basic definition is the total biomass produced per unit of water use.

Factors which influence WUE depend on the definition of WUE. Plant mass can be a function of carbon dioxide assimilation (A), total crop biomass (B) (above or below ground), or crop grain yield (G). Water consumed can be expressed as transpiration (T), evapotranspiration (ET), or total water input to the system (I).

Stanhill (1986) has described water use efficiency at both the hydrological and physiological levels. In a hydrological context, WUE relates primarily to efficiency with which water is used in irrigation, and is defined by Bos and Nugteren (1974) as "the increase in water content of the root zone following irrigation, expressed as a fraction of the total water supplied to the irrigated area". In a physiological context WUE has been defined as the ratio of the weight of crop water loss to the atmosphere to that of its yield or total dry matter production. Stanhill (1986) preferred to use the term transpiration ratio rather than WUE. When the crop water loss includes evaporation from the surface of the soil and crop canopy then Stanhill (1986) used the term evapotranspiration ratio which was expressed on a unit crop area basis and evaporation was related to the fresh weight of the yield component, often as a reciprocal of the evapotranspiration ratio, i.e., kg/ha/mm. Values of transpiration ratio and evapotranspiration ratio are normally within the range 100 to

1000 g/g (Stanhill 1986). In irrigation studies, evapotranspiration ratio is often termed the "water production function" and is related to water application rather than evapotranspiration.

Farquhar *et al.* (1989a) defined the term transpiration efficiency as the total amount of carbon biomass produced per unit water transpired by the plant. The instantaneous ratio of CO₂ assimilation rate of a leaf, (A), to its transpiration rate (E) is given approximately by $A/E = ((P_a - P_i) / 1.6v)$ (Farquhar 1989b) where P_i and P_a are the ratio of partial pressure of CO₂ inside the leaf air spaces and atmosphere, respectively, v is the water vapour pressure difference between the intercellular spaces and the atmosphere, and the factor 1.6 is the ratio of diffusion of water vapour and CO₂ in air.

Because of the difficulties in determining the weight of roots, Gregory (1988) suggested another approach to determining WUE by considering only the above-ground biomass (Ludlow and Muchow 1990). For most crops, only part of the dry matter produced is of economic significance to the farmer (e.g. grain crops), therefore the economic proportion of the total dry matter can be related to the amount of water loss. Turner (1986b) has pointed out the usefulness of WUE based on grain yield, or economic yield per unit of growing season rainfall for the agronomic evaluation of crops.

In semi-arid conditions in which run-off (R), drainage (D), and interception of rainfall by crop canopies (I) are substantial components of the water balance, Gregory (1988) suggests:

$$WUE = \frac{Y_{ec} / T}{1 + [E + R + D + I] / T}$$

where Y_{ec}/T is transpiration efficiency considering economic yield (Y_{ec}), R (run-off), D (drainage), and I (interception of rainfall by crop canopies) are substantial components of water balance. Bielorai (1992a) supported the usefulness of the above equation because it indicates it is possible to improve WUE by increasing Y_{ec} or by increasing T proportionally more than the other water losses. Overall, the transpiration efficiency is a function of plant physiology, while the components E+R+D+I are amendable to soil and crop management.

2.2.3 Factors effecting water use efficiency

2.2.3.1 Plant factors

Plants have different methods to improve their WUE, their mode of carbon fixation being one of them. C4 plants have a physiological advantage over C3 plants at higher temperatures and light intensities (Ehleringer and Mooney 1983). Certain tropical crops have a combination of the Hatch-Slack (C4) photosynthetic pathway, and the Calvin (C3) cycle. This combination appears to be intrinsically more efficient than the C3 cycle alone, at least under high radiation or high temperature (Pearson and Ison 1987). Crassulacean acid metabolism (CAM) plants have a low transpiration ratio because their stomata close during the day and open at night and fix CO₂ in malic acid, causing a gradient from the atmosphere to the leaf. The CO₂ absorbed at night is assimilated by the C3 pathway during the day, in the almost complete absence of transpiration. Schulze (1988) pointed out that the high WUE of CAM plants resulted in their slow growth, because of the dependence of their carbon gain to the size of their vacuoles for malate storage.

The ability of leaves to expand and fully shade the ground, thus decrease water transpired by weeds and evaporated from the soil is an important plant characteristic to increase WUE (Stanhill 1986). Bolger and Matches (1990) noted that differences in leaf area index (LAI) development of sainfoin and alfalfa accounted for differences in evaporation. The higher rate of leaf area development in lucerne than sainfoin in the second harvest resulted in final LAI of 3.2 and 1.3 for lucerne and sainfoin respectively, and compared with lucerne the lower LAI of sainfoin at regrowth harvest caused its greater evaporation and lower season-long WUE than lucerne.

Sinclair (1984) stated that one of the best opportunities to increase WUE is that leaf gas exchange occurs, only when the difference between saturation vapour pressure at the leaf surface (e_L) and vapour pressure of the atmosphere (e) [Vapour pressure deficit "VPD" ($e_L - e$)] is low. The high WUE of sainfoin in the early spring (Bolger and Matches 1990) can be related to the high vegetative growth of sainfoin in the early spring when VPD is less than summer, considering the

$WUE = 1.6c * ((p_a / (e_L - e)))$ (Sinclair *et al.* 1984), and this aspect will be expanded in Chapter 8.

The WUE can be expected to decrease as the ratio of the internal assimilating surface to the transpiring surface of a leaf increases. Nobel (1980) has measured this ratio between extremely xerophytic and mesophytic species, and found greater WUE as a result of increased mesophyll cell wall area to leaf area.

Diurnal change in foliage orientation is also another factor which influences WUE. Lucerne, cowpea, and beans reduce their radiation absorption under conditions of high irradiance by leaf movements which avoid direct solar radiation, this is termed paraheliotropism (Shackel and Hall 1979; Ehleringer and Forseth 1980; Travis and Reed 1983).

2.2.3.2 Environmental factors

The principal environmental factor affecting WUE is atmospheric humidity. Turner (1986a) noted that an increase in the VPD of the atmosphere around a leaf increased transpiration without a commensurate increase in photosynthesis, leading to a decrease in W.

Temperature also affects WUE through its effects on VPD and higher temperatures will increase WUE. Bieloria (1992a) noted for cool-climate plants (mostly C3), that WUE decreased with increasing temperature, whilst (within certain limits) the opposite was true for warm-climate crops (C4). Jones *et al.* (1985) found that transpiration increased with air temperature at two levels of CO₂ concentration (330 ppm and 800 ppm) solely due to the associated increase in saturation vapour pressure deficit and hence vapour pressure gradient in soybean.

Soil moisture content has a direct effect on plant growth and productivity, and therefore low soil moisture is conducive to low WUE. Soil factors exert a direct influence on nearly every phase of the agricultural hydrologic cycle e.g.: determining infiltration into, and runoff from, the soil surface, downward drainage and upward capillary movement through the root zone, as well as availability of stored soil water, the component that is potentially available for crop exploitation in transpiration. Soil factors also indirectly affect WUE through their influence on both static and dynamic

plant processes. For example, the temperature and salinity of the soil can limit water uptake by the root system of the plant and so decrease transpiration from the canopy. Dynamically, the same factors influence the growth of the crop root and canopy systems, and can thus also limit crop transpiration. Low soil moisture increases the stomatal resistance through increasing ABA levels and decreases leaf water potential, and finally increases WUE.

The internal (p_i) and external (p_a) concentration of CO_2 affect the WUE mainly through their influence on photosynthesis. The relationship between CO_2 concentration and WUE is given by $A/E = \{[p_a * (1 - (p_i/p_a)) / (1.6 * v)]\}$, the greater the difference between p_a and p_i the greater the WUE (Farquhar and Richards 1984).

2.2.4 Improving water use efficiency

WUE can be improved by reducing the amount of water used, or by increasing yield. Reduced water loss could result from a higher stomatal resistance, thicker boundary layer, or greater cuticular resistance. A higher stomatal resistance will not only reduce transpiration but will also limit CO_2 exchange, Stanhill (1986) stated "because the total diffusion pathway to water is less than for CO_2 , which has an additional liquid-phase resistance up to the chloroplast, it is to be expected that any increases in the stomatal, cuticular, and boundary layer resistance will reduce transpiration more than carbon exchange, and so increase WUE. Sinclair *et al.* (1984) pointed out that the midday closure of stomata during periods of vapour pressure deficit would be a very useful strategy for increasing WUE.

Alteration of the cropping VPD environment is another way to improve WUE. This could be achieved in two ways. A geographical solution would be to grow plants in regions with a more humid climate where greater WUE results from lower ($e_a - e$). Another approach is by shifting the growing season to periods of lower ($e_a - e$). Koch *et al.* (1972) found that the greater growth of sainfoin at the first cutting in early spring gave a higher WUE than in the other seasons. The WUE of sainfoin and lucerne and their growth pattern over the season will be investigated in Chapter 8.

2.3 Water status of the plant

2.3.1 Water potential

In a transpiring plant that is well supplied with water, the free-energy status of the water declines gradually as it passes through the soil to the root, through the xylem to the transpiring leaves, and into the atmosphere. For water to move against a gradient from a high energy potential to a point of low energy potential a force in the contrary direction has to be applied i.e. work has to be done. The potential energy of water is defined relative to pure water at a specified temperature, and elevation.

2.3.2 Components of water potential

The total water potential (Ψ) of the plant consists of several mutually independent components: the osmotic potential (π), arising from solutes in the water; the turgor or pressure potential (P), arising from hydrostatic forces in the system; and the matric potential, arising from capillarity forces at the water-air interfaces. The water potential is measured in units of energy per unit volume which is dimensionally equivalent to pressure (Turner and Kramer 1980). Since Turner (1986b) has reviewed all water potential components and the techniques for their measurement, only the role of osmotic adjustment and related factors such as turgor potential, root-shoot communications, and stomatal resistance will be discussed.

2.4. Osmotic adjustment

2.4.1 Definition

Osmotic adjustment in higher plants refers to the lowering of osmotic potential arising from the net accumulation of solutes in response to water deficit or salinity. Turner and Jones (1980) recommend that the term osmotic adjustment be used only for the accumulation of solutes in higher plants in response to water deficits and that osmoregulation and turgor regulation be reserved for use in relation to lower plants in response to salinity.

2.4.2 Osmotic potential (π)

In considering the osmotic effect of cell solutes their chemical nature is of secondary interest compared to their concentration. Solute concentration can be related to π using Vant Hoff's equation (Kramer 1983), $\pi = -RTN_s/V_w$ where π = osmotic potential, T= absolute temperature (273+25 °C=298 K), R= gas constant (8.32 ml MPa/K mol), V_w = solution volume (ml), and N_s = moles of solute (Munns and Weir, 1981, Morgan 1992).

The degree of osmotic adjustment is measured as the change in osmotic potential at a particular water potential or water content, for convenience and purposes of comparison. It is usual to measure the degree of osmotic adjustment at either full or zero turgor (Turner and Jones 1980).

In higher plants the osmotic potential and elasticity of the tissue will determine turgor potential at a particular water potential (Turner 1979; Turner and Jones 1980). In addition to turgor, solute accumulation, cell size, osmotic volume and cell wall thickness are effected by both osmotic potential and elasticity (Steudle *et al.* 1977). Barker *et al.* (1993) found the occurrence of osmotic adjustment in response to water deficit in both C3 and C4 grass species, but lower osmotic adjustment for the C3 species. The more elastic cell wall (low modulus of cell wall elasticity) of C3 grasses maintained turgor despite loss of water.

2.4.3 Components of osmotic adjustment

Consideration of specific components of osmotic potential (π) is necessary for a mechanistic understanding of how the process occurs (Barker 1990). The important components are classified into the following groups (Thomas 1991; Munns and Weir 1981): 1) sugar 2) inorganic acids 3) organic acids and 4) amino acids. Munns and Weir (1981) found an increase in sugars accounted for 70-100% of the 0.12-0.34 MPa osmotic adjustment of wheat leaves. In contrast, Morgan (1992) found that sugar accumulation did not account for osmotic adjustment in wheat, but that potassium was the most abundant inorganic ion. Morgan (1992) found that 78% of osmotic adjustment was due to potassium accumulation, with amino acids the only

other important contributor (22%). In contrast, Turner *et al.* (1978) found no change in potassium concentration during osmotic adjustment.

Malate is the organic acid that usually responds to water stress, however, aconitate, citrate, and succinate have also been implicated. Ford and Wilson (1981) found changes in the levels of malate of (40 $\mu\text{mol/g DM}$) in spear grass (*Heteropogon Contortus*).

Accumulation of proline is a widely recognized metabolic response of plants to water stress (Barker 1990). In a study of C3 and C4 species Barker *et al.* (1993) found that the physiological role of proline accumulation was uncertain as even a dramatic increase in leaf proline concentration was unlikely to influence osmotic potential.

2.4.4. Importance of osmotic adjustment in dry conditions

The metabolic cost of using photosynthate for osmotic adjustment in grain sorghum (*Sorghum bicolor* L.) was less than the cost of converting it to new biomass (Richardson and McCree 1985). This suggests that there is no particular cost of osmotic adjustment above that of normal growth. For this reason, and because osmotic adjustment is an inducible trait that occurs only when stress develops, there should be no loss of yield potential.

Turner (1978) suggested that osmotic adjustment has several major advantages and a few limitations. Included in the advantages were (a) maintenance of cell turgor (b) continued cell elongation, (c) maintenance of stomatal opening and photosynthesis, (d) survival of dehydration, and (e) greater soil exploration by roots. Re-watered plants can lose most of their osmotic adjustment within 10 days. A second limitation is the finite limit to adjustment. Ludlow and Muchow (1990) noted that some of the consequences of osmotic adjustment promote dehydration avoidance, and some reduce it. The continued water loss caused by maintenance of green leaf, delay of leaf rolling (Hsiao *et al.* 1984), and stomatal adjustment reduces dehydration avoidance. An inevitable consequence is that leaf water potential falls progressively (Morgan 1984), and this can cause leaf and plant death if critical ψ or RWC are reached, or if the soil water is exhausted, irrespective of the dehydration tolerance

of the species (Ludlow *et al.* 1983). Thus, species like soybean (*Glycine max* L.) and some forage legumes, which have high osmotic adjustment and high dehydration tolerance, die before other species such as cowpea and siratro that lack this attribute (Ludlow *et al.* 1983).

When osmotic adjustment results in greater root growth and exploration, consequently soil water extraction dehydration avoidance is enhanced. Blum (1988) pointed out that osmoregulation is effective in providing plant tolerance to salinity and freezing stress, both of which involve a component of water deficit. Munns (1988) however, concluded that more measurements of leaf water relations and the concentrations of individual solutes are unlikely to provide any useful information, Munns (1988) argued that progress in evaluating the role of osmotic adjustment in plant tolerance of drought and salinity will only occur if the nature of research changes from that of collecting repetitive data to that of testing hypotheses. However, Ludlow and Muchow (1990) recommended osmotic adjustment as a highly desirable characteristic for plants in intermittent and terminal stress environments in modern agriculture. At present more research is needed to fully understand the role of osmotic adjustment in crops, during drought. Further explanation of osmotic adjustment is provided in Chapters 7 and 8.

2.5 Stomatal resistance

In dry conditions when water is the factor limiting growth increasing stomatal resistance may be a useful action for plant survival. On the other hand, the closure of the stomata will limit the plant growth via inhibition of CO₂ diffusion to the mesophyll. The physiological behaviour of stomata and factors affecting stomatal resistance are discussed in this section.

2.5.1 Stomatal resistance and water stress

As water stress develops, physiological responses occur in the plant, such as decreases in leaf water potential, relative water content, photosynthetic rate, and osmotic potential. Typically stomatal closure occurs as a response to water stress. Various stomatal characteristics such as low conductance, high sensitivity to water status and saturation deficit, and abscisic acid (ABA) accumulation, have been suggested as desirable traits to improve the yield of crops in water-limited environments (Jones 1980; Turner 1982, 1986b). All these characteristics reduce water loss and lower the probability of desiccation.

2.5.1.1 Stomatal resistance and transpiration

The role of stomatal resistance to control transpiration has been reviewed recently (Meinzer 1993). Until recently, the idea that stomata play a dominant role in regulating transpiration from extensive stands of vegetation has remained largely unquestioned by plant physiologists and ecologists (Meinzer 1993). In contrast, micrometeorological models often adequately predict canopy transpiration without explicit consideration of stomatal response (Jarvis *et al.* 1986). These results have led to opposing conclusions on the relative importance of environmental variables such as incident radiation and of stomatal movement in individual leaves on the control of transpiration from well-watered vegetation. Regardless of the extent to which stomatal movements ultimately control transpiration, considerable research has documented that stomata sense and respond dramatically to variation in the aerial and soil environment. Here, stomatal response to humidity and soil drying are discussed.

2.5.1.2 Stomatal response to drought and reduced plant water status

Soil drying influences stomatal behaviour through the effect of reduced water uptake reducing the water status of leaf tissue. Rate of drying affects the water potential at which stomata close. Feres *et al.* (1978) showed that sorghum stomata did not close in non-irrigated field conditions when the leaf water potential was as low as -1.0 MPa. However, plants grown with a restricted root zone where the stress developed more rapidly showed closure at -1.4 to -1.6 MPa.

Blum and Johnson (1993) found reduction in stomatal conductance in non-stressed plants when their relative water content and leaf water potential decreased, indicating the control of stomatal resistance was by leaf water status. Turner *et al.* (1978) presented a table of the different water potential values required for stomatal closure and emphasized that there is not a unique water potential value for stomatal closure. Pierce and Raschke (1980) showed that the water potential at which abscisic acid (ABA) was produced corresponded to zero turgor pressure. Other studies have shown that ABA increased linearly with decreasing turgor (Henson 1983, 1985). Turner *et al.* (1985) noted that leaf conductance and carbon assimilation were not closely coupled to the leaf water potential or leaf turgor pressure in sunflower and oleander. It is clear that stomatal resistance is not always related to leaf water potential. One explanation is that stomata do not open because plants respond to the availability of water in the soil and regulate stomatal behaviour accordingly, whatever the water status of their shoots, which suggests a role for phytohormones (discussed in Section 2.5.4). The information about the direct influence of leaf water potential on stomatal resistance requires further information, and this aspect will be discussed in Chapters 3-8.

2.5.2 Stomatal response to humidity

Humidity can control stomatal resistance (Schulze *et al.* 1972; Camacho *et al.* 1974; Pallardy and Kozlowski 1979; Morison and Gifford 1983; Muchow *et al.* 1986; Kaufman 1976 a,b). As the leaf-to-air vapour pressure deficit increases, stomata close and stomatal resistance increases (Hall and Hoffman 1976; Aston 1976). Those

species with a direct response to humidity average larger sub-stomatal cavities than other species (Sheriffi 1977). Lange (1975) noted that the humidity response affected the diurnal stomatal resistance pattern and controlled the midday depression of transpiration and photosynthesis, especially under desert conditions. The response to humidity can also be affected by other environmental factors such as temperature, sun or shade, and whether plants had been previously exposed to stress (Kaufman 1976a).

2.5.3 Stomatal response to CO₂ concentration

The sensitivity of stomata to CO₂ concentration and the greater responsiveness of C₃ plants than C₄ plants has been widely reported (Akita and Moss 1972; Ludlow and Wilson 1971; and Osmond *et al.* 1980). The sensitivity of stomatal resistance to internal and external concentrations of CO₂ in the leaf and the effect of humidity (VPD) on the sensitivity of stomatal conductance to CO₂ was demonstrated by Morison and Gifford (1983). They found the sensitivity of stomatal resistance to CO₂ was linearly proportional to the magnitude of stomatal resistance. Similarly, the sensitivity of stomatal resistance to VPD was linearly proportional to the magnitude of stomatal resistance.

Coordination of gas exchange and chloroplast activity are important to plant performance in water limited environments. Together they allow growth to proceed while minimizing dehydration. Stomatal closure is a primary effect of moderate water stress and the observed decrease in photosynthesis under these conditions is often mainly due to a reduction in the partial pressure of CO₂ inside the leaf (Chaves 1991; Vasey *et al.* 1991). Yves and Markhart (1992) found *Phaseolus acutifolius* had a higher net photosynthetic rate than *P. vulgaris* at a high to moderately low water potential and they found the more rapid decrease in the photosynthetic rate of *P. acutifolius* at low water potential than for *P. vulgaris* was related to increased stomatal closure as water potential decreased. Higher photosynthetic rate at any given internal CO₂ led to a higher WUE in *P. acutifolius* than in *P. vulgaris*.

2.5.4 Stomatal response to phytohormones

Stomata apparently play an active role in regulating leaf water status rather than the more commonly believed converse. The basis for this response appears to be some form of the chemical signalling between the roots and the shoots. The role of stomata in the reduction of transpiration during soil drying often results in a more favourable leaf water status during drought than in well-watered plants (Davies and Jeffcoat 1990; Davies and Zhang 1991). Jensen *et al.* (1989) found no significant changes in water potential or turgor pressure in the roots or the leaves of lupin (*Lupinus cosentinii* Guss. cv. Eregulla) when a small reduction in soil water potential was induced which led to 60% reduction in leaf conductance.

Although some ABA is synthesized in the mesophyll cell of the leaf it is predominantly produced in the roots (Zeevaart and Boyer 1984; Hubick *et al.* 1986b; Cornish and Zeevaart 1985, 1986). ABA then moves into the transpiration stream, where it possibly could act as the communicator between the root and shoot. However, a recent study in which the roots of maize (*Zea mays* L.) plants were split and one half allowed to dry while the other half remained in wet soil, showed stomata began to close as the soil in the un-watered pot dried without any detectable change in the abscisic content of the leaf and a slight increase in leaf turgor pressure (Blackman and Davies 1985). More details of root and shoot communications can be found in reviews by Davies and Zhang (1991) and Davies and Jeffcoat (1990).

Kinetin and zeatin are other phytohormones involved in stomatal behaviour. Incubation of the leaves from plants with partly closed stomata in solutions of kinetin and zeatin induced the stomata to open (Turner 1986b). Hubick *et al.* (1986a) have shown that water deficits induced a reduction in cytokinin as well as an increase in abscisic acid in sunflower (*Helianthus annuus* L.) shoots. They found the decrease in cytokinin levels in the roots resulted not from a reduction in the production of cytokinin by the roots but from an increase in "bound" cytokinin in the root which was not transferred to the shoots.

2.6 Roots and water stress

The effect of drought on plant growth can be reduced by root growth increasing the supply of water. This can be achieved by deeper rooting, by changing

the distribution of the root system, or altering the size of the vascular system (Gregory 1989; Turner 1986b; Passioura 1982, 1983). There is genotypic variation in rooting depth and in the rate of downward growth of the root axes. Genotypic variation in root characteristics of crop plants has been reviewed extensively by O'Toole and Bland (1988). Variation occurs in both monocotyledons and dicotyledons. Many root characteristics have been shown to be under genetic control and, therefore, heritable (Ludlow and Muchow 1990).

2.6.1 Rooting depth

Deeper roots could recover some of the water otherwise lost by deep drainage. Some evidence suggests deep roots may have additional benefits for water extraction and root function. Since water uptake continues at night, an increase may occur in the soil water content of the upper soil layers and presumably of roots in these layers (Richards and Caldwell 1987). This water is available the following day. As well as assisting water extraction, this 'hydraulic lift' could keep roots alive in the upper layers where most of the nutrients and hence, nutrient uptake, occur. Since deep roots moderate the effects of water stress this may also reduce the production of hormone signals, which reduce leaf growth and stomatal conductance (Turner 1986b). The cost of water used for producing deeper roots in comparison to water gained has been questioned. Passioura (1983) claimed the cost of assimilates used for deep root production could have been used for shoot growth and thus increased yield. Other investigators have found the cost for extra deep roots was small compared to the above-ground biomass. For example, sorghum, at maturity can exceed 10,000 kg/ha of above-ground dry weight (Wright *et al.* 1983; Muchow 1988,1989). Ludlow and Muchow (1990) agreed the benefits of greater rooting depth and density in opportunistic situations, out-weighed the possible risk of running out of water and the carbon cost in above-ground growth.

For short-term water stress, control of water loss can be achieved through stomatal closure, however, long term adjustment must come through adaptation of the ratio between effective root surface and leaf area (Loomis, 1983). The Shoot:Root ratio (S/R) is a very plastic character, and tends to decrease with water stress (Fitter

and Hay 1987).

Kummerow (1980) pointed out the S/R does not offer any clues regarding the adaptation of roots to water stress because it can be affected by factors other than water stress e.g. nutrient deficiency. However, Begg and Turner (1976) noted that at a low S/R is a very effective means of plants adaption to water stress. It is easier for the root system of the individual plant to maintain an adequate water supply if the transpiring surface is reduced or root size increased.

Hsiao and Acevedo (1976) found that water stress reduced the above-ground mass of stressed lucerne plants, but did not reduce the root dry mass of water stressed plants compared to control plants. Mayaki *et al.* (1976) reported that under water stress, shoot height of soybean was reduced more than root depth. Some aspects of rooting will be discussed further in Chapter 7.

2.6.2 Root Hydraulic conductance

The hydraulic conductance to water flow in the plant is an important factor influencing water uptake. A high hydraulic resistance between the soil and the shoot will result in a lower leaf water potential than for plants with a low root hydraulic resistance. The hydraulic resistance between the root and shoot varies with species, for example, a four-fold difference between bean (*Phaseolus vulgaris*) and soybean (Boyer 1971). Turner (1986a) noted that any axial resistance to flow in the plant is likely to decrease leaf turgor more than root turgor provided there is not a concomitant decrease in osmotic potential. Therefore, plants with high hydraulic resistance to water are more likely to be sensitive to aerial stress and less sensitive to soil water stress than plants with a low resistance. Turner *et al.* (1984) showed that species with high hydraulic resistance were more sensitive to high vapour pressure deficits than species with low hydraulic resistances. Resistance to water flow in wheat can be increased by either reducing the number of root axes or reducing the diameter of the xylem vessels (Belford *et al.* 1987). Seminal roots may be more important than nodal roots (for water flow) because they sometimes grow deeper in the profile (Belford *et al.* 1987).

2.7 Carbon isotope discrimination

2.7.1 Definition

Carbon isotope discrimination and its application in plant science has been extensively reviewed by O'Leary (1981) and Farquhar *et al.* (1989a). Carbon isotope discrimination and its relationship with the effect of drought on plants and water use efficiency is outlined here.

Atmospheric carbon dioxide contains about 1.1% of the heavier carbon isotope ^{13}C and 98.9% ^{12}C (Carig 1954). Ribulose biphosphate (RUBP) carboxylase-oxygenase reacts more rapidly with $^{12}\text{CO}_2$ than with $^{13}\text{CO}_2$. The estimate of this effect is 1.029 with respect to CO_2 dissolved in water (Roeske and O'Leary 1984), and therefore, 1.030 with respect to CO_2 in air, since there is proportionally less $^{13}\text{CO}_2$ in solution than in the gas phase at equilibrium (Vogel *et al.* 1970). If this were the only source of isotope discrimination, the molar abundance ratio of CO_2 in air ' R_a ' ($^{13}\text{C}/^{12}\text{C}$) would be 1.030 times that in the plant ' R_p ' using the definition of discrimination by Farquhar and Richards (1984), $\Delta = (R_a/R_p) - 1$, $= 30 \times 10^{-3} = 30 \text{‰}$ (Farquhar *et al.* 1987).

The absolute isotopic composition of a sample is not easy to measure directly. Rather, the mass spectrometer measures the deviation of the isotopic composition of the material from a standard, $\delta = (R_p/R_s) - 1$, where R_s is the molar abundance ratio, $^{13}\text{C}/^{12}\text{C}$, of the standard. The reference material traditionally has been carbon in carbon dioxide generated from a fossil belemnite from the Pee Dee formation, denoted PDB [for which $R = 0.01124$ (Graig 1957)]. Using the two equations above we have $\Delta = (\delta_a - \delta_p) / (1 + \delta_p)$. On the PDB scale, free atmospheric CO_2 ($R_a = 0.01115$ in 1988) currently has a deviation (δ_a) of approximately -8‰ and typical C3 plants material ($R_p = 0.01093$) a deviation (δ_p) of -27.6‰ , which yields $\Delta = 20.1\text{‰}$.

2.7.2 Drought, soil strength and discrimination (Δ)

Drought results in diminished stomatal conductance, rate of transpiration and assimilation. Intercellular CO_2 partial pressure usually decreases during photosynthesis, or as stomatal resistance increases. This is revealed in smaller discrimination against ^{13}C (Farquhar and Richards 1984; Jonathan and Ehleringer

1993; Condon *et al.* 1992). The normal gas-exchange pattern is a reduction of conductance, followed by an apparent inhibition of RUBP regeneration capacity, and either at the same time or later, sometimes, an apparent loss of RUBP activity (Caemmerer and Farquhar 1984; Kirschbaum 1987; and Sharkey and Badger 1982).

Masle and Farquhar (1988) showed that transpiration efficiency increased and discrimination (Δ) decreased as soil strength increased. Soil strength increases with increasing bulk density, as occurs with compaction, and also with decreasing soil water content. Thus, some drought effects may be accompanied by effects of increased soil strength, which increase resistance to deformation and, therefore, to root penetration of the soil. There is a more detailed examination of carbone isotope discrimination in Chapter 9.

2.7.3 Water use efficiency and discrimination (Δ)

In a leaf, the instantaneous transpiration efficiency, that is the ratio of net photosynthesis (A) to transpiration (E) is given by $A/E = [P_a(1-P_i/P_a)]/[1.6(e_i-e_a)]$ (Farquhar 1989). Integrated over the life of plant, the transpiration efficiency (W) is $W = [P_a(1-P_i/P_a)*(1-\phi)]/[1.6(e_i-e_a)]$ (Farquhar *et al.* 1989a). The vapour pressure difference between the leaves and air is (e_i-e_a) and losses of carbon or water not associated with CO₂ uptake through the stomata are represented by ϕ . The partial pressures of carbon dioxide inside and outside the leaves are P_i and P_a , respectively.

The discrimination in C3 plants is, like W, determined by the ratio of internal to ambient CO₂ pressure. Farquhar and Richards (1984) predicted discrimination with the following equation: $\Delta = (4.4 + 22.6 P_i/P_a) * 10^{-3}$. If we rearrange this equation and substitute for P_i/P_a into the previous equation we have the expression where W is dependent on Δ : $W = [P_a(0.027 - \Delta)(1 - \phi)]/[0.036(e_i - e_a)]$ and the negative relationship between W and Δ is clear. This relationship has been confirmed practically in peanut (Hubick *et al.* 1986a, 1988), barley (Hubick and Farquhar 1989), cotton (Hubick and Farquhar 1987), sunflower (Virgona *et al.* 1990), crested wheatgrass (Mayland *et al.* 1993), wheat (Araus and Buxo 1993, Condon *et al.* 1992), rice (Dingkuhn *et al.* 1991), and cowpea (Ismail and Hall 1992). More information about W and Δ can be obtained from Farquhar *et al.* (1989a) and Farquhar (1990).

2.8 Sainfoin (*Ononbrychis viciifolia* Scop.)

Sainfoin originated from the Near East which includes the regions of Iran, Turkey, Iraq, the Caucasian Mountains and the area east of the Caspian Sea (Vavilov 1951). However, the present distribution of sainfoin is wide, covering the Mediterranean, Central Europe, western Asia, and central Asia from the Caspian Sea to Lake Baykal (Kernick 1978). The morphological characteristics of sainfoin have been extensively explained by Sheely (1977), Hume (1981), Kon (1982), and Fortune (1985). The potential of sainfoin as a forage legume and its water relations are reviewed here.

2.8.1 Sainfoin: potential as a forage legume

Sainfoin has a high nutritive value and does not cause bloat in ruminant livestock due to the presence of tannin. It is highly palatable to all classes of livestock (Hanna *et al.* 1975). Carleton *et al.* (1968) found that at the same stage of maturity, sainfoin was higher in N-free extracts, total digestible nutrients (TDN) and phosphorus than lucerne. The N-free extracts of sainfoin at full bloom were 46 and 43.5 % for irrigated and dryland conditions compared with 35.5 and 34.6 for lucerne at 10% bloom under similar conditions, indicating that the total available energy of sainfoin forage is equal to and possibly superior to that of lucerne. Karnezos and Matches (1991) in a study of sainfoin and wheatgrass found that grazing by sheep of wheatgrasses mixed with sainfoin produced greater average daily weight gain, feed conversion, and intake compared to a monoculture of wheatgrass. In a sheep grazing trial over a five year period in Alberta (Canada) while the water table was 1.5 to 2.5 m below the surface, it was found that the dry matter production of sainfoin (Eski) and lucerne was similar and that sainfoin was more palatable than lucerne. The yield of sainfoin ranged from 5175 to 10045 kg/ha/year and for lucerne was from 5115 to 8690 kg/ha/year (Smoliak and Hanna 1975).

The important quality of sainfoin forage when grazed by or fed to ruminants is its non-bloating attribute. The anti-bloating characteristic of sainfoin herbage is due to condensed tannin (Kendall 1966; Jones and Lyttleton 1971; and Jones *et al.* 1973)

which inhibit the production of foam in the rumen (Reid *et al.* 1974). Gutek *et al.* (1974) found large quantities of condensed tannin in sainfoin in all seasons and growth stages.

2.8.2 Agronomy of sainfoin

Sainfoin is predominantly used for forage production from early-spring to mid-summer (Hanna *et al.* 1972; Koch *et al.* 1972; Griggs and Matches 1991; Mowrey and Matches 1991). The regrowth, persistence, and competition against weeds of sainfoin is often poor and has been widely studied. Studies on the effect of plant growth stage at defoliation, and the intensity of defoliation on the persistence of sainfoin have shown that sainfoin defoliated at the bud stage, or flowering stage, under light or medium intensities of defoliation gives acceptable stand persistence over five years under irrigation systems (Mowrey and Matches 1991). Cutting of sainfoin at the pre-flower bud and pink flower bud stages was significantly superior to cutting at the full-flowering stages over three years (Evans 1961). Percival and McQueen (1980) found increased dry matter yields when defoliation occurred up to the full bloom stage. Frequent defoliation and low cutting height have been found to reduce plant productivity. Percival and McQueen (1980) found that although cutting to 3 or 10 cm had little effect on productivity, 8 week cutting intervals gave higher dry matter yield than at 6 or 4 weeks.

The storage of nitrogen in the root has been investigated as a factor affecting regrowth and persistency of sainfoin. Inferior regrowth and stand persistence of sainfoin may be due, in part, to its inability to obtain sufficient nitrogen by fixation. The nitrogen fixed by sainfoin is not sufficient for plant requirements (Schneiter *et al.* 1969; Sims *et al.* 1968; Hume 1985). Application of nitrogen to nodulated plants has increased yields of sainfoin (Koter 1965; Jenson and Sharp 1968). Meyer (1975) found that sainfoin vigour and regrowth were enhanced by nitrogen fertilization, but there was little effect of phosphorus and potassium on regrowth and persistence.

Total non-structural carbohydrate concentration in the roots showed that sainfoin stores all energy reserves for winter survival during autumn growth (Mowrey and Matches 1991) thus plant persistence is possibly reduced by grazing during

autumn (Mowrey *et al.* 1992).

There is a linkage between regrowth ability and longevity apparent in the different sainfoin types. The two-cut type of sainfoin has better regrowth yield during the season but its longevity is poor. In contrast, the one-cut type has poorer regrowth but its longevity is better than the two cut type. Cooper and Watson (1968) have shown that for a one-cut type (e.g. Eski) total available carbohydrate (TAC) in the roots did not reach peak levels until seed maturity. Karal and Delaney (1982) also found that levels of TAC in sainfoin were low compared to those of lucerne. These two results suggest that regrowth will be affected by TAC reserves, and that frequent severe cutting may result in depletion of these reserves.

The mixture of sainfoin with other plant species is useful for increasing the nutritional value of the forage, reducing bloat, and increasing the productivity of the forage (Cooper 1973; Smoliak and Hanna 1975; Hanna *et al.* 1977; Scott 1979; Griggs and Matches 1991), but it will not help the persistence of sainfoin. Kilcher (1982) found that heavy grazing reduced the competition from the grass component in a mixture of sainfoin and Russian wild ryegrass and this apparently enabled the sainfoin to persist.

The following reasons for the poor regrowth ability of sainfoin were found in a series of glasshouse and field experiments by Fortune (1985): a) poor development of any new shoots to provide a starting point for regrowth, b) little leaf area remaining at the base of the plant after harvest to provide a photosynthetic surface, and c) losses of root and nodule tissue after defoliation which were subsequently replaced, possibly at the expense of top growth.

2.8.3 Sainfoin in dry conditions

Sainfoin is found in regions with hot, dry summers (e.g. Mediterranean). The morphological features and time of maximum growth of sainfoin demonstrate its adaptation to drought (Koch *et al.* 1972). The deep root system of sainfoin has been cited as a reason for its drought resistance (Bland 1971). Two *Ononbrychis* species *O. echidna* Lips. and *O. cornita* (L.) Desv. from central Asia have been described as xerophytic (Kul'tiasov 1961), but not sainfoin. Some sainfoin leaflet characters such

as: thickened epidermal cell walls, thick cuticle, and water-bearing cell layer under the epidermis (Dalenvoa 1962) reveal that it might have both avoidance and tolerance mechanisms like many arid and semi-arid plants (Chabot and Bunce 1979), but benefit from these mechanism have yet to be demonstrated. The lower specific leaf area (SLA) of sainfoin compared to lucerne (Sheehy *et al.* 1978; Sheehy and Popple 1981) is another useful character for dry conditions.

The slow regrowth of sainfoin, particularly of one-cut types, could contribute to drought tolerance. Shain (1959) has reported lower drought tolerance from multi-cut sainfoin types. In addition to drought tolerance, sainfoin has drought avoidance strategies, such as its ability to grow at low temperatures and thereby have a short growing season (Young *et al.* 1970). Sainfoin yield potential in the spring is higher than in summer (Bolger 1988), and Bolger and Matches (1990) found sainfoin produced 58-63% of its total yield in the spring. The greater production of sainfoin in spring causes greater water use efficiency, a useful attribute for areas with dry summers.

In a field study of sainfoin and lucerne, Sheehy and Popple (1981) found that sainfoin leaf water potential remained remarkably high throughout the regrowth period. Furthermore sainfoin solute potential decreased and turgor increased, whereas lucerne leaf water potential and osmotic potential decreased as plants increased in size, and turgor decreased. The high turgor potential of sainfoin even with decreasing osmotic potential, during the regrowth period is a useful feature in dry conditions.

Sainfoin showed only a 20% decrease in yield when the interval time of irrigation was increased from 10 to 20 days (Koocheky 1984). Rizzov and Giorgio (1982) found under insufficient water supply in a dry conditions in Italy the dry matter yield of sainfoin over a 3 year period was greater than for tall fescue, phalaris, and cocksfoot, indicating an ability of sainfoin to grow in water limiting conditions.

Chapter 3

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Adaptation of sainfoin cultivars and lucerne to water stress.

*Modified from a paper presented at the XVII International Grassland Congress, at
Palmerston North, February 1993*

3. Adaptation of sainfoin cultivars and lucerne to water stress

3.1 Abstract

Sainfoin (*Onobrychis viciifolia* Scop.) is of particular interest as a pasture crop because it is a productive legume in dry areas but does not induce bloat in ruminant animals. In this indoor study three sainfoin cultivars (Fakir, Melrose and Grasslands G35) and one lucerne (*Medicago sativa* L.) cultivar 'Grasslands Oranga' were compared during a drying phase when water was withheld after flowering commenced. A randomized complete block design with four replicates was used. Transpiration rate (Tr) declined as volumetric soil water content (VSWC) was reduced. Stomatal resistance (Rs) increased and relative water content (RWC) decreased when VSWC decreased. Critical VSWC for Rs, Tr and RWC was 8 and 12% for lucerne and sainfoin, respectively. Lucerne showed lower Tr, and higher Rs and RWC than sainfoin during water stress. Root dry weight varied significantly, with lucerne and G35 having the lowest and highest weight, respectively. Leaf and stem dry weight were not significantly different between cultivars.

3.2 Introduction

Sainfoin (*Onobrychis viciifolia* Scop.) is regarded as a drought resistant forage legume (Kozyr 1948). Its advantages compared to lucerne (*Medicago sativa* L.) have been described by Hume (1981) and include : a) high nutritional value, b) non-bloating characteristics, and c) resistance to some lucerne pests such as the alfalfa weevil (*Hypera postica*) (Eslick, 1968; Hanna *et al.* 1977) and spotted alfalfa aphid (*Therioaphis maculat*) (Lance 1980). Sainfoin is reported to be more palatable than lucerne (Chapman and Carter 1976). Osborn *et al.* (1966) found the voluntary intake of sainfoin by sheep was greater than for lucerne or red clover (*Trifolium repense* L.). Smoliake and Hanna (1975) found that sheep preferred to graze sainfoin rather than lucerne or cicer milkvetch (*Astragalus cicer* L.).

Quantification of the responses to water stress of sainfoin is important for understanding forage production in drought areas. Bolger and Matches (1990) found sainfoin yield potential and water use efficiency (WUE) were higher in sainfoin than lucerne during spring water deficit. Sainfoin extracted soil moisture from 1.8m depth (Koch *et al.* 1972) and the specific leaf area (SLA) of sainfoin was approximately half that of lucerne, though leaf weights were similar in both species (Sheehy and Popple 1981).

A glasshouse experiment was carried out a) to gain familiarity with a range of sainfoin cultivars and lucerne under glasshouse conditions and b) to compare the responses of mature plants of sainfoin cultivars and lucerne to water depletion.

3.3 Materials and Methods

3.3.1 Experimental

Seed of the sainfoin cultivars Melrose, Fakir, and Grasslands G35, and the lucerne cultivar Grasslands Oranga was germinated on 29 September 1990 in petridishes in a solid agar medium, and three seedlings were transplanted to pots (20 cm diameter and 20 cm height) containing 9 kg of a mixture comprised of 33% river sand and 66% soil, on 5 October 1990. The soil was a Haplquept (fine, loamy,

mixed, mesic). The soil surface was covered with plastic chips to prevent evaporation after the seedlings were transplanted.

The experiment was conducted in a glasshouse at Palmerston North from 5 October 1990 to 6 January 1991 with day/night temperatures of 28°C/20°C, 15 hours natural day light and with a mean average relative humidity of 63%. A randomised complete block design with four replicates was used.

Moisture was kept at pot capacity by top watering at frequency increasing from 7 to 14 times weekly as leaf area increased, until watering ceased at flowering (15 December 1990).

3.3.2 Measurements

- 1) Transpiration rate (T_r) calculated by difference in pot weight on alternate days.
- 2) Volumetric soil water content (VSWC) to 15 cm was measured by time domain reflectometry (TDR) (Topp *et al.* 1980, 1984, Zegelin *et al.* 1989).
- 3) Stomatal resistance (R_s) of two fully expanded leaves per pot was measured by porometer Mk3 (Delta-T devices).
- 4) Relative water content (RWC): Ten disks (100mm diam) of fully expanded leaves were weighed immediately (fresh weight, FW) soaked in distilled water 4 h in a dark and cool environment, then surface water was removed by paper towel and the turgid weight (TW) measured. After drying leaf samples at 80 °C for 24 h (Henson *et al.* 1989b) dry weight (DW) was measured and RWC calculated by the ratio of $((FW-DW)/(TW-DW))*100$.
- 5) At the end of the experiment plants were separated into leaf, stem and root fractions. Roots were washed free of soil using a 2mm screen. Dry weight of all parts was determined after 24 h drying at 80 °C.

3.4 Statistical analysis

The data of morphological characters (e.g. yield) were examined by analysis of variance using the General Linear Model (GLM) procedure of SAS (SAS Institute Inc. 1991). Repeated measures analysis was used for physiological measurements repeated on the same experimental unit over time (Rowell & Walters 1976).

3.5 Results and Discussion

Root DW was significantly different between cultivars, with lucerne having the lowest and G35 the highest weight (Table 3.1). Thus, in moisture limiting conditions sainfoin could have more capacity to absorb soil water. Lucerne had the highest harvest index (above ground DW/total DW) because of its lower root weight (Table 3.1).

Table 3.1: Root, leaf and above ground dry matter (g/pot) for three sainfoin cultivars and lucerne after 100 days, with no watering over the last 30 days.

Character	Fakir	G35	Melrose	Lucerne	Pr>F
Leaf DW	4.46	4.91	6.10	4.02	0.1511
Root DW	6.88ab	8.90a	6.91ab	4.65b	0.0406
Above ground DW	13.44	14.02	14.31	12.58	0.9244

*Numbers with the same letter(s) are not significantly different ($P < 0.05$).

Stomatal resistance was significantly greater for lucerne than sainfoin at two observations (Fig. 3.1). The R_s increased significantly when the VSWC was below 8% for sainfoin and below 12% for lucerne.

Differences in T_r for high VSWC were less than those occurring below 8% VSWC for sainfoin and 12% for lucerne. The only significant difference between

Fakir and lucerne.

Relative water content did not change significantly until VSWC fell below 8% for sainfoin and 12% for lucerne. At 8% of VSWC, RWC decreased suddenly to 43% and 48% for sainfoin and lucerne respectively (Fig. 1). At low VSWC (12%) the average values of RWC for lucerne, Fakir, Melrose and G35 were 75.82, 69.23, 60.84 and 57.26% respectively ($P < 0.05$). The results showed that R_s , T_r and RWC were largely unresponsive to VSWC above 8% and 12% of VSWC for sainfoin and lucerne respectively, these values providing critical targets for irrigation.

At 12% VSWC, R_s of lucerne was about 10 s/cm but for sainfoin it is still 2 s/cm. Several factors could influence the later response to water stress in sainfoin than in lucerne. Inhibitors like abscisic acid (Davies and Zhang 1991) could affect R_s in lucerne sooner than for sainfoin or osmotic adjustment (Turner 1978) in companion cells of lucerne stomata might close stomata sooner than for sainfoin.

Alternatively, the greater root weight of sainfoin than of lucerne would make more water available at similar values of VSWC (Gregory 1989). The lower SLA of sainfoin (Sheehy and Popple 1981) means that with equal leaf DW sainfoin has less leaf surface, and therefore possibly less T_r in the same conditions. These characteristics of sainfoin will be particularly desirable when water is limiting.

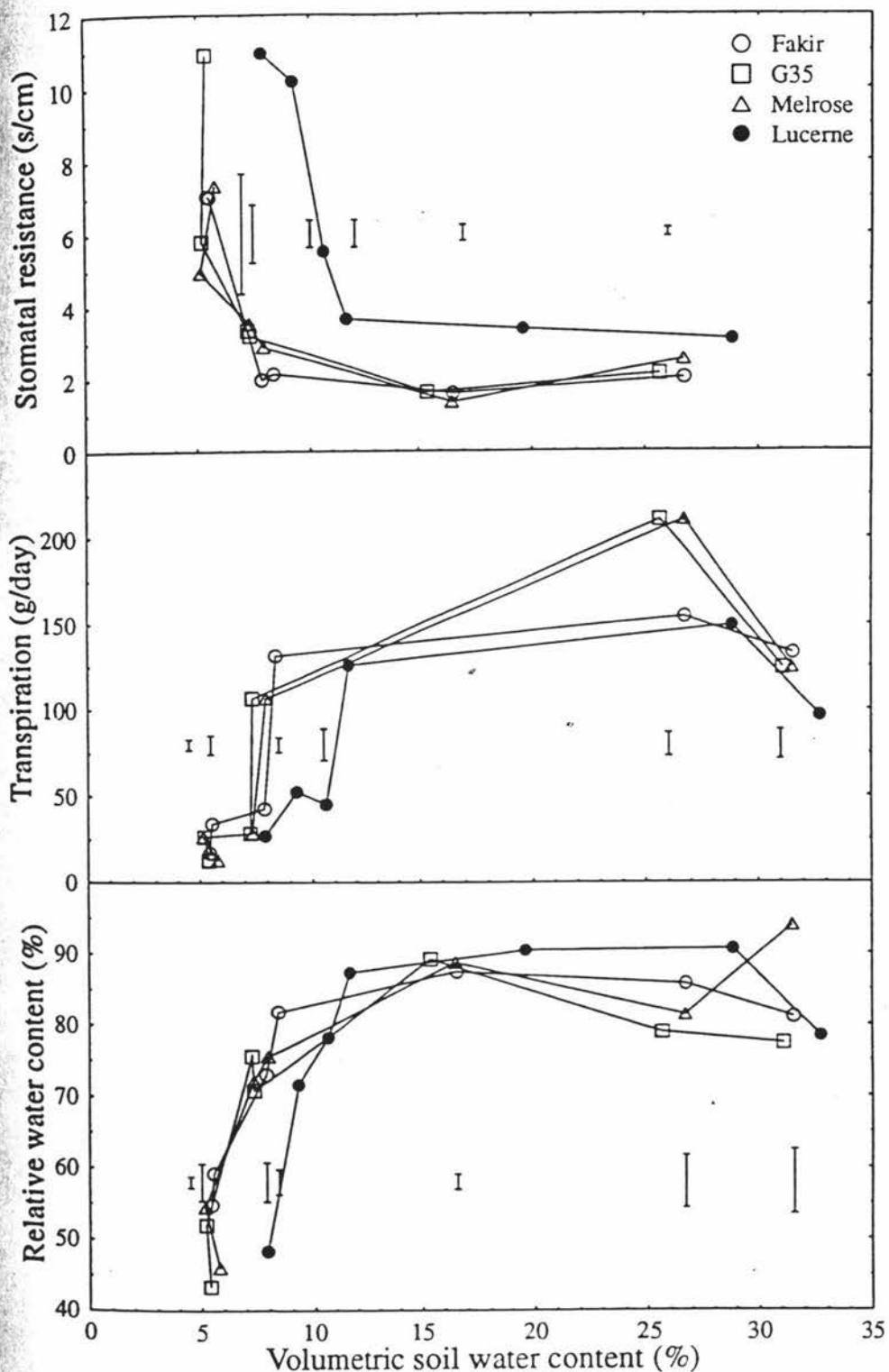


Fig. 3.1: Stomatal resistance, transpiration and relative water content during 31 days water stress. Symbols are the average of four replicates for three sainfoin cultivars and lucerne. Bars show \pm SEM

Chapter 4

Physiological and morphological responses of lucerne to soil moisture stress

Modified from a paper presented at the International Grassland Conference at Inner Mongolia China (16-20 August) 1993.

4. Physiological and morphological responses of lucerne to soil moisture stress

4.1 Abstract

Lucerne (*Medicago sativa* L.) is a productive plant which can supply quality forage even during extreme climatic conditions. Water limitation in arid and semi-arid zones will, however, reduce production from this species. Our objective was to determine relationships between soil moisture and physiological, morphological characters, and relative shoot and root development of the plant. In this glasshouse study, three levels of soil moisture were imposed with soil water contents of 22, 15, and 12% (g/g) as control, moderate, and severe stress, respectively. Water stress reduced the transpiration rate of control plants from 500 ml/day to 140 ml/day in severely stressed plants. Stomatal resistance was constant over time and was higher for severe stress than for control plants in early growth. The osmotic potential of the plants at dawn was lowest for severely stressed plants and highest for control plants.

Soil moisture had significant effects on total dry weight of shoot, root, and leaf, root length, and leaf area. The shoot dry weight in severely and moderately stressed plants decreased to 71 and 58% of that of the control plants. Root length and root dry weight of the severely and moderately stressed plants were 33% and 66% of the control respectively. The leaf-to-stem ratio was 0.59, 0.89, and 1.45 for control, moderately, and severely stressed plants respectively. The shoot-to-root ratio of the control, moderately, and severely stressed plants was 1.38, 1.2, and 0.94 (g/g) respectively, suggesting that the relative allocation of carbohydrate to roots increased under water stress.

4.2 Introduction

Lucerne (*Medicago sativa* L.) is often included in crop rotations in dry areas since it produces high quality forage and fixes large quantities of nitrogen. In spite of, being deep rooted, water deficit can reduce lucerne's growth in arid and semi-arid zones. Water deficit decreases dry weight accumulation (Carter and Sheaffer 1983a), and reduces stem number and stem diameter, internode length, and leaf size (Cowett and Sprague 1962). Also, net photosynthesis typically declines with drought due to both stomatal and non-stomatal factors and their effects on inhibition of net photosynthesis (Chiara *et al.* 1988).

Despite the abundant literature on water deficit effects there is still a need for studies on the effect of different soil moisture levels on the physiological and morphological responses during the development of the plant, so that the relative importance of the adaptations by lucerne to drought can be determined. Nevertheless, the physiological responses of lucerne to water stress are better understood than those of sainfoin so lucerne is used as a reference plant in this thesis. In this chapter the responses to water stress of the lucerne cultivar Grasslands Oranga were examined using a comprehensive set of water stress measurement techniques. This was the basis for lucerne being used a reference. Lucerne was grown for a prolonged period (4 months) at different levels of soil water content to evaluate :

- a) the functional relationships between available soil moisture and plant physiological characters, and
- b) the relative shoot and root development of the plant at different soil moisture levels.

4.3 Materials and Methods

4.3.1 Experimental

Seeds of lucerne 'Grasslands Oranga' were germinated on 15 June 1991 in petridishes in a solid agar medium, and 5 seedlings were transplanted on 20 June 1991 to pots (20 cm diameter and 20 cm height) containing 9 kg of a mixture comprising 50% river sand and 50% soil.

Plants were grown in a glasshouse until 30 October 1991 with day/night temperatures 28/20 °C, natural daylight, and daylength and a mean average relative humidity of 63%. A layer of plastic chips on top of the soil prevented evaporation.

Initially, soil moisture was maintained at pot capacity until plants were well established. Then three levels of soil moisture were imposed one month after transplanting (20 July), as follows:

- 1) control, 22% water content (g/g).
- 2) moderately stressed, 15% water content.
- 3) severely stressed, 10-12% water content.

The available soil water content (ASWC) in the severe and moderate stress treatments was 50 and 25% of the control treatment, respectively. The ASWC was calculated as the difference between pot capacity (22% g/g) and wilting point (9% g/g) determined from a soil moisture retentivity curve at soil water potentials of -0.0114 and -1.5 MPa, respectively. The three soil moisture levels were replicated four times in a randomised complete block design.

4.3.2 Measurements:

4.3.2.1 Transpiration (Tr)

Pots were weighed and watered every night using a 30 kg electronic balance with an accuracy of 0.001 kg. The Tr was calculated as $W_1 - W_2$ where W_1 was the pot weight after irrigation and W_2 was the weight before watering on the following day.

Evaporation was monitored from two identical extra pots without plants, but

no evaporation was detected.

4.3.2.2 Relative water content (RWC)

Ten disks (100 mm²) from fully expanded leaves were removed weekly at midday, weighed immediately (fresh weight, FW) using an electronic balance with an accuracy of 0.0001 g, and then soaked in distilled water for 4 h in a dark and cool environment. Afterwards, surface water was removed by paper towel and the turgid weight (TW) measured. Leaf samples were dried at 80°C for 24 h. Dry weight (DW) was measured and relative water content (RWC) calculated by the ratio of $((FW-DW)/(TW-DW))*100$.

4.3.2.3 Stomatal resistance (Rs)

Two fully expanded leaves from the top of the canopy in each pot were used at midday each week to measure Rs of the abaxial and adaxial surface of the leaves using an automatic porometer Mk3 (Delta-T devices).

4.3.2.3 Leaf water potential (Ψ), Leaf osmotic potential (π)

A leaf disk (80 mm²) was taken from a fully expanded leaf near the top of the canopy, and loaded into the chamber of a Wescor HR33T Dew Point psychrometer (Brown 1972, Rawlings 1972, Campbell and Barlow 1973) and leaf water potential (Ψ) measured. The same sample was then frozen in liquid air and reloaded into the chamber to measure osmotic potential (π). These factors were measured weekly at dawn.

4.3.2.4 Leaf area development, and leaflet number

The leaves of all plants were counted, and the LA of two randomly selected plants per pot was estimated every 2 weeks. Five leaf area classes were chosen, and the number of leaves in each area class was recorded (Appendix 4.1) (Williams *et al.* 1964). At the end of the experiment, actual leaf area was measured by leaf area meter (Li-Cor Inc, model 3100). At the same time 10 plants were chosen randomly and their LA was estimated as above. The regression equation relating the estimated

LA to actual LA at the end of experiment was $Y=-110+1.10X$; $R^2=0.86$.

4.3.2.5 Plant height and relative stem elongation

The total length of the stem and the length of the branches of the plants were measured every 2 weeks and the relative stem elongation rate (RSE) calculated from the total length of the stems and the branches as follows: $RSE=(\ln L_2-\ln L_1)/(T_2-T_1)$ where L is the total stem and branch length per pot at time T (Radford 1967).

4.3.3 Plant harvest

At the end of the experiment, plants were harvested and separated into leaf, stem, and root fractions. Roots were washed free of soil using a 2 mm screen and root length was measured by Comair Root Length Scanner. The dry weight of all plant components was determined after 24 h drying at 80°C.

4.4 Statistical analysis

Data from the final harvest were analyzed by analysis of variance using the General Linear Models (GLM) procedure of SAS (SAS Institute, Inc., 1991). Physiological data presented in Table 4.2 exhibited no trend over time when analyzed by repeated measures analysis (Rowell and Walters 1976) so the mean for all measurement times was subjected to analysis of variance. When repeated measures analysis showed there was a significant interaction between soil moisture levels and time the results are presented in figures. The fitted functions in Figures were generated with FITLS (Mr. C. Pugmire, Industrial Research Ltd, Pers comm) using the biggest significant R^2 .

4.5 Results

Total stem plus branch length of the plants from all treatments increased with time (Fig. 4.1). Control plants were tallest and severely stressed plants the shortest. The total stem plus branch length of control, moderately, and severely stressed plants at the end of the experiment were 1.3, 0.9, and 0.4 m, respectively. The RSE of the severely stressed plants was initially less than that of the moderately stressed and control plants (Fig. 4.2). The RSE of the plants in all treatments approached zero towards the end of the trial but this occurred earlier in the severely stressed plants (Fig. 4.2). The development of estimated LA over time followed a similar pattern to stem length. Control plants always had the largest, and severely stressed plants the smallest, LA during the experiment (Fig. 4.3). The LA did not increase after the flowering stages in any treatment. Water stress affected the leaf numbers on the plants significantly ($P < 0.01$), with the final leaf numbers per pot for the control, moderately, and severely stressed plants being 601, 351, and 293, respectively (Fig. 4.4).

There were significant effects ($P < 0.01$) on total shoot dry weight (DW), root DW, stem DW, leaf DW, root length, and LA (Table 4.1). The shoot DW of the plants in the severe and moderate stress treatments was 71 and 58% of the control treatment respectively. Stem DW of the treatments was significantly different ($P < 0.01$) with stem DW of the plants under severe and moderate stress being reduced to 82 and 52 % of the control, respectively (Fig 4.5). LA of the moderate and severe stress treatments was 34 and 60% of the control. Total leaf dry weight was reduced to 54 and 27% of the control in severe and moderate stress treatments, respectively. The specific leaf area (SLA) was significantly different between the treatments. SLA of control and moderately stressed plants was higher than that of the severely stressed plants (Table 4.1). There were significant differences between the root DW and root length of the plants with the control and severely stressed plants having the largest and smallest root system, respectively (Fig 4.6). The mean root DW and root length of the control plants was 37.8 g and 1288 m, respectively, while the severely and moderately stressed plants had root DW and length approximately 33% and 66% of the control respectively.

Table 4.1: Dry weight (g DW) of total shoot, stem, leaf, and root, root length (m), leaf area (cm²), specific leaf area (cm²/g), and leaf number (LNO.) per pot of lucerne at different levels of soil water available (133 DAS)

Treatment	SHOOT DW (g)	STEM DW (g)	LEAF DW (g)	ROOT DW (g)	ROOT L (m)	LA (cm ²)	SLA (cm ² /g)	L NO.
Control	52.18a	32.84a	19.33a	37.85a	1288.8a	4381a	218.3a	601a
Moderate	29.84b	16.02b	13.81b	24.76b	756.1b	2908b	213.3a	351ab
Severe	14.48c	5.89c	8.59c	15.27c	361.05c	1396c	171.19b	293b
SEM	3.15	2.71	1.34	1.44	105.5	301.5	12.01	83.9

-Means with same letters within columns are not significantly different.

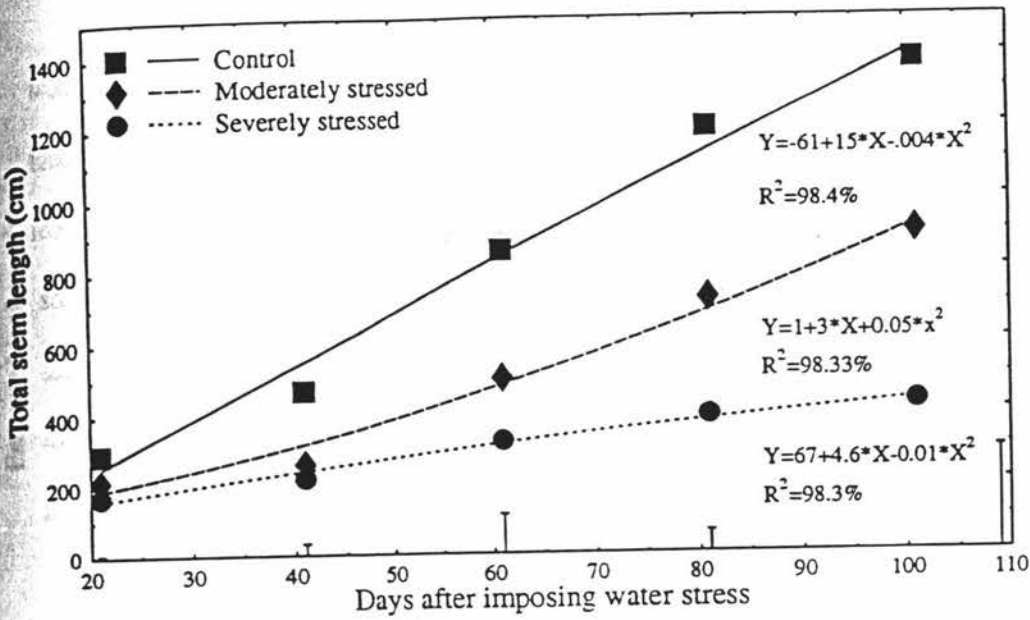


Fig 4.1: Lucerne height at different levels of soil water available. Vertical bars show \pm SEM.

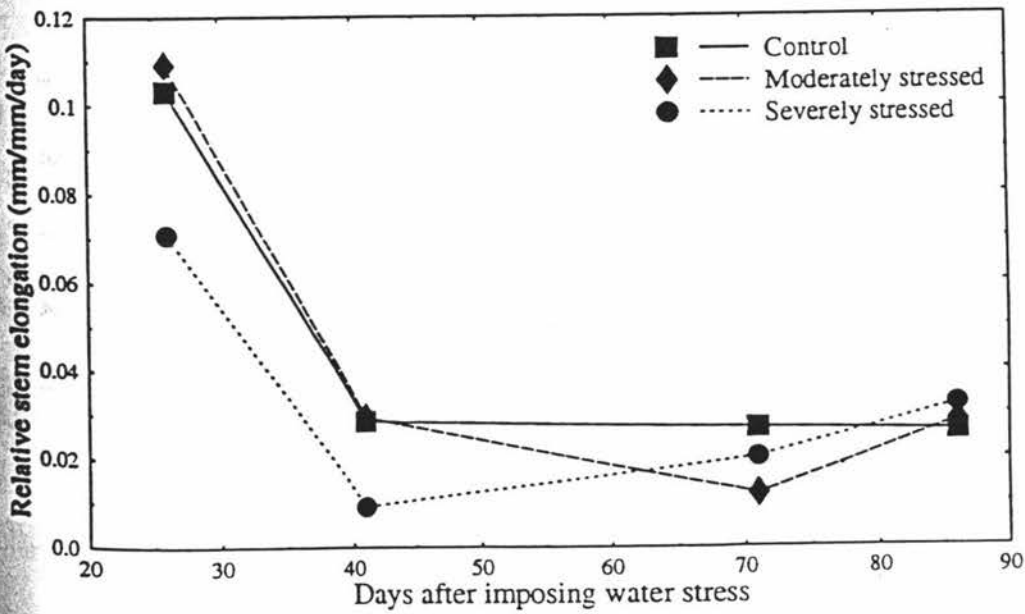


Fig. 4.2: Lucerne relative stem elongation per pot at different levels of soil water available.

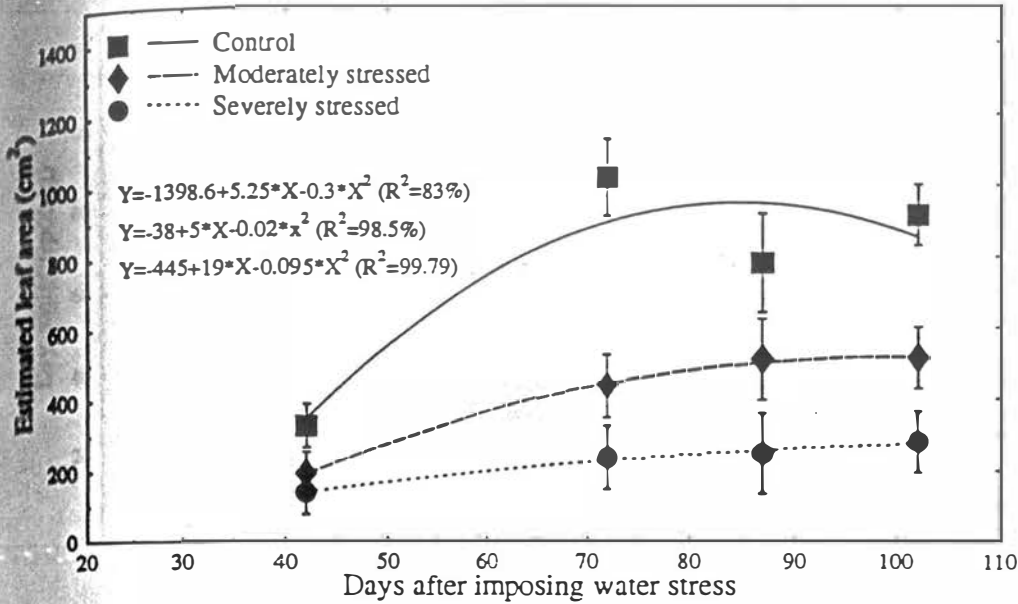


Fig 4.3: The estimated leaf area per plant for lucerne at different levels of soil water available. Vertical bars show \pm SEM.

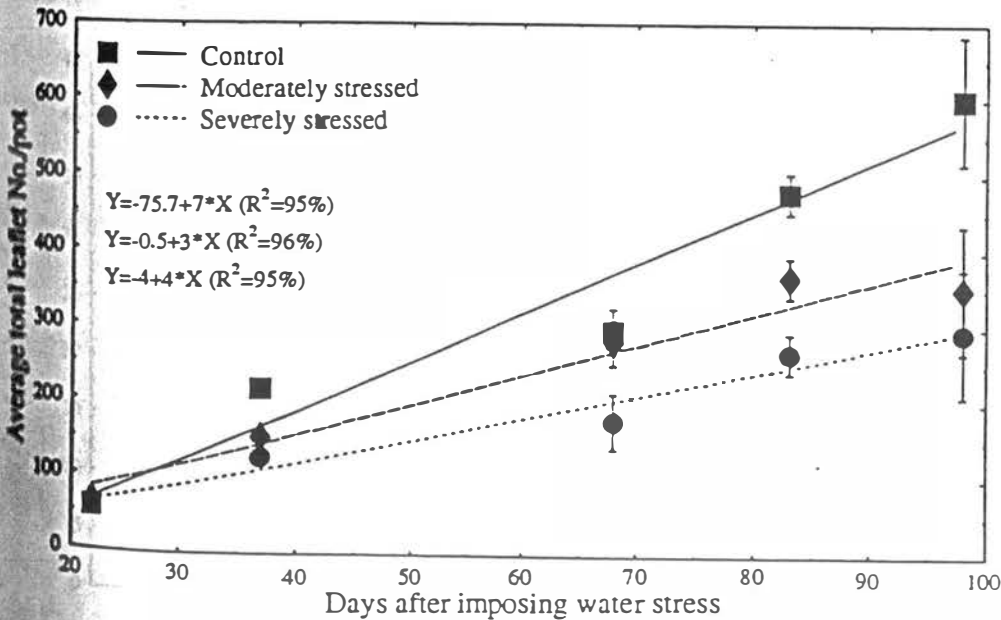


Fig 4.4: The leaflet numbers per pot for lucerne at different levels of soil moisture available. Vertical bars are \pm SEM.

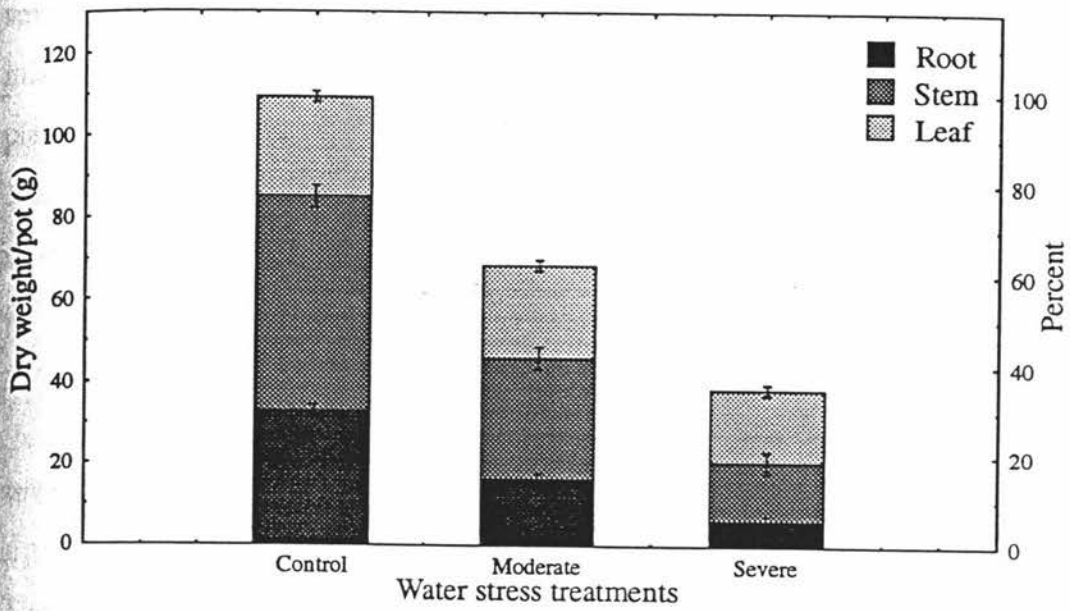


Fig. 4.5: The total dry weight components per pot for lucerne at different levels of soil moisture available. Vertical bars show \pm standard error of the means.

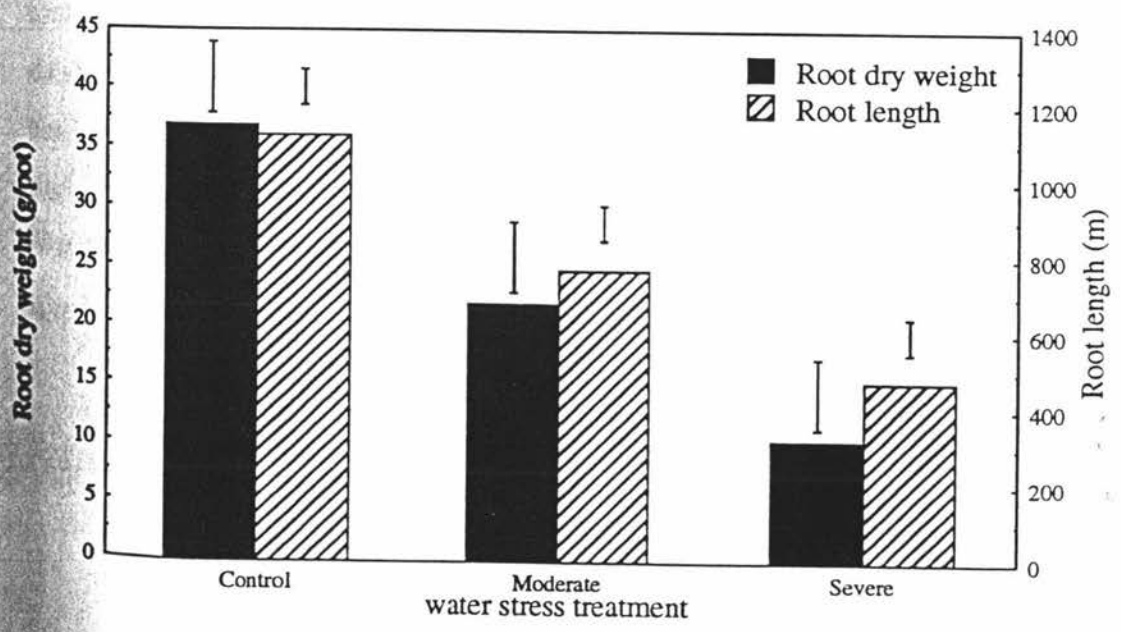


Fig. 4.6: The root dry weight and root length per pot for lucerne at different levels of soil moisture available. Vertical bars show \pm standard error of the mean.

The average transpiration rate (ml/pot/day) of the control, moderate, and severe stress treatments at the end of the experiment were 503, 276, and 140 ml/d respectively (Table 4.2). The ratio of the transpiration rate of the control plants to the moderate and the severely stressed plants treatments were 1.8 and 3.6, respectively which were similar to the ratios of the LA of the control to moderate (1.5) and to severe stress plants (3.1) at the end of experiment (Fig 4.3). Increase in transpiration rate during the experiment in all treatments paralleled the increase in LA (Fig 4.7).

The mean of ten measurements of R_s was significantly higher for the severely stressed plants than for the control plants (Table 4.2). There was no significant difference between the R_s of the abaxial and adaxial leaf surfaces. The R_s of the moderately and severely stressed plants was similar.

The mean RWC, based on six measurements times, of the control, moderately, and severely stressed plants was 85, 83, and 74%, respectively. The mean of eight times of measurements at dawn of total Ψ over time showed that Ψ of the control and moderately stressed plants was different from that of the severely stressed plants (Table 4.2). The Ψ of severely and moderately stressed plants was similar. The mean of the eight times of measurements showed that π was significantly more negative for severe stress than for the control and moderately stressed plants (Table 4.2). The difference between the π of the moderately and severely stressed plants was always non-significant.

Table 4.2: Stomatal resistance (Rs), relative water content (RWC), transpiration rate (Tr), dawn leaf water potential (Ψ), and osmotic potential (π) of lucerne at three soil moisture levels.

Treatment	Rs (s/cm)	RWC (%)	Tr (ml/pot/day)	Ψ (-MPa)	π (-MPa)
Control	1.59 ^b	84.7a	502.8a	1.13b	1.59b
Moderate	1.57b	83.3a	275.5b	1.24b	1.63b
Severe	3.15a	73.9b	140.8c	1.50a	1.85a
Pr>F	0.0067	0.0195	0.0001	0.0164	0.0077
SEM	0.25	2.07	16	0.07	0.04

¹ Means with same letter(s) within columns are not significantly different. Numbers are the mean of four replicates.

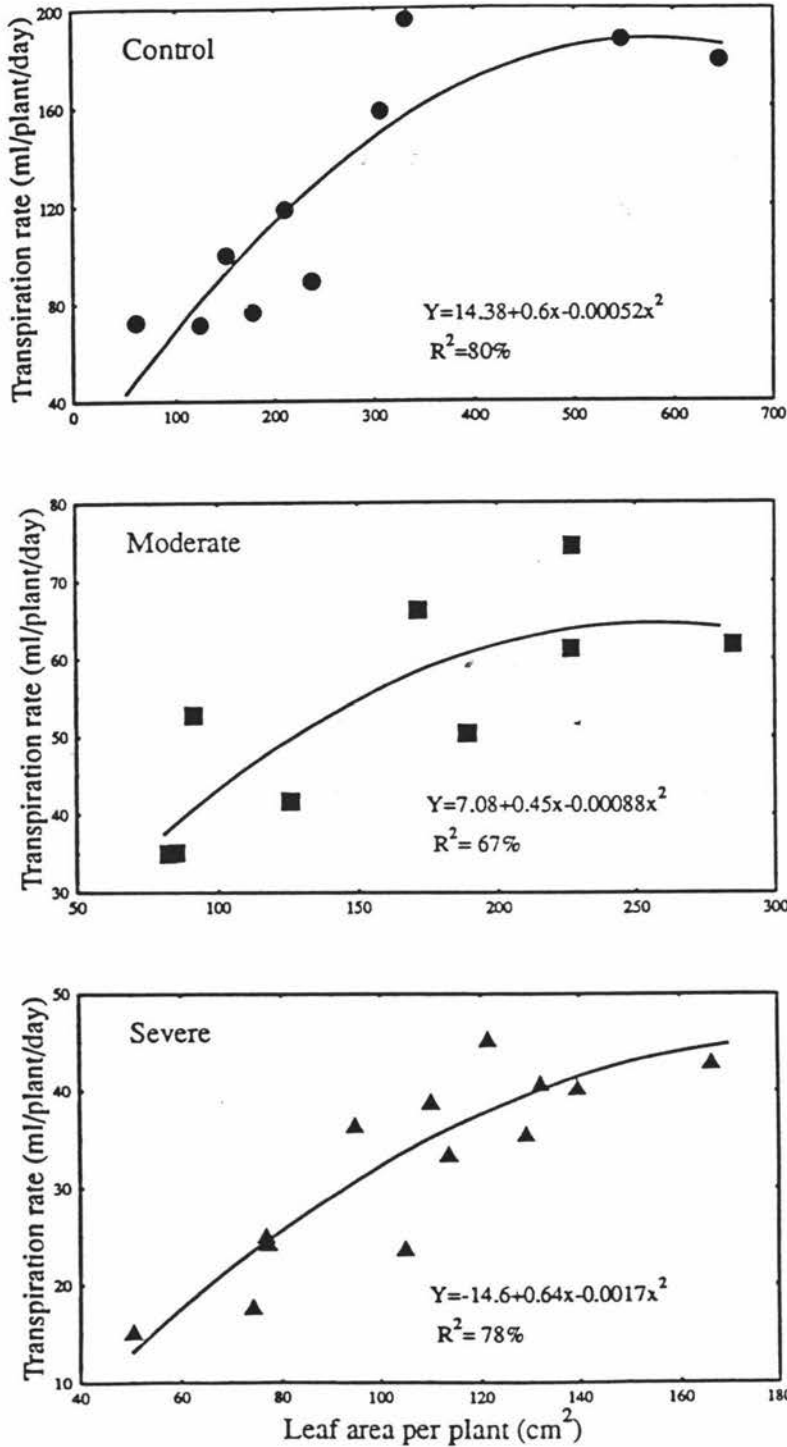


Fig 4.7: The relationship between transpiration rate and leaf area at different soil moisture levels.

4.6 Discussion:

4.6.1 Physiological responses

Some physiological characters were unresponsive to moderate water deficit. In particular, RWC and stomatal resistance did not differ significantly between the control and moderately stressed plants. These results supported the results of the previous experiment (Mir-Hosseini-Dehabadi *et al.* 1993a) (Chapter 3) which showed there was no difference in the Rs and RWC of lucerne when volumetric soil water content was more than 12% (cm^3/g).

The effect of moderate stress in reducing plant growth was not likely to result from changes in RWC or Rs since there were no significant changes between these factors during the latter part of the experiment. However, the greater stomatal resistance of the severely stressed plants in the early growth stages reduced transpiration and maintained cell turgor.

The osmotic potential was more negative for the severely stressed plants than for the moderately stressed and control plants. Since RWC was significantly different between treatments the decreased π was consistent with both solute accumulation (Slout 1980), and solute concentration by dehydration. The more negative osmotic potential of the severely stressed plants could have helped to maintain turgor and thus enable cell expansion to continue (Turner and Jones 1980).

The patterns of increase in leaf area and transpiration rate were similar, and suggested that leaf area had a strong effect on transpiration rate. There was a quadratic relationship between transpiration rate and leaf area for all treatments which suggests that the decline in transpiration rate per unit leaf area at the higher leaf area was due to the shading of the lower leaves (Fig. 4.7).

4.6.2 Morphological responses

Palatability and herbage quality of lucerne are related to the leaf-to-stem ratio (Halim *et al.* 1989; Hall *et al.* 1988; Brown and Tanner 1983). This ratio was 0.59, 0.89, and 1.45, for control, moderate, and severe stress treatments, respectively. The stem height/stem DW ratio of the control, moderate, and severe stress treatments was

314, 381, and 513 (cm/g) respectively. Thus water stress caused thinner stems which may have increased palatability and herbage quality.

The severely stressed plants were smaller than the other treatments and produced stem and branches at a relatively slower rate. Lower net assimilation rate or lower leaf area per length of stem plus branches could be considered as factors limiting RSE in the severely stressed plants. The earlier cessation of stem elongation suggests that neither physiological nor morphological adaptation allowed the severely stressed plants to grow for as long as the other plants.

Water stress reduced root dry weight and root length, of the severely stressed plants relative to the control plants. This result contrasted with the results of Carter *et al.* (1982) who found no significant difference in the root length of lucerne plants at three different soil moisture. This difference in results may have been caused by different experimental conditions. Carter *et al.* (1982) used large metal tubes (150 cm depth and 30 cm diam.) while we used plastic bags (20 cm depth and 20 cm diam.). The pots of Carter *et al.* (1982) may have held enough water for growth for a long period after watering ceased, whereas the soil moisture of our pots decreased quickly to target stress levels. The herbage yield of severely stressed plants in our experiment was 25% of the control, whereas in the experiment by Carter *et al.* (1982) it was 54% of the control, thus the extreme treatment of our experiment suffered from greater water deprivation. Furthermore, the cultivars used in the experiment of Carter *et al.* (1982) were stated to have a large root system, able to scavenge soil water and avoid moisture stress. There is no similar information for Grasslands Oranga which was bred under New Zealand conditions.

The shoot to root ratio of the control, moderately, and severely stressed plants was 1.38, 1.2, and 0.94 (g/g), respectively. Therefore, the relative allocation of carbohydrate to roots increased under water stress, in agreement with the shoot-to-root ratio observed by Carter *et al.* (1982) of 1.75, and 1.38, for moderately and severely stressed plants, respectively.

4.7 Conclusion

The responses of severely stressed plants can be summarized as follows:

a) at the physiological level, increased stomatal resistance and lower (more negative) osmotic potential allowed plants to reduce transpiration, and to possibly maintain turgor, respectively.

b) at the morphological level, relative dry weight changes of the plant components showed that stems were a lower priority than roots and leaves for the allocation of carbohydrate, since the DW reduction of the severely stressed plants relative to the control plants was 82% for stem, but was 56% and 71% for leaf and root, respectively. Leaves and roots may be of greatest benefit to the plant for photosynthesis and water uptake, and appeared to be "protected" relative to the stems.

Chapter 5

Comparison of sainfoin cultivars and lucerne, with an emphasis on sainfoin responses to water stress.

Modified from paper presented to the Agronomy Society of New

Zealand, Auckland (23-27 August 1993)

5. Comparison of sainfoin cultivars and lucerne, with an emphasis on sainfoin responses to water stress.

5.1 Abstract

Although sainfoin has been recognized as a possible alternative to lucerne in New Zealand, comparative information on cultivar performance is sparse. The objectives of this study were to compare eight sainfoin cultivars with lucerne under non-limiting water conditions in the glasshouse, and to examine the water stress responses of a single sainfoin cultivar in the field. Lucerne leaf area, leaf weight and stem weight were greater than for sainfoin cultivars. Significant differences between root, leaf, and stem weight, and leaf area of sainfoin cultivars were observed. Cotswold-Common and Remont had the lowest leaf area among sainfoin cultivars. Melrose and Remont had significantly greater leaf and stem dry weight than Cotswold-Common. In the field study plant water status, relative water content and stomatal resistance were measured weekly at midday for the sainfoin cultivar Remont. There were significant differences between stomatal resistance and relative water content of stressed (rain-out shelter) and non-stressed (rain-fed control) plants. Water stress reduced LA to 25%, and total dry weight (leaf+stem) to 62%, of control plants. Relative water content of sainfoin was more sensitive to soil moisture than leaf water potential or stomatal resistance. The practical significance of the physiological and morphological responses of sainfoin to water stress is discussed.

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5.3 Materials and Methods

5.3.1 Glasshouse experiment

Seeds of six sainfoin cultivars (Grasslands G35, Remont, Cotswold-Common, Melrose, Eski, Pola), *O. tanaitica*, *O. transcaucasica*, and lucerne (Grasslands Oranga) were planted individually in pots (15 cm diameter and 18 cm height) on 26 December 1991, and subsequently thinned to 2 seedlings/pot on 2 January 1992. The media was 50% peat, 50% sand, and fertilizer (Osmocote+® 14-6-12, 2.25 g/l; superphosphate 0-9-0-11, 1.5 g/l; lime 1.5 g/l; dolomite 3.0 g/l; Micromix® 0.6 g/l). Plants were grown in a glasshouse at Palmerston North with natural daylight, natural daylength, and day/night temperature 25/15°C (Plate 5.1). Pot moisture was maintained at field capacity by top-watering automatically up to four times daily. At harvest (1 March 1992), leaves were separated from stems and leaf area (LA) measured with a planimeter (Li-Cor Inc, model 3100). Leaf (LDW) and stem (SDW) dry weight were determined after drying at 80°C for 72 h. Soil was washed from roots and root dry weight (RDW) determined as previously. A randomized complete block design with four replicates was used.

5.3.2 Field experiment

The sainfoin cultivar Remont was used to study water stress effects in the field. Seed was germinated in "peat pots" in the glasshouse (25 October 1992) and seedlings transferred to the field (1 November). The soil was a Tokomaru silt loam (Fragiaqualf, gleyed yellow-grey earth). There were two adjacent experiments (rain-fed control and water stressed) each comprised of three replicates. Between 3 November 1992 and 2 March 1993 a fully automatic rain-out shelter moved to cover the stressed experiment within 30 s of the onset of rain. A plastic sheet was buried to 1 m to prevent lateral flow of soil water into the stressed experiment.

Volumetric soil moisture content (VSWC) (cm^3/cm^3 , %) of the control and stressed experiments was measured weekly in the zones 0-15, and 50-70 depth. A time domain reflectometer (TDR) (Topp *et al.* 1980; Chanasyk and Neath 1988) measured the surface VSWC with a single pair of vertical 15 cm probes per pot. The

deeper VSWC was calculated from the difference between TDR readings of the probes at 70 and 50 cm depth. Approximately weekly measurements were made of a) petiole water potential (Ψ) by pressure bomb (Model 3005 Soil Moisture Equipment Corp) (Scholander *et al.* 1965; Boyer 1967; Tyree and Hammel 1964,1972; Turner 1981) (8 times, 22 December - 2 March) and b) stomatal resistance (R_s) using a promoter (Delta) (5 times, 20 December - 2 March), on two fully expanded leaves at midday from near the top of the canopy. Each week (6 times, 22 December - 2 March) ten leaf disks were cut at midday, weighed immediately (FW), soaked in distilled water for 4 h in a cool and dark environment blotted to surface dryness by paper towel, re-weighed (TW), dried 24 h at 80°C, re-weighed (DW) and relative water content (RWC) calculated as $(FW - DW)/(TW - DW)\%$. Plants were harvested on 3 March 1993 and LA, LDW and SDW determined as for the glasshouse experiment.

Mean LA, SDW, LDW, and SLA from the water stressed and the control experiments were compared by t-test. Physiological data from the control and the stressed experiments were combined to derive a single relationship with VSWC. Equations were chosen which gave high R^2 , using the program FITLS option of GLE (Mr. C. Pugmire, Industrial Research Ltd, Pers comm).



Plate 5.1: A sainfoin (left), and lucerne plant (right) 60 days after planting.

5.4 Results

5.4.1 Glasshouse experiment

The LDW and SDW for lucerne were significantly greater than for all the sainfoin cultivars and species (Table 5.1) ($P < 0.05$). For both LDW and SDW Eski, Melrose, and Remont were significantly greater than Cotswold-Common (Table 5.1). There was no significant difference between RDW of lucerne and the sainfoin cultivars and species (Table 5.1). There was no significant difference in the specific leaf area (SLA) with the result that the relative differences in LA were similar to LDW (Table 5.1).

Table 5.1: Leaf area (LA), leaf dry weight (LDW), stem dry weight (SDW), root dry weight (RDW), and specific leaf area (SLA) of eight glasshouse-grown sainfoin cultivars and species, and lucerne, at 65 days after planting.

Cultivar	LA (cm ² /plant)	LDW (g/plant)	SDW (g/plant)	RDW (g/plant)	SLA (cm ² /g)
lucerne (Oranga)	1484 a	8.79 a	10.91 a	5.24 a	172.4 a
Grasslands G35	1220 ab	5.10 bc	5.43 bc	4.27 a	236.6 a
Eski	1143 abc	6.21 ab	5.58 b	5.30 a	188.5 a
Melrose	1003 abc	5.89 bc	5.52 b	3.87 a	170.5 a
Pola	947 abc	5.75 bc	4.42bc	4.17 a	165.3 a
<i>O. tanaitica</i>	815 bc	4.73 bc	4.61 bc	3.97 a	176.4 a
<i>O. transcaucasica</i>	741 bc	4.18 bc	4.82 bc	4.16 a	176.2 a
Remont	669 c	4.41 bc	6.41 b	4.64 a	150.3 a
Cotswold- Common	615 c	3.29 c	2.01 c	2.04 a	188.2 a
Pr>F	0.05	0.028	0.003	0.528	0.192
S.E.M.	188.7	0.96	1.15	0.97	19.28

Numbers are the mean of four replicates,
Within a column, numbers with same letter are not significantly different.

5.4.2 Field experiment

Initial VSWC was similar for both the control and stressed experiments. Subsequently, as the plants developed, topsoil moisture (0-15 cm depth) decreased for the water stressed experiment but did not change appreciably at 50-70 cm depth (Fig. 5.1). The VSWC of the control experiment was always close to field capacity.

Significant differences were found between LA ($P < 0.05$), LDW ($P < 0.01$) and SLA ($P < 0.05$) of the stressed and non-stressed Remont (Table 5.2). Non-stressed plants had a higher LA and LDW, but larger SLA than the stressed plants (Table 5.2).

Significant differences in the midday Ψ between the water stressed and non-stressed plants were only found at the last two observations, when VSWC was less than 15%. Variation in Ψ was related to VSWC by an exponential equation that showed rapid change (decrease) in Ψ below 19% VSWC (Fig. 5.2).

Measurement of the RWC of stressed plants began when the top-soil VSWC (0-15 cm) in the stressed experiment was less than 25%. The RWC of water stressed Remont was significantly lower than for non-stressed Remont, at all observations. A cubic function relating RWC to VSWC (including stressed and control plants) accounted for 93% of the variation in RWC and showed appreciably lower RWC when the VSWC was less than 32%. RWC was unchanged between 32-46% VSWC (Fig. 5.2).

Initial observations of the adaxial and abaxial R_s of leaves of stressed and non-stressed Remont were similar, but R_s was significantly greater for stressed plants once VSWC was less than 21%. Exponential equations relating the adaxial and the abaxial R_s to VSWC showed a rapid increase in R_s when VSWC was less than 20% (Fig. 5.2).

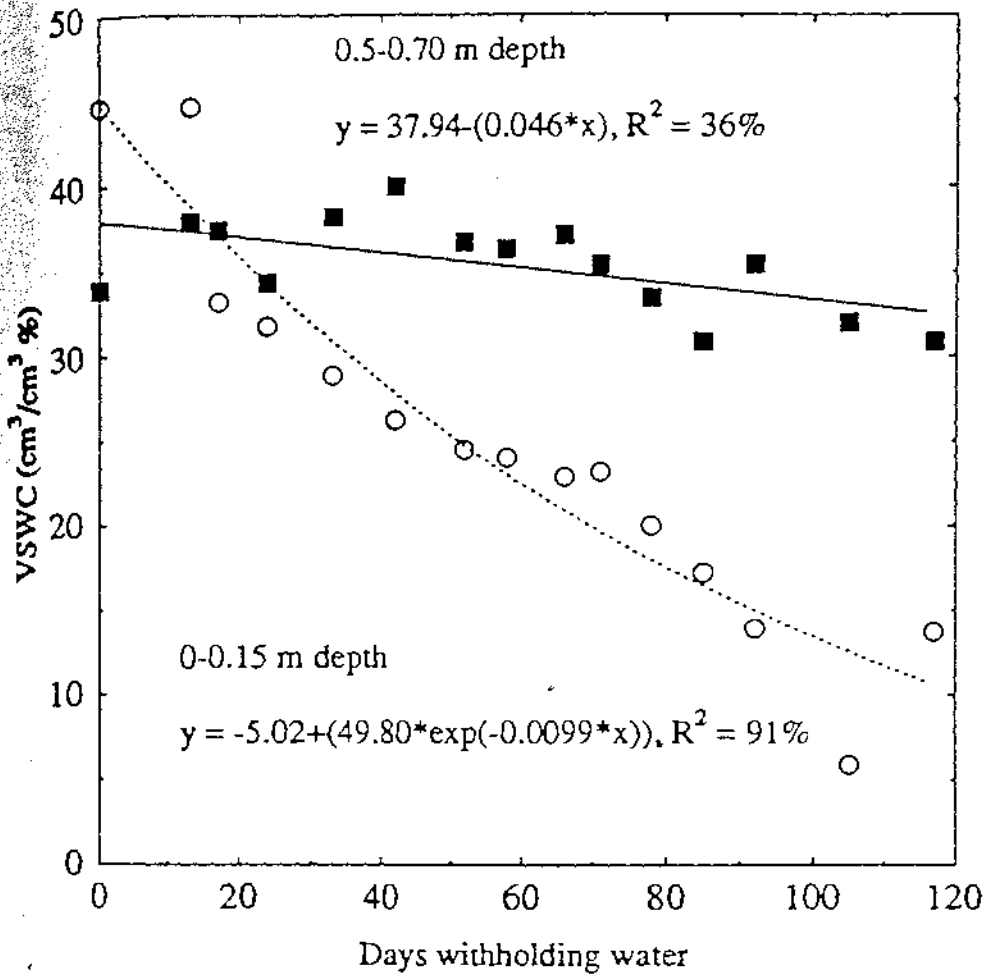


Fig. 5.1: Volumetric soil water content (VSWC, cm^3/cm^3 %) for 0-0.15 (○), and 0.5-0.7 (■) m depth under a rain-out shelter. Symbols are means of three replicates.

Table 5.2: Leaf area (LA), leaf dry weight (LDW), stem dry weight (SDW), and specific leaf area (SLA) of field grown Remont, for stressed (rain-out shelter) and non-stressed (rain-fed control) treatments.

Treatment	LA (cm ² /plant)	SDW (g/plant)	LDW (g/plant)	SLA (cm ² /g)
Stressed	1037.5	26.81	16.66	62.27
Control	4237.9	29.72	40.06	105.78
T-Test	**	NS	**	*

Numbers are the mean of three replicates,

*, ** significantly different at the 0.05 and 0.01 probability levels, respectively.

NS= non significant at 5% of probability

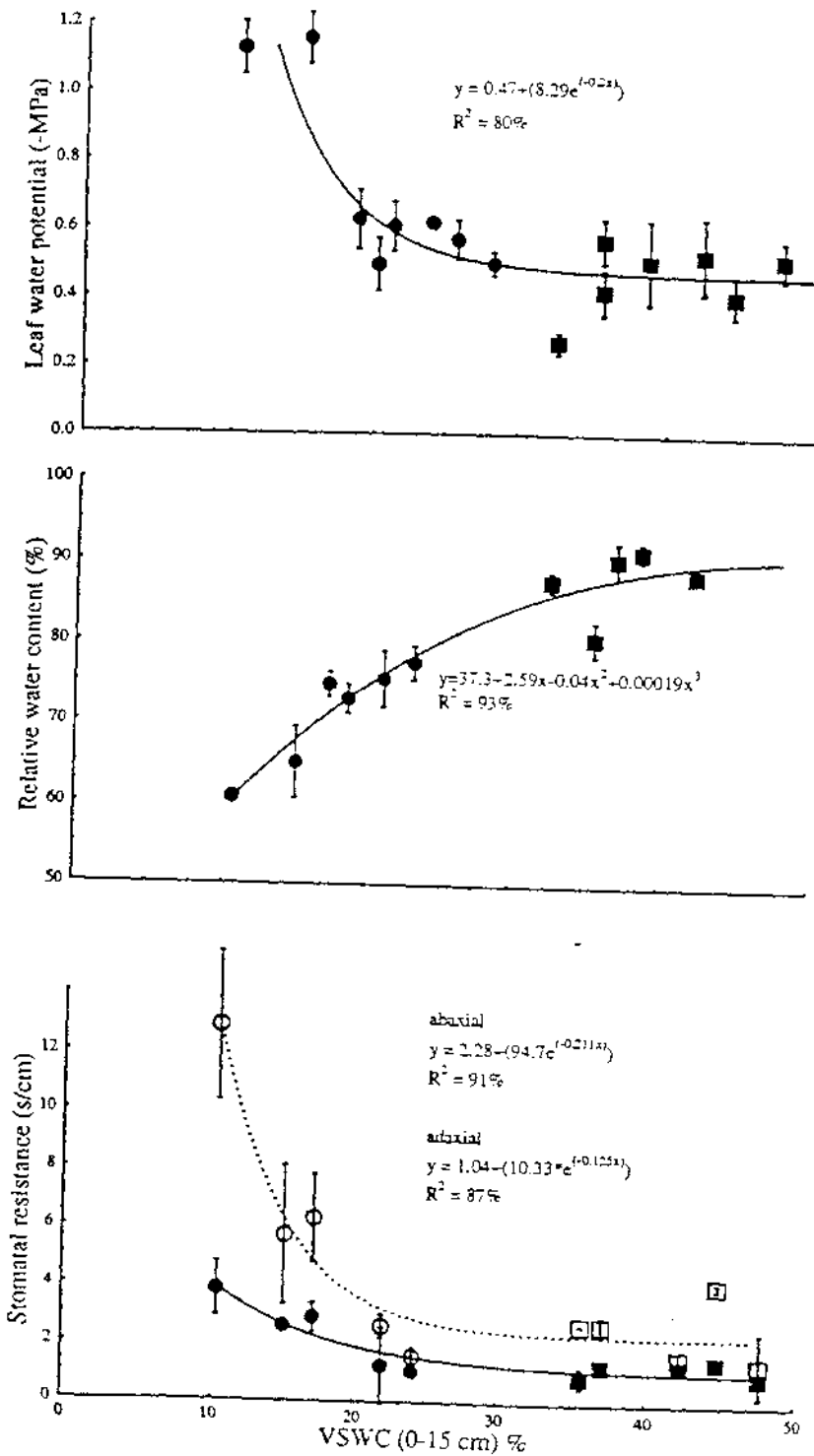


Fig. 5.2 Relationships between Remont petiole water potential (Ψ , -MPa), relative water content (RWC, %), or stomatal resistance (adaxial surface, closed symbols; abaxial surface, open symbols) and volumetric soil water content (0-15 cm, VSWC, cm^3/cm^3 %), for rain-fed (non-stressed) plots (\square, \blacksquare) and stressed plots (\circ, \bullet). Symbols are means of three replicates. Vertical bars show \pm SEM.

5.5 Discussion

5.5.1 Glasshouse experiment

Lucerne was more productive than sainfoin, with a higher leaf area, leaf weight, and stem dry weight, in agreement with the results of Sheehy and Popple (1981). The overall mean LA and above-ground dry weight of sainfoin were 895 cm²/plant, and 9.8 g/plant, respectively, whereas LA and above-ground dry weight for lucerne were 1484 cm²/plant and 19.7 g/plant, respectively. This faster growth of lucerne compared to sainfoin was also reflected in its development. Lucerne reached the bud stage approximately a week earlier than most sainfoin cultivars.

Among the sainfoin cultivars, Remont and Cotswold-Common had the lowest LA (Table 5.1). Reasons for this, however, were probably different for the two cultivars. Remont is representative of "two-cut" sainfoin types which show earlier growth and maturity than "one cut" types (Carleton and Delaney 1972). The low yield of Cotswold-Common was in agreement with Rumball (1982) who found Cotswold-Common was less productive than Pola, Remont, and Melrose. The lower LA of Remont may have resulted from senescence, since it matured sooner than the other sainfoin cultivars and lost leaves prior to harvest. In the case of Cotswold-Common, the low LA was probably due to slower growth. This cultivar did not flower during the experiment. The rapid growth of Remont might be a useful attribute for escaping drought, when soil moisture is limited for growth late in the growing season.

Root dry weight of sainfoin cultivars and lucerne was similar, in contrast to the results of Mir-Hosseini-Dehabadi *et al.* (1993a) (Chapter 3) who showed that the root dry matter of sainfoin was greater than for lucerne. These contrasting results were probably due to a smaller pot size in this study. The shoot/root ratio of sainfoin cultivars was 2.39 and that of lucerne was 3.78. The greater relative allocation of carbohydrate to roots by sainfoin suggested that root size was a character that might aid survival of sainfoin under dry conditions.

The leaf area/plant weight ratio (LAR) of sainfoin (91.2 cm²/g) was higher than for lucerne (75.3 cm²/g). This greater leafiness of sainfoin shoots relative to

Lucerne shoots resulted from a lower stem dry weight. The SLA of sainfoin cultivars (mean $181 \text{ cm}^2/\text{g}$) and lucerne ($172 \text{ cm}^2/\text{g}$) were similar. In contrast, Sheehy and Pople (1981) found in a field experiment that the SLA of sainfoin was half that of lucerne. A possible explanation for this difference was the different environmental conditions of the field and glasshouse, for example, air temperature, relative humidity, and light intensity in the field were not as constant as in the glasshouse. In our field experiment the SLA of Remont was 43% of that in the glasshouse.

5.5.2 Field experiment

Leaf area and yield of Remont decreased as VSWC decreased (Table 5.2). Leaf dry weight, LA, stem dry weight, and SLA decreased 58, 76, 10, and 47% respectively under water stress. The greater decrease in LA was probably due to decreased RWC when VSWC was less than 32%. Low RWC would impair cell elongation (Begg and Turner 1976).

Relative water content of Remont decreased in response to VSWC earlier than for Ψ or R_s . Ψ was less sensitive to water stress than RWC and R_s which suggested that maybe osmotic adjustment occurred in the water stressed sainfoin leaves (Begg and Turner 1976).

Stomatal resistance R_s of the adaxial leaf surface (2.2 s/cm) for sainfoin was lower than for the abaxial (8.1 s/cm) leaf surface which was in contrast to the usual situation for most plants. Stomatal resistance for the adaxial and abaxial leaf surfaces of lucerne, for example, are equal (Carter *et al.* 1982; Mir-Hosseini-Dehabadi *et al.* 1993b). The adaptive significance of the relatively lower adaxial R_s of sainfoin was not apparent though possibly leaflet folding under water stress would result in lower transpiration loss due to high humidity on the adaxial surface.

5.6 Conclusion

Lucerne out-yielded all sainfoins cultivars and species under non-limiting soil moisture conditions. Nevertheless, sainfoin exhibited a greater relative leafiness and a lower shoot/root ratio than lucerne. Costwold-Common yielded less than all other sainfoins. Remont grew faster than the other sainfoins and matured sooner. A relatively large root system (low S/R ratio) is a possible mechanism that could assist sainfoin survival under water stress. Relative water content appeared to be more sensitive to water stress than leaf water potential and stomatal resistance and possibly osmotic adjustment occurs in sainfoin. The stomatal resistance of the abaxial surface of sainfoin leaves was higher than that of the adaxial surface in stressed and non-stressed conditions, but any adaptive significance of this difference was not apparent.

Chapter 6

Plant water status, and shoot and root growth of sainfoin cultivars at constant water stress levels.

6. Plant water status, and shoot and root growth of sainfoin cultivars at constant water stress levels.

6.1 Abstract

Study of sainfoin (*Onobrychis viciifolia* Scop.) in previous Chapters showed the physiological and morphological adaptation of sainfoin to soil moisture stress when a progressive water stress was imposed. To compare the responses of different sainfoin types to limitation of soil moisture, an indoor experiment was planned to investigate the morphological and physiological responses of sainfoin at different soil moisture levels .

Four sainfoin cultivars Cotswold-Common, Eski, Fakir, and Remont were used with a randomised complete block design with four replicates. Three soil moisture levels of 22, 15, and 11 % (g/g) were imposed, as control, moderately, and severely stressed treatments, respectively.

Water stress decreased leaf area, leaflet number, root mass, and root length, yield, leaf water potential, turgor potential, osmotic potential, relative water content and transpiration and photosynthesis rates, but increased stomatal resistance, water use efficiency, and specific leaf area.

Remont was more sensitive to water stress than Eski, Fakir, and Cotswold-Common, but Eski was more tolerant to water stress than the other three cultivars, through its greater root mass and higher leaf water potential and turgor potential.

6.2 Introduction

The results from Chapters 3 and 5 and work by other researchers suggest that the physiology and the morphology of sainfoin respond to water stress in a number of ways that alleviate the effects of stress. Different growth patterns for the sainfoin cultivars were found in the absence of soil moisture stress (Mir-Hosseini-Dehabadi *et al.* 1993c) (Chapter 5). Remont grew faster than Eski, and the growth and yield of Cotswold-Common was lower than the other cultivars. Rumball (1982) found Fakir was the highest yielding among a wide range of sainfoin cultivars.

Thomson (1938) has classified sainfoin into two taxonomically distinguishable types. "One-cut" or "common" type and a multi-cut or "giant" type, according to the growth behaviour after about the six leaf stage. In the one-cut type, (e.g. Eski) stem elongation is limited during the establishment year. Flowering usually occurs in the second year and is once a year (Spedding and Diekmahns, 1972). In the multi-cut (e.g. Remont) type stems tend to be longer and leaflets larger (Spedding and Diekmahns, 1972), with stem elongation and flowering occurring in the establishment year (Thomson 1938).

The early growth and relatively high yield of the first cut of sainfoin resulted in similar dry matter yields to lucerne in Idaho under dry conditions (Murray and Slinkard 1968). Hume (1981) mentioned sainfoin generally yielded more than lucerne at the first cut and less at the second, and appeared to have a comparative advantage where conditions enabled only one cut per year. Roath and Graham (1968) found that under dryland conditions, hay yields of sainfoin compared favourably with lucerne, red clover (*Trifolium pratense* L.) and cicer milkvetch (*Astragalus cicer* L.).

These investigations showed the drought resistance of sainfoin was from both drought tolerance and drought escape strategies. The high root mass of sainfoin can obtain water deep in the soil, and the high productivity of sainfoin in early spring (Bolger and Matches 1990) and at the first cut (Roath and Graham 1968) can help the plant to escape from the following drought in a dry summer.

The different sainfoin types have different responses to dry conditions. The

one-cut type grows slower than the two-cut type and can, therefore, save soil moisture in spring and be more tolerant of a dry summer. On the other hand, the two-cut type can be more productive in spring when soil moisture is adequate, and be less active in a dry summer.

In this study we used Remont, Eski, Cotswold-Common, and Fakir with the objective to: a) compare the different sainfoin types at different soil moisture levels, and b) investigate the morphological and physiological responses to water stress of sainfoin grown at different soil moisture levels.

6.3 Materials and Methods

6.3.1 Experimental

Seed of four sainfoin cultivars: Eski (E), Remont (R), Fakir (F), and Cotswold-Common (C), was germinated (15 June 1991) in petri dishes in a solid agar medium, and five seedlings were transplanted on 20 June 1991 to pots (20 cm diameter and 20 cm high) containing 9 kg of a mixture comprising 50% river sand and 50% soil. The soil was a Haplquept (fine, loam, mixed, mesic). Plastic chips were placed on the soil surface to minimize evaporation.

Plants were grown in a glasshouse with day/night temperatures $25/16 \pm 2^\circ\text{C}$, 12 h natural daylight, and a mean average relative humidity of 63%. On 30 October 1991 pots were transferred to a fully controlled climate room with day/night temperature $25/15^\circ\text{C}$, day length 14 h, relative humidity $50 \pm 5\%$, and light intensity $706 \mu\text{mol}/\text{m}^2/\text{s}$ (Warrington *et al.* 1978) (Plate 6.1). A factorial randomized complete block design with five replicates was used. The fifth replicate was harvested when plants were transferred to the climate room.

Initially, soil moisture was maintained at pot capacity until plants were well established (5 August 1991) and then three levels of soil moisture were imposed, as follows:

- 1) non-stressed, $22 \pm 2\%$ water content (g/g).
- 2) moderately stressed, $15 \pm 2\%$ water content, and
- 3) severely stressed, $11 \pm 2\%$ water content.

Soil moisture adjustment was based on Time Domain Reflectometer (TDR) measurements and pot weight.

The available soil water content (ASWC) of severely and moderately stressed treatments was 50 and 25% of the non-stressed treatment, respectively. The ASWC was calculated as the difference between pot capacity (22% g/g) and wilting point (8.05% g/g) determined from a soil moisture retentivity curve at soil water potentials of -0.0114 and -1.5 MPa, respectively (Fig. 6.1). Nutrient solution was applied twice weekly using 100 ml stock solution 2 M for PO_4^{3-} , Ca^{2+} , and Mg^{2+} . The pH was 6.8. Plants were inoculated with rhizobium NZPS4S4 and showed no symptoms of nitrogen deficiency.

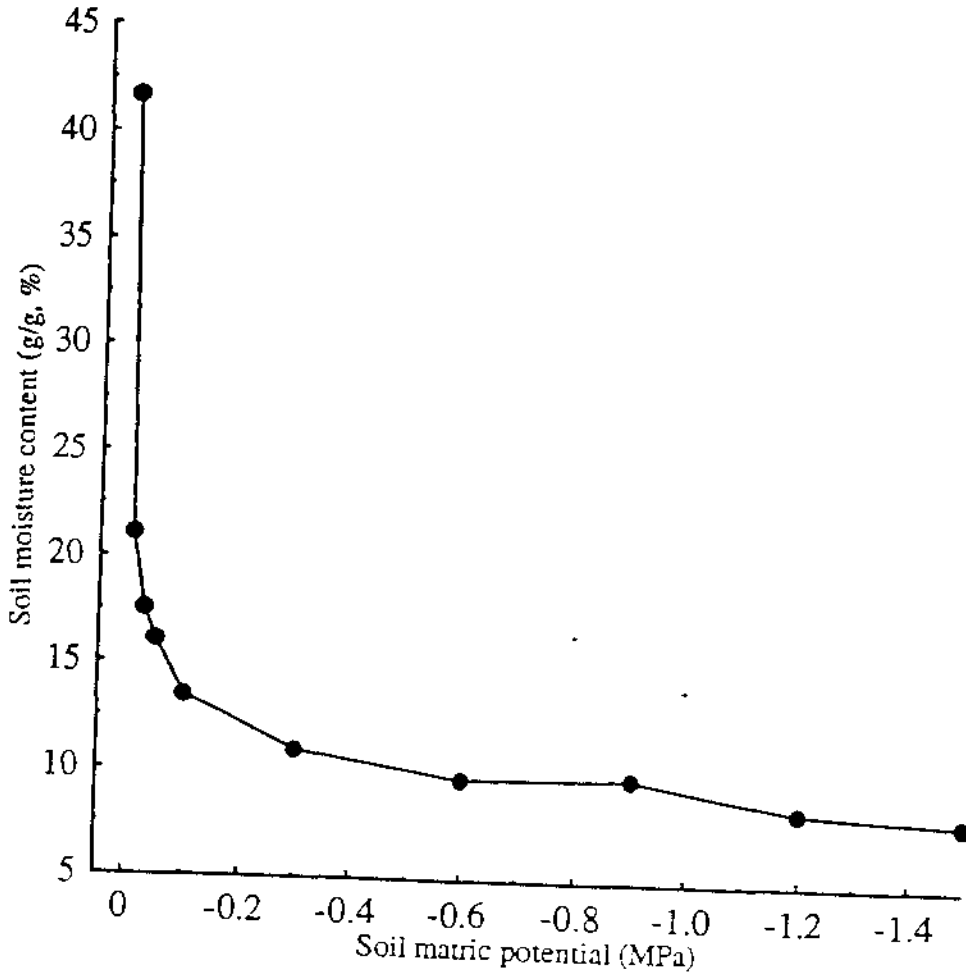


Fig. 6.1: Soil water retentivity curve for the growth medium.

6.3.2 Measurement

6.3.2.1 Transpiration rate (Tr)

Pots were weighed and watered every night and midday (as required) using a 30 kg capacity electronic balance with an accuracy of 0.001 kg. Daily Tr was calculated as $W_1 - W_2$ where W_1 was the pot weight after irrigation and W_2 was the weight before watering on the following day plus watering at midday. Evaporation was determined as negligible in two identical extra pots without plants.

6.3.2.2 Relative water content (RWC)

During the climate room phase, ten disks (100 mm²) from fully expanded leaves were removed weekly at midday, weighed immediately (fresh weight, FW) using an electronic balance with an accuracy of 0.0001g, and then soaked in distilled water for 4 h in a dark and cool environment. Afterwards, surface water was removed by paper towel and the turgid weight (TW) measured. Leaf samples were dried at 80°C for 24 h. Dry weight (DW) was measured and relative water content (RWC) calculated by the ratio of $((FW - DW) / (TW - DW)) * 100$.

6.3.2.3 Stomatal resistance (Rs)

During the climate room phase, two fully expanded leaves from near the top of the canopy in each pot were used at midday each week to measure Rs of the abaxial and adaxial surface of the leaves using a Porometer MK3 (Delta-T devices). The leaf stomatal resistance was found by comparing the counts with those from a moulded polypropylene calibration plate with six diffusion resistances of known value. A calibration graph of these resistances plotted against their corresponding count, was used to convert counts obtained from leaf measurements into diffusion values. The calibration was done under the same conditions as leaf measurement and was repeated when temperature changed.

6.3.2.4 Leaf water potential (Ψ), leaf osmotic potential (π)

A leaf disk (80 mm²) was taken from a fully expanded leaf near the top of the canopy, and loaded into a chamber of a Wescor HR33T Dew Point micro-voltmeter (Brown 1972, Rawlings 1972, Campbell and Barlow 1973) and Ψ measured. The same sample was then frozen in liquid air, reloaded into the chamber and π measured. Chambers were calibrated weekly with a range of NaCl solutions of known potential eg: 0.05, 0.2, 0.4, 0.6, 0.8, and 1 M. Samples Ψ was calculated using a regression equation between microvolt and Ψ . Temperature effects were removed by identifying π_v for each chamber according to the instructions. Ψ and π were measured weekly at "dawn" in the climate room and the glasshouse. Additional measurements were made weekly at midday during the last month of the experiment (Plate 6.2). Turgor potential (P) was calculated as the difference between Ψ and π for both leaves and roots and was not adjusted for possible dilution by apoplastic water.

Midday total leaf water potential (Ψ) was measured by pressure bomb (Scholander *et al.* 1965; Boyer 1967; Tyree and Hammel 1972; and Turner 1981). Two fully expanded leaves from near the top of canopy, per pot in the climate room were excised and placed in a pressure bomb (Soil Moisture Equipment Corp. Model 3005), and the pressure recorded when sap appeared at the cut surface.

6.3.2.5 Photosynthesis

At least three fully expanded leaflets at the top of the canopy in each pot were measured weekly at midday using a Li-Cor 6200 Primer with 0.25 l chamber volume. The boundary layer resistance of the plant was measured using Watman paper of similar size and shape to the leaflets, as described in the manual for Function 41 of the Li-Cor 6200. The regression equation of leaflet size and corresponding boundary layer resistance was $Y = 0.51 + 0.02 * X$ where Y is boundary layer resistance and X is leaflet area. This equation was loaded in function 41 of Li-Cor 6200 with A3= constant (0.5) and A8= slope (0.02). After finishing photosynthetic measurements LA of the leaflets was measured by leaf area meter (Li-Cor Inc model 3100), entered into the Li-Cor 6200, and photosynthetic rate re-computed.

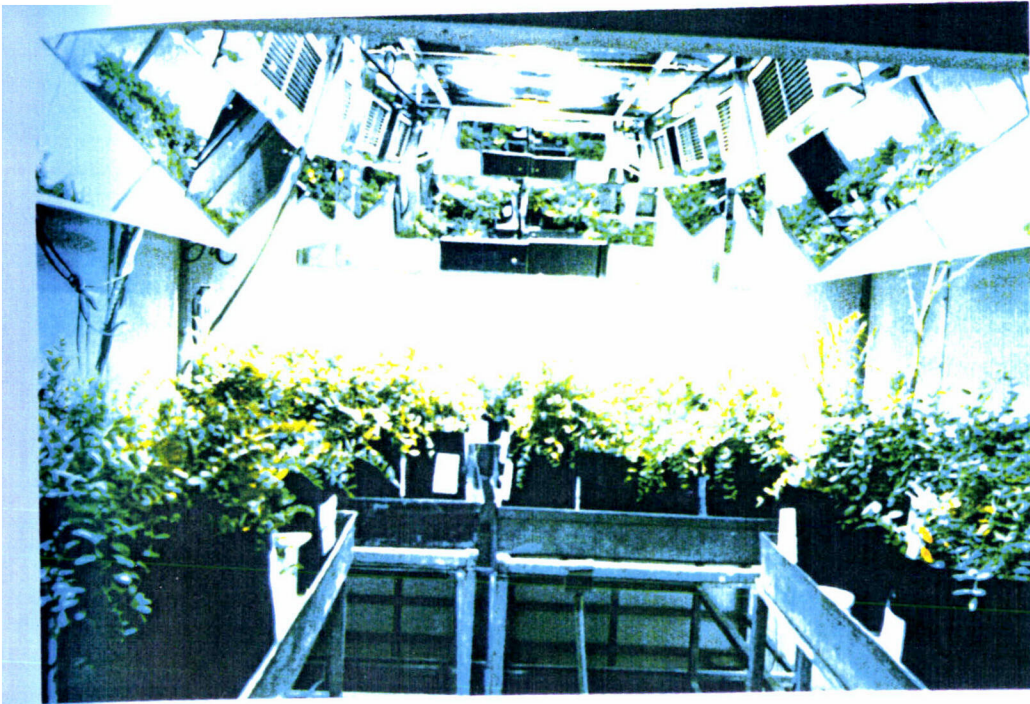


Plate 6.1: A view of sainfoin in the climate room.



Plate 6.2: Measurement of total and osmotic potential by Wescor.

6.3.2.6 Leaf area development, and leaflet number

The number of leaves per plant was counted every 2 weeks, and at the same time, leaf area (LA) of two randomly sampled plants per pot was estimated using the technique of Williams *et al.* (1964). Six leaflet area classes were chosen (Appendix 6.21), and the number of leaflets in each area class recorded. At the end of the experiment, actual leaf area was measured. At this time 10 plants were chosen randomly and their LA estimated as above to test the accuracy of estimation.

The regression equation was $Y=38.4 + 0.56*X$ where Y =Actual leaf area and X =Estimated leaf area, and $R^2=0.92$

6.3.2.7 Plant harvest

At the end of the experiment (31 December 1991), plants were harvested and separated into leaf, stem, and root fractions. Roots were washed free of soil using a 2 mm screen, divided into thick (diameter > 3 mm) and fine roots (diameter < 3 mm) and root length was measured by Comair Root Length Scanner (Voorhees *et al.* 1980). Dry weight of all plant components was determined after 24 h at 80°C (Plates 6.3, 6.4).

Cumulative yield was calculated using the yield at harvesting time plus adjusted dead leaf weight. Dead leaves were collected and counted at 15 d intervals. As collected dead leaves did not include all the leaflets, dead leaf weight was estimated from the dead leaf number and the ratio of leaf dry weight/leaf number for related treatments at the end of the experiment.

6.3.2.8 Water use efficiency (WUE)

WUE was calculated as follows:

- 1) WUE1 = Above ground dry matter at end of experiment/total water use.
- 2) WUE2 = As for WUE1, but also including the estimated dead leaf weight.
- 3) WUE3 = As for WUE2, but also including root dry matter/total water use.
- 4) WUE4 = As for WUE1, but also including the actual dry weight of dead leaves.
- 5) WUE5 = As for WUE4, but also including root dry matter.

6.3.2.9 Specific leaf area (SLA)

Two plants per pot were chosen randomly, and at harvest time, leaflets were separated from petioles, their area and dry weight determined and SLA calculated as area per dry weight.

6.3.3 Statistical analysis

The data for measurements made at the final harvest, for example: root dry matter, were analyzed by analysis of variance using the General Linear Model (GLM) Procedure of SAS (SAS Institute Inc. 1991). A log transformation of root length data did not alter the interpretation. Repeated measures analysis was used to analyze the weekly measurements made on the same experimental unit e.g. Ψ , π , estimated LA, etc (Rowell and Walters 1976).



Plate 6.3: Control (left), moderately (middle), and severely (right) stressed sainfoin.

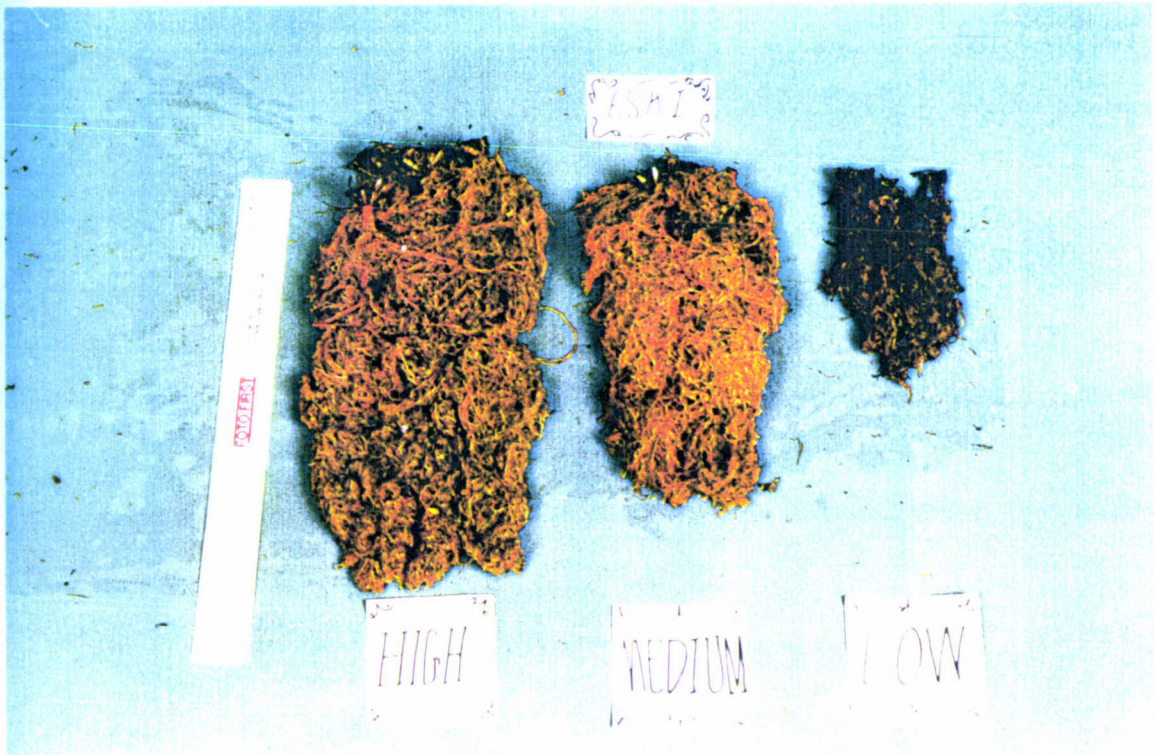


Plate 6.4: Roots of Eski for control (left), moderately (middle), and severely (right) stressed treatments.

6.4 Results

6.4.1 Roots

Soil moisture treatments had different effects on thick (TR) and fine (FR) roots (Table 6.1). Although there were no significant differences for TR, FR varied significantly ($P < 0.01$) between moisture treatments. Control, moderate, and severely stressed plants averaged 1985, 1271, and 835 m, respectively.

The root fractions varied between cultivars. Fakir (3.22 m) and Cotswold-Common (1.88 m) had the greatest and the smallest TR length, respectively. Eski had the smallest TR DW after Cotswold-Common even though it had the largest FR length. Although the TR length of Eski was the largest its TR DW was ranked third of the four cultivars. Eski (1660 m) and Cotswold-Common (1020 m) had the greatest and smallest total root length, respectively.

The cultivar by soil moisture interaction was significant only for total root length (Table 6.2) and showed cultivars Cotswold-Common, and Fakir were more impaired by water stress.

6.4.2 Yield

The yield and cumulative yield varied significantly between the three soil moisture levels (Table 6.3). Control and severely stressed treatments had the highest and lowest yield and cumulative yield, respectively. Water deprivation reduced cumulative yield of moderately and severely stressed plants by 22 and 49%, respectively, compared to that of the control. The cumulative yield was not significantly different for cultivars but cultivar yield at the harvesting time was significantly different. The interaction between cultivar and soil moisture was not significant ($P < 0.05$).

Table 2.1: The length and dry weight of fine and thick roots of four seasons cultivars in three levels of soil moisture (100, 50 and 25% of field capacity).

Cultivar	Fine RL ¹ (m)	Fine R DW (g)	Thick RL (m)	Thick R DW (g)	Total RL (m)	Total R DW (g)
C.Common	1015.5b ²	16.36b	1.887c	3.44b	1017.4c	19.81b
Eski	1661.3a	25.66a	2.148bc	4.55a	1663.5a	30.22a
Fakir	1443.6ab	20.45ab	3.227a	5.53a	1446.8b	25.98ab
Remont	1325.0ab	18.36ab	0.754ab	4.59a	1327.8c	22.95ab
Control	1982.7a	28.08a	2.318a	4.32a	1985.0a	32.36a
Moderate	1268.8b	21.04b	2.797a	5.05a	1271.6b	26.00a
Severe	832.7c	11.54c	2.397a	4.21a	835.1c	15.76b
Cu (Pr>F)	0.0106	0.0182	0.0075	0.0009	0.0206	0.0098
W (Pr>F)	0.0001	0.0001 ¹	0.4237	0.1023	0.0001	0.0001
W*Cu (Pr>F)	0.2089	0.2474	0.3466	0.0762	0.04407	0.1991
SEM	144.3	2.23	0.25	0.32	144.4	2.4

¹ RL= Root length, Cu= cultivar, W= soil moisture, SEM= standard error of the mean

² Data are means of the four replicates. Numbers with same letter(s) within columns are not significantly different.

* Transformed data were used for statistical analysis, similar results were found for untransformed data.

Table 6.2: The interaction between cultivars and soil moisture for total root length (m) (SEM = 288.7) ($P < 0.05$).

Cultivar	Control	Moderate	Severe
Cotswold-C.	1883	761	407
Eski	2292	1476	1221
Fakir	2107	1539	693
Remont	1658	1308	1017

Table 6.3: Yield, cumulative yield (Cum yield), and leaf area (LA), of four sainfoin cultivars at different soil moisture levels, 200 days after planting (DAP)

Cultivar	Yield (g/pot)	Cum Yield (g/pot)	LA (cm ² /pot)
Cotswold-C.	21.29b ¹	41.73b	1475.30b
Eski	24.42a	50.00a	1770.80a
Pakir	21.29b	44.84ab	1513.90b
Remont	20.49b	45.23ab	1216.80c
Control	30.97a	59.69a	2334.40a
Moderate	20.64b	46.38b	1281.27b
Severe	14.30c	30.29c	866.87c
P>F W ² .	0.0001	0.0001	0.0001
P>F Cu	0.0299	0.1500	0.0003
P>F W*Cu	0.0780	0.3800	0.0930
WATER SEM	0.89	2.16	68.20
CULTIVAR SEM	1.03	2.50	78.75
W*Cu SEM	1.79	4.30	136.40

Data are mean of four replicates. Number(s) within columns with the same letter are not significantly different.

W= soil moisture levels, Cu= cultivars, SEM= standard error of the mean.

6.4.3 Leaf area

The LA of plants was significantly different between soil moisture levels (Table 6.3), with control and severely stressed plants having the highest and lowest LA, respectively ($P < 0.01$) (Table 6.3). The LA of cultivars was significantly different ($P < 0.01$). Eski and Remont had the highest and lowest LA respectively. The LA of severely stressed Remont, Fakir, Cotswold-Common, and Eski, was 76, 70, 68, and 55 %, of control plants, respectively.

6.4.4 Specific leaf area (SLA)

The SLA (cm^2/g) of plants was significantly different between soil moisture levels (Table 6.4 a). Control plants had significantly higher SLA than moderately and severely stressed plants ($P < 0.01$). There was no significant difference between the SLA of cultivars, however, the interaction of soil moisture by cultivar was significantly different. Fakir had the highest and the lowest SLA for control and severely stressed plants, respectively. The highest SLA for moderate and severely stressed plants was for Remont and Eski, respectively (Table 6.4 b)

Table 6.4 (a) Leaf dry weight (DW), leaf area (LA), and specific leaf area (SLA) of two sampled plants per pot.

Cultivar	Leaf DW (g)	LA (cm)	SLA (cm/g)
Cotswold-C.	2.1 ¹	238	112
Eski	2.4	300	127
Fakir	1.9	256	122
Remont	2.0	259	131
Control	2.7a	394a	145a
Moderate	1.9b	227b	119b
Severe	1.6b	168b	104b
P>F Water	0.0016	0.0001	0.0003
P>F Cultivar	0.5100	0.6000	0.3100
P>F W*Cu ²	0.8600	0.1300	0.0300
Water SEM	0.21	28.6	6.42
Cultivar SEM	0.23	33.0	7.40
W*Cu SEM	0.41	57.2	12.85

¹ Data are mean of four replicates. Numbers within a column with the same letter are not significantly different.

² W= Soil moisture, Cu= Cultivar, and SEM= standard error of the means.

Table 6.4 (b): Interaction of cultivar by soil moisture treatment for SLA (SEM= 12.85) (P < 0.05).

Cultivar	Control	Moderate	Severe
Cotswold-C.	129.8	106.7	100.0
Eski	134.7	126.1	120.8
Fakir	180.2	106.2	79.7
Remont	138.5	137.4	117.5

6.4.5 Relative water content (RWC)

Repeated measures analysis showed a significant effect for soil moisture levels ($P < 0.01$) over time but not for cultivars or their interactions with soil moisture (Appendix 6.18). The RWC of severely stressed plants decreased over time whereas the RWC of the control and moderately stressed plants did not (Appendix 6.1). The mean for seven measurements of RWC of leaves was significantly different between soil moisture levels ($P < 0.05$) and cultivars ($P < 0.05$) but not their interaction. Control and severely stressed plants had the highest and the lowest RWC respectively (Fig. 6.2).

Cotswold-Common and Fakir had the highest (80%) and Remont had the lowest (77%) RWC. Independent ANOVA for each measurement time showed a significant effect of soil moisture treatment at all but the second measurement. Cultivars and their interaction with soil moisture were sometimes significant but no consistent trend was found (Appendix 6.1).

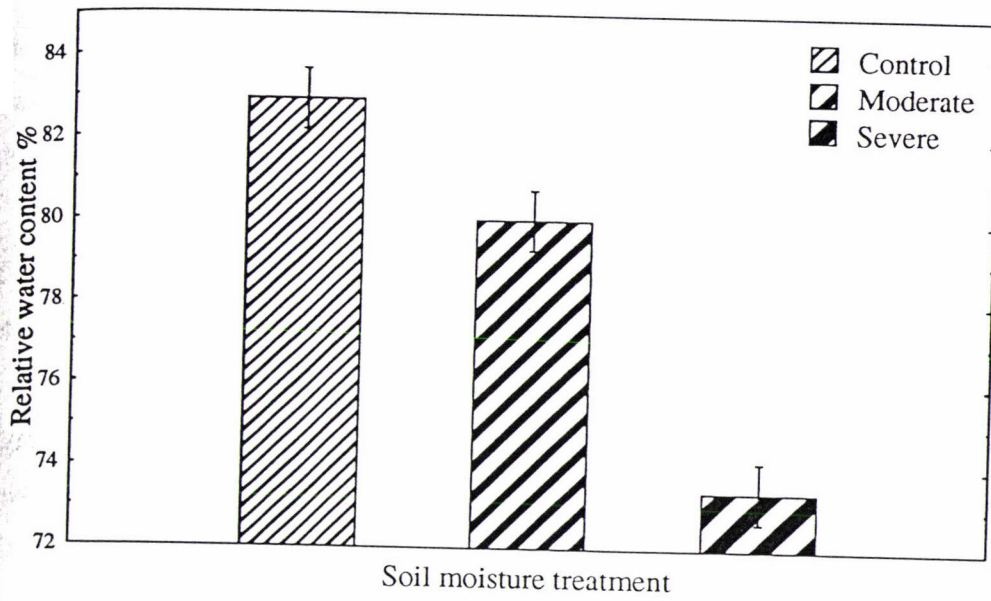


Fig. 6.2 (a): Relative water content of sainfoin at three soil moisture levels. Bars show \pm standard error of the mean.

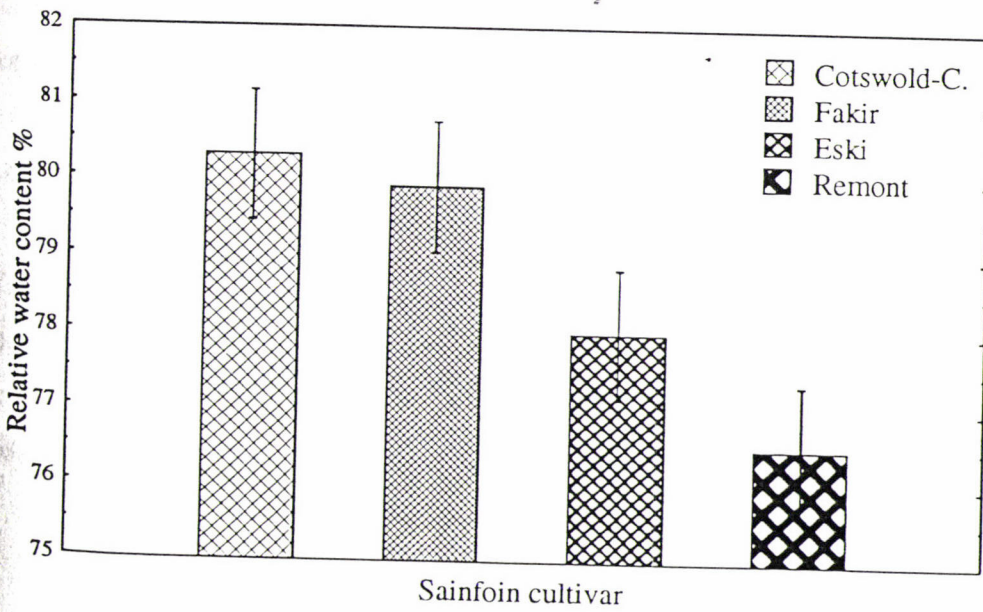


Fig. 6.2 (b): Relative water content of four sainfoin cultivars. Bars show \pm standard error of the mean.

6.4.6 Stomatal resistance (Rs)

The Rs of the abaxial leaf surface (19 ± 3.2 s/cm) was higher than the adaxial leaf surface (3.35 ± 0.5 s/cm) (Fig. 6.3). The average stomatal resistance of the adaxial surface of the control and severely stressed plants was 2.3 and 4.7 s/cm, while that of the abaxial surface was 17, and 22 s/cm respectively.

Repeated measures analysis for each leaf surface showed no significant effects of soil moisture treatment, cultivar, or their interaction for both sides of the leaf over time (Appendix 6.18).

The mean of the six measurements of abaxial leaf surface Rs was not significantly different for cultivar, soil moisture level, or their interaction. In contrast, however, adaxial surface Rs was significantly different for soil moisture levels but not for cultivar or their interaction with soil moisture. Control and severely stressed plants had the lowest and the highest Rs respectively (Fig. 6.3).

Independent ANOVA for each measurement time for the abaxial Rs of the surface showed no significant effect for cultivar, soil moisture, or their interaction. The Rs of the adaxial leaf surface was significantly increased by soil moisture stress, but the cultivar effect and the interaction of soil moisture treatment by cultivar were not significant (Appendix 6.3).

6.4.7 WUE

Water stress significantly increased plant WUE ($P < 0.0001$) (Table 6.5). Water stress increased the WUE of severely and moderately stressed plants by 34, and 15%, respectively relative to the control plants. The interaction of soil moisture by cultivar was not significant, as the cultivars responded similarly at each soil moisture level.

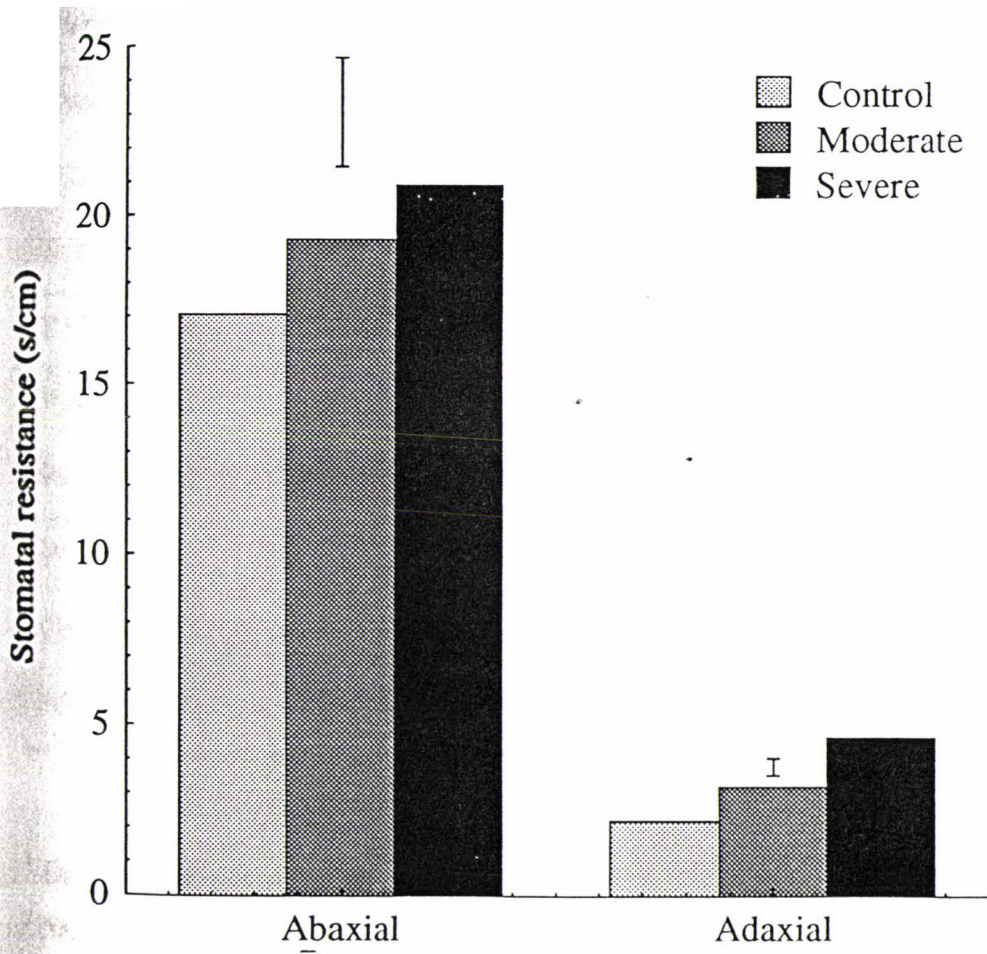


Fig. 6.3: Stomatal resistance of abaxial and adaxial surfaces of the leaf. Error bars are standard error of the means.

Table 6.5: Water use efficiency (g DM/kg H₂O) (by 5 methods, see text) of four sainfoin cultivars at three soil moisture levels.

Cultivar	WUE1	WUE2	WUE3	WUE4	WUE5
Cotswold-C.	0.97 ¹	1.77	2.60	1.11	1.90
Eski	0.97	1.88	3.00	1.16	2.30
Fakir	0.90	1.77	2.80	1.10	2.10
Remont	0.81	1.70	2.60	1.10	1.94
Control	0.07c ²	1.35c	2.09a	0.92c	1.67b
Moderate	0.09b	1.80b	2.80b	1.10b	2.14a
Severe	1.10a	2.18a	3.30c	1.29a	2.40a
P>F W ³	0.0001	0.0001	0.0001	0.0001	0.0001
P>F Cu	0.5200	0.5000	0.1600	0.5400	0.1800
P>F W*Cu	0.8800	0.6000	0.4000	0.7100	0.7200
W SEM	0.04	0.073	0.135	0.036	0.11
Cu SEM	0.046	0.084	0.156	0.042	0.128
W*Cu SEM	0.081	0.141	0.27	0.072	0.22

¹ Data are the mean of four replicates.

² Numbers with the same letter within column are not significantly different.

³ W= soil moisture, Cu= cultivar, and SEM= standard error of the mean.

6.4.8 Leaf water potential at dawn

6.4.8.1 Glasshouse

Repeated measures analysis of the water potential at dawn showed no significant effect over time for soil moisture treatment, cultivar, or their interaction (Appendix 6.18). The mean for the six measurements of dawn Ψ was significantly affected by soil moisture and cultivar ($P < 0.05$) but their interaction was not significant.

The average of the six measurement of dawn Ψ of control, moderately, and severely stressed plants was -0.49, -0.56, and -0.78 MPa respectively (Fig. 6.4a). Cotswold-Common and Fakir (-0.7 and -0.53 MPa) had the lowest (most negative) and the highest Ψ , respectively.

Independent ANOVA for each measurement time showed Ψ was significantly effected by soil moisture level. At each time, Ψ was lowest (most negative) in the severely stressed plants and the highest (least negative) in the control plants, respectively (Appendix 6.4).

6.4.8.2 Climate room

Repeated measures analysis showed no significant changes over time in Ψ due to soil moisture and the interaction of soil moisture by cultivars, however, cultivar effects were not consistent over time (Appendix 6.18). Cotswold-Common Ψ was consistently lower (more negative) than for Remont, Fakir and Eski (Appendix 6.7). The mean of seven measurements of dawn Ψ in the climate room was significant for soil moisture level ($P < 0.001$) but not for cultivar or the interaction with soil moisture. Control and severely stressed plants had the highest (least negative) and the lowest (more negative) Ψ respectively (Fig. 6.4 b).

Independent ANOVA of each of the seven measurements showed that, except at the first time, Ψ was significantly lower (more negative) in the severely stressed plants than the control plants. The cultivar and the cultivar by soil moisture interaction were sometimes significant, but no consistent trend was apparent.

6.4.9 Osmotic potential (π) at dawn

6.4.9.1 Glasshouse

Repeated measures analysis showed no significant changes over time in π due to soil moisture level, cultivars, or their interaction (Appendix 6.18).

The mean of the six measurements of dawn π showed soil moisture, and cultivars significantly affected π , but not their interaction ($P < 0.001$). Severely stressed and control plants had the lowest (-0.88 MPa) and the highest (-0.69 MPa) π respectively (Fig. 6.4 a).

Independent analysis of each of the measurement times showed that except for the first measurement, π of the severely stressed plants was significantly ($P < 0.01$) lower (more negative) than moderately stressed or control plants (Appendix 6.5). The cultivar and the cultivar by soil moisture interaction were significant at some measurement times but no consistent trend was apparent.

6.4.9.2 Climate room

Repeated measures analysis showed no significant effects on π of cultivars, and soil moisture levels over time, but there was a significant response to the interaction of soil moisture by cultivars by time (Appendix 6.18). Cotswold-Common π was consistently lower (more negative) than for Remont, while Eski, and Fakir π tended to be inconsistent.

The mean of the seven measurements, of dawn π was significantly different for soil moisture levels and cultivars, but not their interaction. These results showed severely stressed and control plants had the lowest (more negative) and the highest (less negative) π respectively (Fig. 6.4-b). The average π of control, moderately and severely stressed plants was -1.01, -1.10, and -1.18 MPa, respectively.

Remont had higher (-1.03 \pm 0.02) π than the other three cultivars. The average dawn π of Eski, Fakir, and Cotswold-Common during the climate room period was -1.10, -1.10, -1.15 respectively.

Independent analysis of the seven measurement times showed that, except for

times 2 and 4, there were significant differences between π of the soil moisture treatments ($P < 0.05$). Severely stressed and control plants had the lowest (most negative) and highest (least negative) π at all the times in the climate room at dawn (Appendix 6.8). Although cultivar and the cultivar by soil moisture interaction were significant at some of the measurement times, no consistent trend was apparent.

6.4.10 Turgor potential (P)

6.4.10.1 Glasshouse

Repeated measures analysis showed P was significantly affected by soil moisture levels over time, but no consistent trend was found for any soil moisture level (Appendix 6.18).

The mean of the six measurement times showed P was significantly affected by soil moisture ($P < 0.05$), but not cultivar or their interaction with soil moisture level. Dawn P of severely stressed and control plants had the lowest (0.1 MPa) and the highest (0.21 MPa) P, respectively (Fig. 4.1-a).

Independent ANOVA at each of the six measurement times showed that dawn P for the soil moisture levels and the interaction of cultivar by soil moisture were only significantly different at the first measurement time (Appendix 6.6).

6.4.10.2 Climate room

Repeated measures analysis showed no significant changes in turgor potential over time for soil moisture, cultivar, and their interaction (Appendix 6.18).

The mean of the seven measurement of the climate room dawn P showed significant effects of soil moisture level and the interaction between soil moisture and cultivar ($P < 0.05$), however cultivars were not significantly different. Control, moderately, and severely stressed plants had mean P of 0.29, 0.25, and 0.17 MPa respectively (Fig. 6.4-b). The P of severely (0.17 ± 0.03 MPa) and moderately (0.2 ± 0.03 MPa) stressed plants of Fakir were lower than for the other three cultivars, but in the control treatment it was highest (0.35 ± 0.03 MPa).

Independent analysis of each of the seven measurement times showed that P was occasionally significantly different for cultivar, soil moisture, and their

interaction but no consistent trend was apparent (Appendix 6.9) .

6.4.11 Total, osmotic, and turgor potential of the leaf at midday

6.4.11.1 Leaf water potential

Repeated measures analysis showed no significant effect over time for midday Ψ for soil moisture and cultivar, but significant changes for their interaction over time.

The mean of the four measurement times for midday Ψ varied significantly for soil moisture treatment ($P < 0.01$) (Fig. 6.4-c), and the interaction between soil moisture and cultivar ($P < 0.05$), however cultivars were not significantly different. The mean Ψ of the control, moderately and severely stressed plants was -0.87 , -1.10 , and -1.30 MPa respectively (Fig. 6.4-c). Cotswold-Common had the highest (-1.1 ± 0.05 MPa) (least negative) Ψ in severely stressed and the lowest (most negative) midday Ψ in control (-0.95 ± 0.05 MPa) or moderately (-1.3 ± 0.05 MPa) stressed plants. Remont had the lowest (1.5 ± 0.05 MPa) (more negative) Ψ in severely stressed plants and the highest (-0.81 ± 0.05) (less negative) Ψ in the control plants.

Independent ANOVA for each measurement time showed a significant effect of soil moisture on midday Ψ at all times of measurement ($P < 0.01$). Severely stressed and control plants had the lowest (more negative) and the highest (less negative) Ψ , respectively. There was no significant effect of cultivars at any time of measurement, however the cultivar by soil moisture interaction was significantly different at some times but no significant trend was apparent (Appendix 6.13).

6.4.11.2 Osmotic potential

Repeated measures analysis showed no significant changes in π over time due to soil moisture, cultivar, and their interaction.

The mean for the four measurement times of midday π was significantly affected by soil moisture but not by cultivar and their interaction (Fig. 6.4 c). Control and severely stressed treatments had the lowest (more negative) and the highest (less negative) Ψ , respectively.

Independent ANOVA for each of the measurement times of midday π showed

significant effects for soil moisture at all times of measurement, however, there, were no significant effects for cultivar or their interaction with soil moisture ($P < 0.05$) (Appendix 6.11). The control and severely stressed treatment had the highest (less negative) and the lowest (more negative) π , respectively. Mean π for times 1 to 4 was -1.22, -1.35, -1.3, and -1.25 MPa, respectively.

6.4.11.3 Turgor potential

Repeated measures analysis for midday P showed no significant changes over time due to cultivars, soil moisture or their interaction.

The means of the four measurement times showed midday P was significantly different for soil moisture treatments ($P < 0.05$) and their interaction with cultivar ($P < 0.05$), but not for cultivar. Control and severely stressed treatments had the highest (0.27 MPa) and the lowest (0.09 MPa) P, respectively (Fig.6.4 c). Remont had the highest (0.3 ± 0.08 MPa) and lowest (0.1 ± 0.08 MPa) P in the control and severely stressed treatments. Cotswold-Common had the highest (0.2 ± 0.08 MPa) P in the severely stressed treatment. In the moderately stressed treatment Eski and Cotswold-Common had the highest (0.26 ± 0.08 MPa) and the lowest (0.09 ± 0.08 MPa) midday P, respectively .

Independent ANOVA of each measurements time only showed significant effects due to soil moisture at times 2 and 4 ($P < 0.05$). There was no significant difference between cultivars or their interaction with soil moisture (Appendix 6.12).

6.4.11.4 Midday leaf water potential by pressure bomb

Repeated measures analysis showed significant changes over time ($P < 0.01$) for soil moisture treatment, but not for cultivar or their interaction with soil moisture treatment.

The mean for the seven measurement times for midday Ψ were affected significantly by soil moisture but not cultivar, or their interaction with soil moisture level. Control and severely stressed plants had the lowest (most negative) and the highest (least negative) Ψ , respectively ($P > 0.01$) (Appendix 6.13).

Independent ANOVA at each of the measurement times showed significant

effects ($P < 0.01$) of soil moisture levels for times 1-7 (Appendix 6.13). Control and severely stressed treatments had the lowest (more negative) and the highest (less negative) Ψ respectively. Cultivars were significantly different only at time 2 with Cotswold-Common and Remont having the lowest (more negative) and the highest (less negative) Ψ , respectively ($P < 0.05$).

6.4.11.5 Leaf water potential: Wescor vs Pressure bomb

The relationship between Ψ measured by pressure bomb and by Wescor (Fig. 6.5) was $Y=0.18+1.25X$ where Y is Ψ measured by Wescor and X is Ψ measured by pressure bomb ($R^2=0.90$).

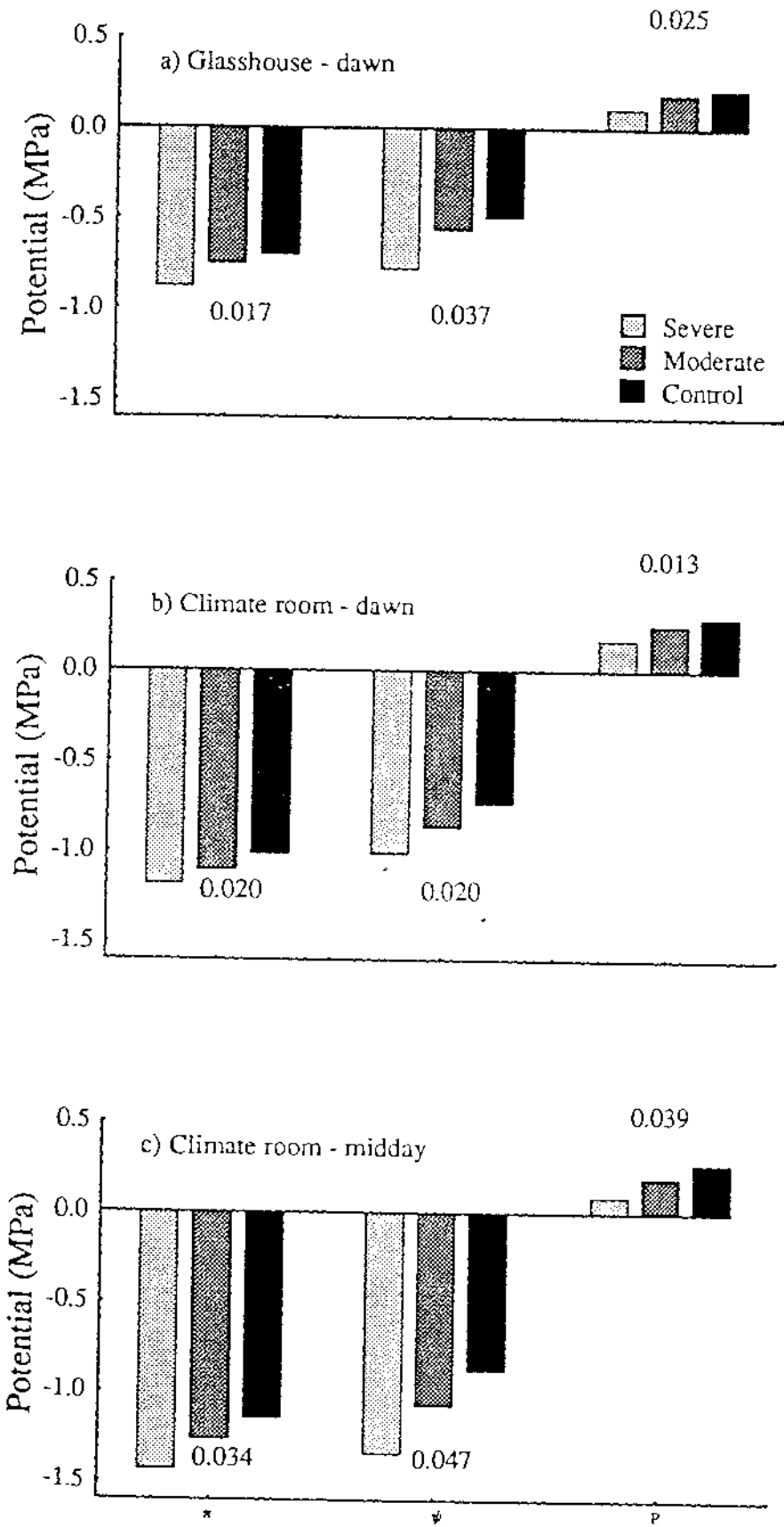


Fig 6.4: Osmotic potential (π), Leaf water potential (ψ) and turgor potential (P) of sainfoin at three soil moisture levels. Numbers show SEM.

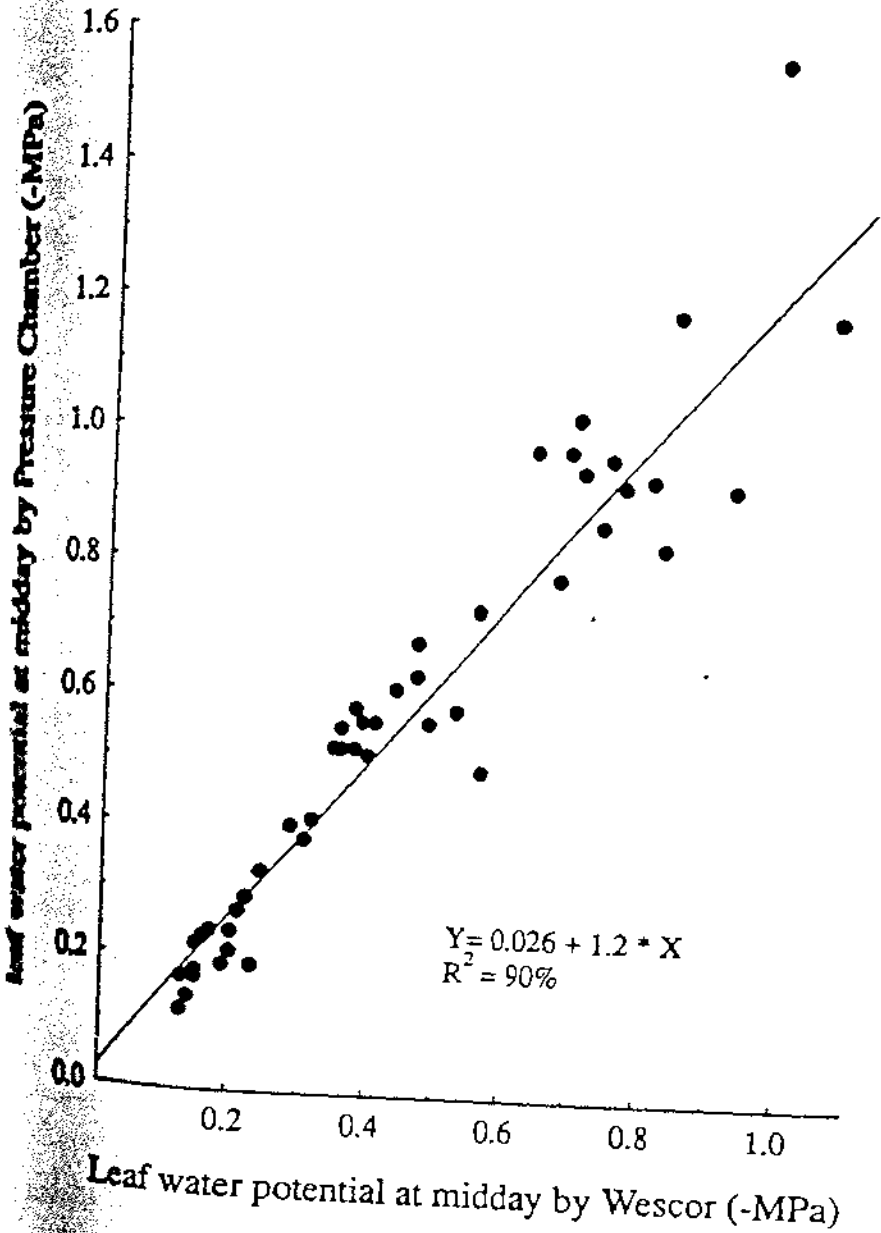


Fig 6.5: Relationship between leaf water potential measured by Pressure Chamber and Wescor.

6.4.12 Photosynthesis (P_n)

Repeated measures analysis of P_n over the seven measurement times found no significant changes over time for soil moisture treatment, cultivar and their interaction.

The mean of seven measurements of P_n showed significant effects for soil moisture ($P < 0.001$) (Fig. 6.6), but not for cultivars or their interaction with soil moisture. Severely stressed and control treatments had the lowest and the highest photosynthetic rate, respectively. There was no significant difference between the photosynthetic rate of the control and moderately stressed treatments. The average photosynthetic rate of the cultivars was $11.1 \mu\text{molCO}_2/\text{m}^2/\text{s}$.

Independent analysis of the seven measurement times showed significant effects of soil moisture on P_n for all the times ($P < 0.05$), whereas there were no significant effect of cultivars or their interaction with soil moisture (Appendix 6.14).

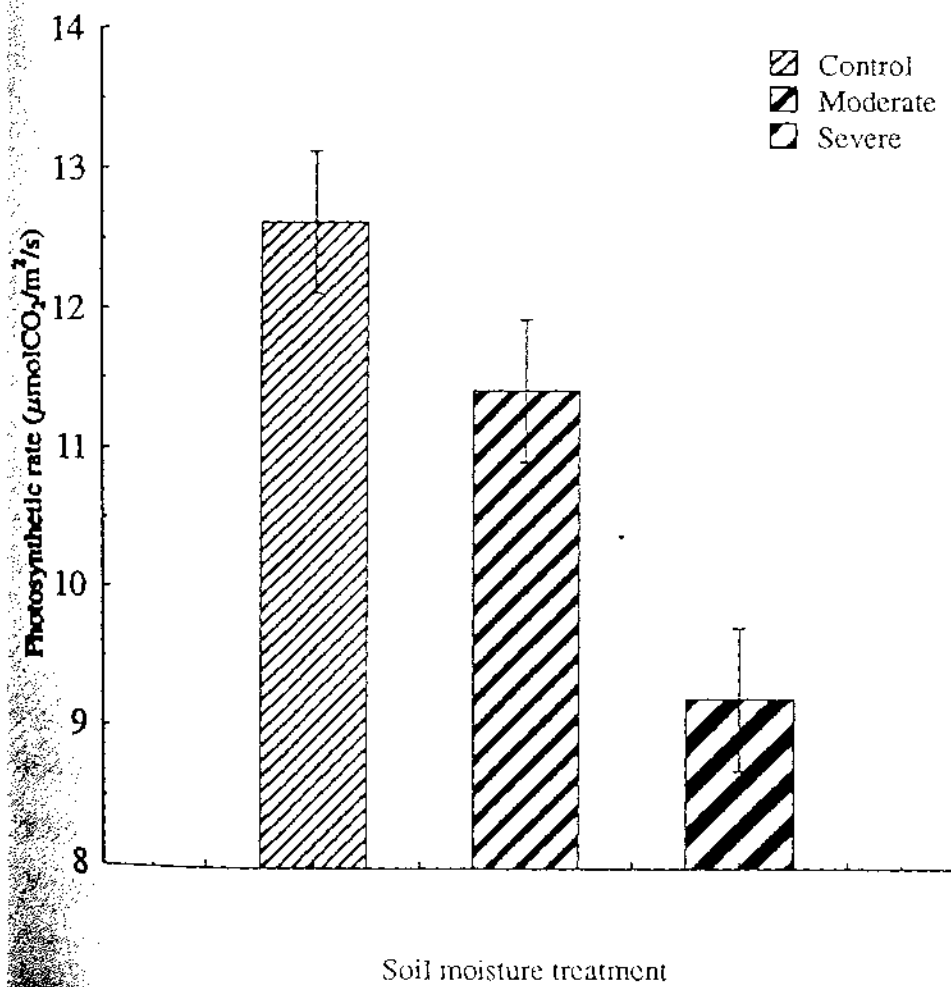


Fig. 6.7: Photosynthetic rate of sainfoin at three soil moisture levels. Bars show \pm standard error of the mean

6.4.13 Leaflet number

Repeated measures analysis showed significant change in leaflet number due to soil moisture over time but there was no significant effect due to cultivars and their interaction with soil moisture levels (Appendix 6.18).

Independent analysis of the seven measurement times showed significant differences between leaflet numbers in the soil moisture treatments for all the times except time 1 (Appendix 6.15).

Control and severely stressed plants had the largest and smallest number of leaflets, respectively (Fig 6.7 a). Cultivars had different leaflet numbers only at first time of measurement. Remont and Cotswold-Common had the largest, while Eski and Fakir had the smallest number of leaflets. There was no significant interaction between soil moisture and cultivar at any time.

6.4.14 Estimated leaf area

Repeated measures analysis showed a significant effect of soil moisture on leaf area over time, however LA of cultivars and their interaction with soil moisture were not significantly different over time (Appendix 6.18).

Independent analysis of seven measurement times showed the effect of the soil moisture treatment was significant for all times, except first time (Fig. 6.7 b). Control and severely stressed plants had the highest and the lowest LA respectively. Relative to the control plants, water stress reduced LA of moderately and severely stressed plants 42 and 58%, respectively (Appendix 6.16).

The LA of cultivars was significantly different only at two times of measurements. Initially, Remont had the largest LA whereas Eski had the largest LA at time 4. The interaction of soil moisture and cultivar was not significant at any time.

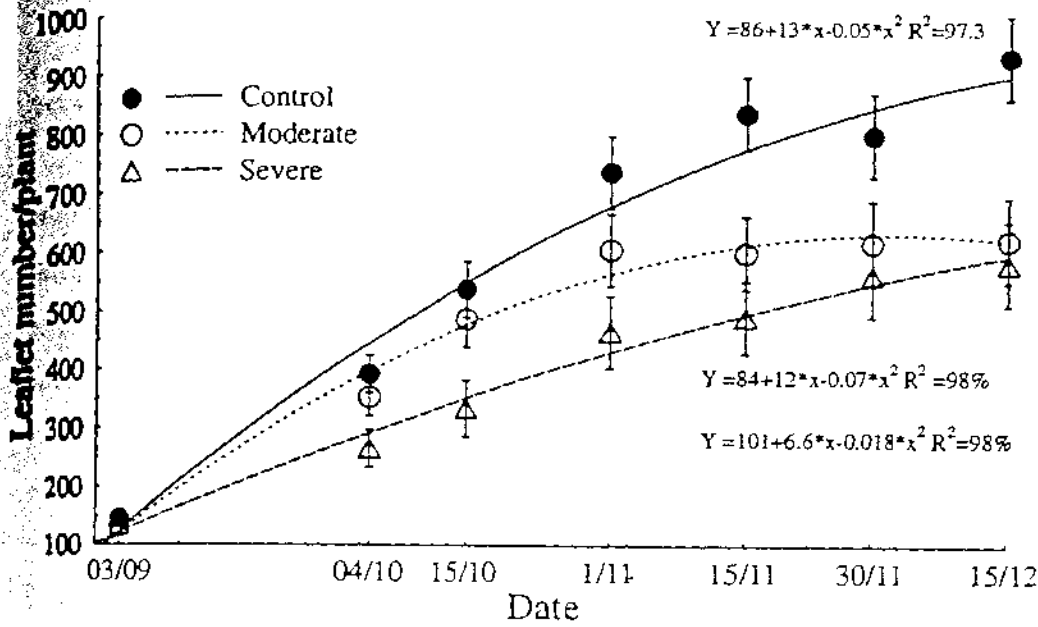


Fig 6.7 (a): Leaflet number of sainfoin at three soil moisture levels. Vertical bars show \pm SEM. Markers are mean of four replicates.

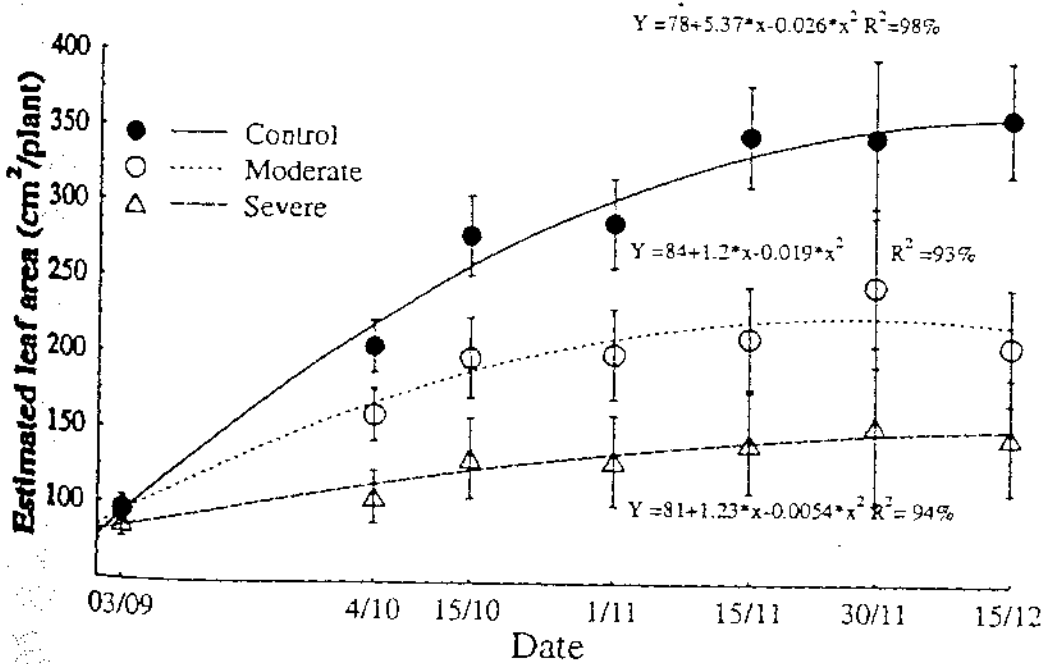


Fig 6.7(b): Estimated leaf area of sainfoin at three soil moisture levels. Vertical bars show \pm SEM. Markers are mean of four replicates.

6.4.15 Transpiration rate

Repeated measures analysis showed that soil moisture and the interaction between cultivar and soil moisture significantly affected transpiration rate over time.

Independent analysis of the five measurement times showed significant differences between the transpiration rates in the soil moisture treatments. Severely stressed and control plants had the lowest and the highest transpiration rate, respectively (Fig. 6.8-a,b). Relative to control plants water stress reduced transpiration rate of moderately and severely stressed plants 59 and 66 %. The transpiration rate of the cultivars was significantly different at the first four measurements.

Initially, Remont had a higher transpiration rate than Eski, but at later times Eski had the higher transpiration rate than Remont (Appendix 6.17). At the end of the experiment there was no significant difference between the cultivars. The interaction between soil moisture and cultivars was not significantly different at all times.

The high initial T_r of Remont could have due to the faster early growth of this cultivar relative to the Eski as was clear from the LA development of Remont (Appendix 6.16). Since R_s of the adaxial leaf surface was affected by soil moisture more than that of the abaxial leaf surface the R_s of the adaxial surface was plotted against transpiration rate to understand the relationships of R_s of upper surface transpiration rate (Fig. 6.9 a,b).

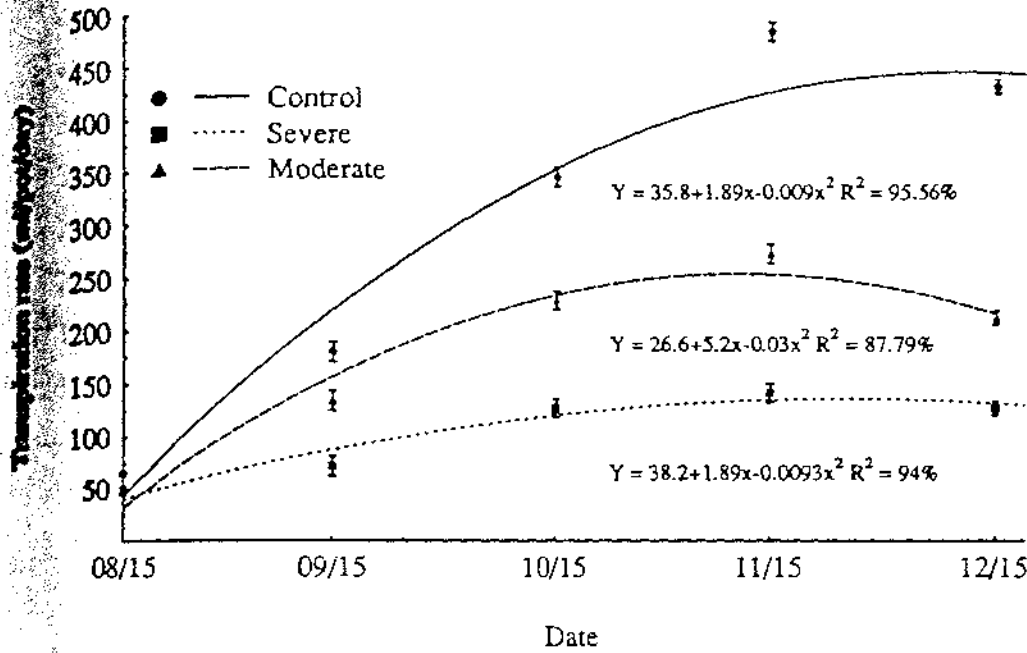


Fig 6.8 (a): Transpiration rate of sainfoin at three soil moisture levels. Vertical bars show ±SEM. Markers are mean of four replicates.

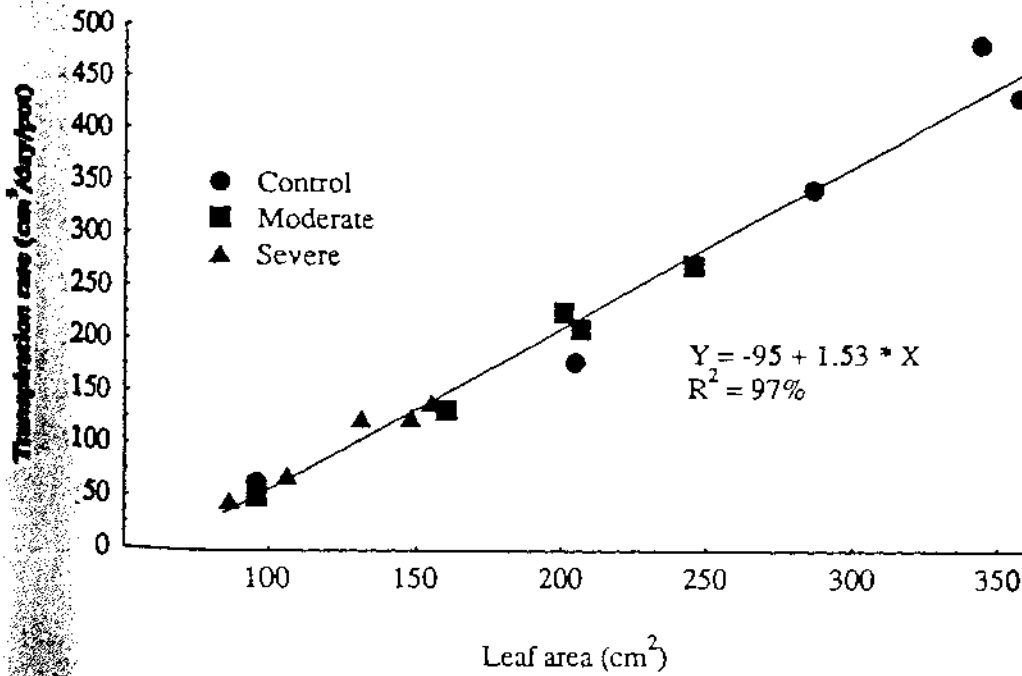


Fig 6.8 (b): Relationship between leaf area and transpiration rate. Markers are mean of four replicates.

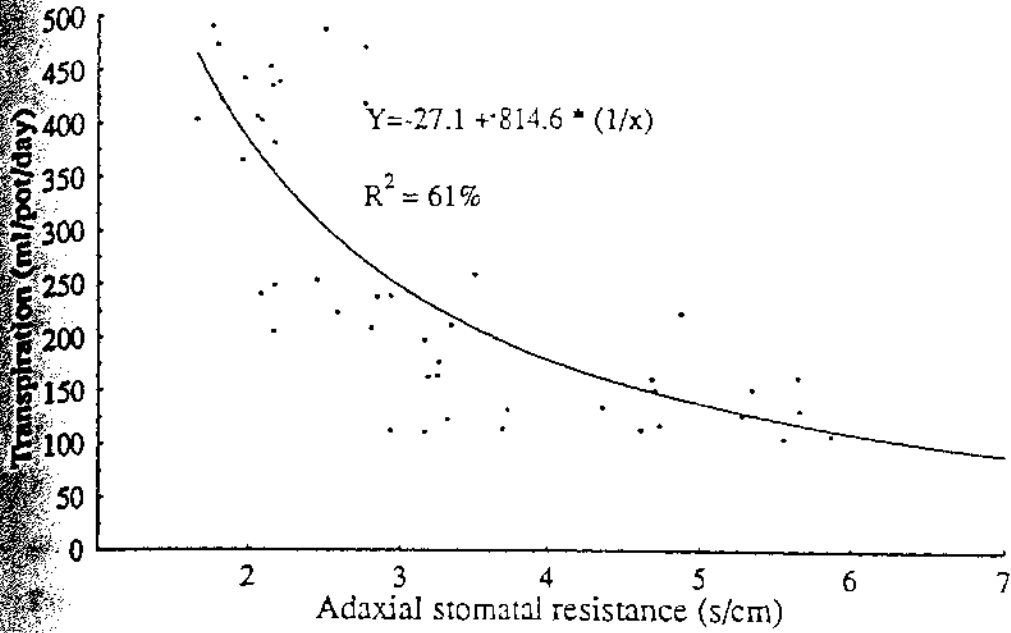


Fig. 6.9 (a): Relationship between adaxial stomatal resistance of sainfoin and transpiration.

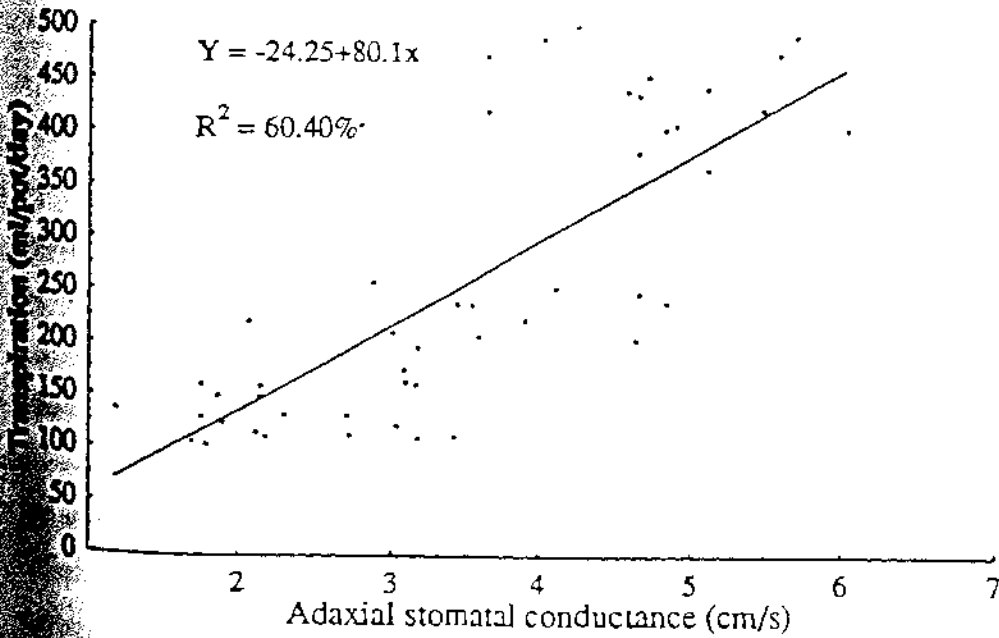


Fig. 6.9 (b): Relationship between adaxial stomatal conductance of sainfoin and transpiration.

6.5 Discussion

6.5.1 Yield and its components

Water stress decreased sainfoin cumulative yield of the stressed plants by 49% relative to the control. This decrease was largely due to a decrease in leaf area. No significant difference was found between the leaflet number of the moderately and severely stressed plants while the leaf area was different for these two soil moisture treatments. It can be assumed that the decrease in leaf area was from smaller leaflets rather than a decrease in leaflet number.

Decreased LA due to water stress has been reported in sainfoin (Bolger 1988) and other plants (Chu 1979; Richardson *et al.* 1985; McCree 1986; Munns and Termat 1986; Richard and Twenley-Smith 1987; White *et al.* 1990; and Acosta-Gallegos 1991). The decline in leaf area was strongly related to the severity of the water stress treatments, with the LA of severely and moderately stressed plants decreased to 63% and 45% of the control plants respectively.

The SLA of severely stressed plants was up to 28% less than the control plants lower SLA reduced the LA more than leaf weight and increased the WUE. Moderately stressed and control plant SLA was similar. Fakir had the highest and lowest SLA and LA in the control and severely stressed treatments, respectively (Table 6.4b). Decreased SLA was found in previous water stress studies: for example in Chapter 4 the SLA of stressed lucerne was 22% less than that of the control plants, and in Chapter 5 stressed Remont had only 50% of the SLA of the control plants. Decreased SLA of stressed plants might have been due to different responses of cell elongation and division to water stress (Vaadia *et al.* 1961; Gates 1964; Clements 1964; Salter and Goode 1967; Slatyer 1967; and Hsiao 1973).

The SLA of water stressed Remont in the field (Chapter 5) was greater than for stressed Remont in the present study. Reasons for this could be the different environmental conditions of temperature, relative humidity, light intensity and air movement in the field compared to in the climate room.

6.5.2 Relative water content

Relative water content of severely and moderately stressed sainfoin was

significantly lower than control plants. A decreased RWC due to water stress has been shown in other plants, for example: wheat (Richie *et al.* 1990), lupin (Henson *et al.* 1989a), sorghum (Bennett *et al.* 1987), centrosema (Ludlow *et al.* 1983), and soybean (Sloane *et al.* 1990).

Remont had a significantly lower RWC than the other cultivars while Cotswold-Common had the highest (Fig. 6.2 b). The RWC of the Remont was decreased more in the severely stressed plants relative to the control than the other three cultivars. The RWC reduction of severely stressed plant compared to the control for Remont was 17% whereas for Cotswold-Common, Eski, Fakir it was 8.6, 10.9, and 9.5% respectively. The RWC of Remont in Chapter 5 was 62, 69, and 80% for VSWC of 10, 15, and 23%, respectively, while in the present experiment the Remont RWC at these VSWC was 68, 80, and 83% respectively.

The trend of a higher RWC of Remont in this experiment may have been due to different soil types used in the two experiments. Different soil types can have a different soil potential at a similar VSWC. The media used in this experiment was mixed with river sand (50%) which made it lighter than the soil used in the field and gave a lower soil potential at the same VSWC relative to the field soil. In severely and moderately stressed plants higher water demands (transpiration) than water supply (soil water) resulted in a decrease in the RWC of the leaves at midday. This was confirmed by P , Ψ , and π results for the leaves at midday.

Stomata closure at midday following decreased RWC possibly helped to maintain plant P . The low P of Remont at midday relative to other cultivars could have been due to lower RWC of this cultivar at midday. Also the higher P of the severely stressed Cotswold-Common was probably due to the smaller reduction in RWC of severely stressed Cotswold-Common relative to other cultivars. High RWC could be considered a positive factor for plant growth in dry conditions. Although attributes such as root mass and root length, WUE, π , R_s , and cell elasticity, also determine yield. The higher RWC, P and Ψ of Cotswold-Common, in the climate room, than the other cultivars was probably due to the different phenological development of the cultivars. Cotswold-Common matured later than other three cultivars.

6.5.3 Water status of sainfoin

Ψ , π and P were affected by the soil moisture level in both the glasshouse and climate room at dawn and midday. These effects have been recognised in other studies for other plants (Turk and Hall 1980; Turner *et al.* 1987; Flower *et al.* 1990; Khalil and Grace 1993). More negative Ψ can result from reduction in π or P, and both these effects occurred in this study. All components of water potential were higher at dawn than at midday. At dawn, stomatal closure reduced transpiration so that plant and soil moisture potential could equilibrate. At midday, higher transpiration than water supply (from soil through roots) caused the Ψ and π of stressed plants to become more negative (Fig. 6.4-c). The more negative π of the severely stressed plants at both dawn and midday might have been caused by dehydration of severely stressed plants leaves or solute accumulation in the leaves of severely stressed plants (Morgan 1984).

Remont showed a different response to soil moisture treatments than the other cultivars, with the lowest Ψ and P in the severely and moderately stressed treatments but the highest Ψ in the control treatment. Remont π was higher (less negative) at dawn than for the other cultivars. These responses indicated that relative to the other cultivars, Remont might perform poorly in dry conditions. Nevertheless, the higher (less negative) Ψ of the control plants of Remont relative to other cultivars showed Remont was probably better adapted to non-stressed conditions than the other cultivars. In contrast to Remont, Cotswold-Common had the highest Ψ and P in the water stress treatments, but for other attributes associated with water stress tolerance such as root length, Cotswold-Common performed poorly compared to other cultivars.

A deep rooted plant can take up water from deep in the soil and thereby avoid drought to some extent. Cotswold-Common grew slower than Remont, and had a smaller root mass and root length, thus it would probably not be as a drought tolerant as Remont despite having a less negative Ψ . Eski had the largest root length and root mass and also maintained P better than Remont. The slower growth of Eski than Remont, plus its greater rootiness, would save water during early growth and allow water uptake from depth.

The measurement of Ψ and π at both dawn and midday can be useful to understand plant water status. Measurement of midday π shows the responses of cultivars to extreme environments (the cultivars less tolerant of midday conditions are less likely to survive). Measurement of dawn π could show the over-night recovery of plants. Although the dawn π of Remont was significantly lower (more negative) than for other cultivars, this effect was not apparent at midday. It seems the over-night recovery of π varied between Remont and the other cultivars. Recovery between night and early morning is a drought tolerance mechanism.

Although measurements of midday Ψ in the climate room measured by pressure bomb and Wescor were similar ($R^2 = 0.90$), the interaction of soil moisture and cultivar was not similar for the two instruments. This may have been due to the duration of the measurement period. The pressure bomb measurements were undertaken during the entire climate room phase, whereas the Wescor measurements were only made in the last month of the climate room phase. Measurement of Ψ by pressure bomb was faster and easier than by Wescor. Since the Wescor required more time for each sample, difficulties occurred when a large number of measurements were needed. Measurements delayed by only 1-2 h may have been different to those at midday. As there was a good relationship between the results of the pressure bomb and the Wescor the pressure bomb results were preferred.

6.5.4 Stomatal resistance

The stomatal resistance of sainfoin leaf surfaces were different, with a higher R_s for the abaxial than the adaxial surface. For lucerne, the R_s of both sides of the leaf is equal (Carter and Sheaffer 1983b). Henson *et al* (1989b) found that the abaxial stomata of lupin closed more readily than those on the adaxial surface in response to both drought and exogenous ABA. Their results are in contrast with the results for sainfoin in this study which showed higher response of adaxial stomata than stomata of abaxial surface to water stress. Relative to control plants water stress increased R_s of abaxial and adaxial surfaces of the severely stressed plants 29 and 120% respectively. Koller and Thorne (1978) noted that in soybean (c.v. Amsoy 71) the R_s of the adaxial leaf surface increased to approximately four times the normal R_s within 48 hours after pod removal whereas there was a very small increase in abaxial leaf surface R_s . In sainfoin the adaxial leaf surface R_s of severely stressed plants increased 53% relative to controls whereas R_s of abaxial leaf surfaces increased only 19% relative to control plants (Fig. 6.3). The higher R_s of the abaxial surface in the sainfoin leaf could be due to differences in stomatal density, size, or cuticle thickness for each side of the leaf. These factors will be investigated in Chapter 8.

Water stress increased the R_s of moderately and severely stressed plants. Moderate water stress has been shown to induce stomatal closure in maize (Bennet *et al.* 1987), soybean (Finn and Brnn 1980), lucerne (Carter and Sheaffer 1983b), lupin (Henson *et al.* 1989b), Centrosema (Ludlow *et al.* 1983), wheat (Shimshi and Ephrat 1975), and sunflower (Snow and Tingey 1985). Mir-Hosseini-Dehabadi *et al.* (1993c) (Chapter 5) found that the adaxial R_s of Remont was about 2.0, 2.5, and 4 s/cm at VSWC of 10, 15, and 24% cm^3/cm^3 respectively which is in agreement with the results presented here. Lucerne had a higher R_s at the same VSWC compared to sainfoin (Mir-Hosseini-Dehabadi *et al.* 1993b) (Chapter 4). The R_s of lucerne at VSWC of 11, 15, 22% was 3.15, 1.51, and 1.59 s/cm for severely, moderately stressed, and control plants, respectively. The R_s of sainfoin for the same soil moisture treatments at the same VSWC was 4.7, 3.2, and 2.2 s/cm respectively. These results were in contrast to those of Mir-Hosseini-Dehabadi *et al.* (1993a) (Chapter 3) who found a greater for R_s of lucerne than for sainfoin under the same

conditions. These differences may have been caused by the different environmental conditions for both experiments. Sainfoin in the present experiment received a lower light intensity and relative humidity and a greater temperature than in the earlier experiment.

It is now well accepted that ABA produced in roots can increase R_s (Davies and Jeffcoat 1990, Khalil and Grace 1993). No relationship was found between R_s and the water status of the mildly water stressed plants (Bennett *et al.* 1987, Drubbe *et al.* 1978). Henson *et al.* (1989b) found that R_s was correlated with P, but not in lupin and concluded that R_s could be mediated by ABA. Other studies (Beanet *et al.* 1984, Baldocchi *et al.* 1985) suggested that stomatal resistance increased only after a threshold leaf water potential was attained (-2 MPa). Sainfoin in this study showed a significant increase R_s between the control and the moderately stressed plants as well as the severely stressed plants. The mean Ψ of the moderately stressed plants, measured by pressure bomb was -0.4 MPa, yet the R_s of the moderately stress plants was still greater than the R_s of the control plants. Therefore, the R_s of sainfoin leaves appears to be more sensitive to Ψ than leaves of soybean and maize.

6.5.5 Transpiration and stomatal resistance

Transpiration rate (ml/pot/day) increased over time for all three soil moisture treatments, except at the last measurement and there was an inverse relationship between T_r and R_s (Fig. 6.9 a). This was in agreement with the results of Shimshi and Ephant (1975), Turner (1974), Rosenthal *et al.* (1987) and O'Toole *et al.* (1984) who found a similar relationship between stomatal resistance and transpiration rate. A linear relationship was found between stomatal conductance and transpiration rate (Fig. 6.9 b).

Transpiration rate was linearly related to leaf area (Fig. 6.8b) Water stress controlled transpiration rate by decreasing leaf area and leaf number. Leaf extension has been affected by water deficits more than transpiration rate (Acevedo *et al.* 1971; Hsiao *et al.* 1976), however this was not found in this study, since severe stress decreased the leaf area of severely stressed plants 63% relative to the leaf area of control plants while decreasing the transpiration rate by 66% relative to the control plants.

6.5.6 Photosynthesis and WUE response to water stress

Photosynthetic rate was affected by soil moisture level. Reduced photosynthetic rate from water stress has been reported by many investigators (Johns 1973; Boyer and McPherson 1976; Begg and Turner 1976). Reduced photosynthetic rate due to water stress can be caused by stomatal and non-stomatal factors (Christiansen and Charles 1982). Begg and Turner (1976) pointed out that the initial reduction of photosynthesis is due to stomatal closure and reduced CO₂ uptake. The increased stomatal resistance of severely stressed plants relative to control plants, was 25% compared with a 25% reduction in P_n (Fig. 6.6), indicating the strong influence of stomatal closure on the reduction in photosynthesis by severely stressed plants.

Water use efficiency of sainfoin was increased by water deficit. This was in agreement with Singh and Kumair (1981), Aggarwal and Sinha (1983), and Misra and Chaudhary (1985), who found an increased WUE due to water stress. The increased WUE of the severely stressed plants was probably due to the increased stomatal resistance of the severely stressed plants. Sinclair *et al.* (1984) pointed out that stomatal sensitivity to water stress could be important for improving water use efficiency. Water use efficiency can be defined as $WUE = 1.6c \cdot p_a / (e_L - e)$ where $c = 1 - (p_i/p_a)$, p_i and p_a are internal and atmospheric partial pressure of CO₂, and e_L and e are the saturated vapour pressure at leaf temperature and vapour pressure of the atmosphere respectively (Sinclair *et al.* 1984). It is obvious from the above equation that stomatal closure will increase c and as a result increase WUE.

6.5.7 Cultivar response to water stress

Sainfoin cultivars showed different responses to water stress. The shoot-root ratios of Eski and Cotswold-Common were the highest and the lowest, respectively of the four sainfoin cultivars, indicating the greater rootiness of Eski compared to other cultivars. Shoot-root ratio of severely water stressed sainfoin was higher than that of control plants, as found by Mir-Hosseini-Dehabadi *et al.* (1993b) (Chapter 4) for water stressed lucerne. Both studies showed a reduction in absolute root mass and length by water stress. Water stress decreased fine root length but not thick root length. The greater root length of Eski relative to the other cultivars was due to its

greater fine root length (diam<3mm). The effect of water stress on the root mass and length of Cotswold-Common was more severe than for the other cultivars. The decrease in root dry weight was up to 60% for Cotswold-Common but only 39, 36, and 33% for Remont, Eski, and Fakir, respectively. The Remont control plants had a shorter root length compared to the other cultivars.

Although the yield of the sainfoin cultivars was significantly different at the final harvest, the cumulative yield of cultivars was similar. This is in agreement with results of Cooper (1972b) who found Eski had a lower yield than Remont at the first harvest but a greater yield at the second harvest, and a similar total yield for the season. Eski is representative of "one-cut" types and Remont representative of "two-cut" types of sainfoin.

The LA of the cultivars varied with Eski and Remont being the highest and lowest LA, respectively, although initially Remont LA was higher than the other cultivars (Appendix 6.16). Similarly, Krall *et al.* (1971) and Carleton and Delang (1972), found Remont began growth earlier, and matured earlier than Eski. Remont lost considerable leaf material during the experiment, from senescence, but the greater LA of Eski was not related to leaflet number, since leaflet number was not significantly different between the cultivars at the final measurement (Appendix 6.15).

6.6 Conclusion

Water stress decreased leaf area, leaflet number, root mass, and root length, yield, leaf water potential, turgor potential, osmotic potential, relative water content, transpiration rate, and photosynthetic rate, and specific leaf area but increased water use efficiency, and stomatal resistance of sainfoin. Transpiration rate was highly controlled by leaf area, and stomatal resistance of the adaxial leaf surface was lower than the abaxial surface.

Remont was more sensitive to water stress than Eski, Fakir, and Cotswold-Common. Between the four sainfoin cultivars, Eski, was more tolerant to water stress than the other three sainfoin cultivars through its greater rootiness and higher Ψ and P under water stress conditions.

Cumulative yield was similar for all cultivars, but Eski yielded more than others at the final harvest and Remont leaf production was initially faster than the other cultivars.

The results of the experiment indicate that the osmotic potential of sainfoin decreased under water stress conditions. This decrease in osmotic potential was caused by dehydration of leaves, or by solute accumulation in the leaf.

Chapter 7

Root and shoot responses of sainfoin and lucerne to water stress

7. Root and shoot responses of sainfoin and lucerne to water stress

7.1 Abstract

Plant factors affecting water availability include root size and distribution. We compared the responses of two sainfoin (*Onobrychis viciifolia Scop.*) cultivars Eski and Grasslands G35, with lucerne (*Medicago sativa L.*) cv. "Grasslands Oranga" in 1 m tall pots in the glasshouse at three harvests. Parallel methods of soil moisture measurement were compared which showed that TDR gave a good estimation of soil moisture. In addition, it was found that an even distribution of soil moisture through the soil profile could be achieved for water-limiting treatments by adjustment of soil moisture at seven depths, based on TDR measurements at three depths.

The physiological and morphological responses of lucerne and sainfoin to decreasing soil moisture, and to constant but limiting soil moisture, were investigated.

Lucerne and sainfoin tolerated water stress by solute accumulation and postponed water stress effects by growing roots to greater depths. Relative to lucerne sainfoin showed more rapid root growth to greater depth. Water stress increased the root mass and root density of the stressed treatments at deeper depths. Relative to the non-stressed treatment the herbage masses of lucerne, Eski, and Grassland G35 were reduced by up to 34%, 12%, and 14% respectively by water stress. Eski was affected less by water stress than Grasslands G35. Sainfoin had a higher root:shoot ratio than lucerne in both the water stressed and the control treatments. Shoot and soil water relationships of sainfoin and lucerne are discussed.

7.2. Introduction

Plant factors affecting water availability include root size and distribution. In addition to the uptake of water, roots absorb macro elements and micro elements for plant nutrition. Root development during drought stress is of particular significance to the plant. When soil moisture is insufficient, one approach is for the roots to osmotically adjust to help maintain turgor. Osmotic adjustment of roots has been observed, for example, in peas (*Pisum sativa* L.) (Greacen and Oh 1972) and corn (*Zea mays* L.) as soil moisture decreased and mechanical resistance of soil increased (Sharp and Davies 1979).

Plant tissue can maintain turgor under dry conditions by postponement of dehydration, or by tolerance of dehydration or both (Kramer 1983). The postponement of dehydration is accompanied by greater rooting depth and volume (Burch *et al.* 1978; Boyer *et al.* 1980; Kasper *et al.* 1984). Dehydration tolerance includes osmotic adjustment, or the accumulation of solute in response to water stress (Radin 1983).

Two critical areas with respect to water flow through the soil-plant-atmosphere continuum are the soil-root and the leaf-air interfaces. Relative to the information available about leaf adaptation to water stress, little is known about the adaptations by roots, because direct observation is difficult.

Sainfoin has a thick (up to 5cm diameter) tap-root which normally extends to a depth of 1 to 2 m but it can be to 10 m (Piper 1924; Whyte *et al.* 1953; Andreev 1963; Spedding and Diekmahns 1972). The roots of sainfoin may penetrate to a greater depth than those of lucerne in open, dry subsoils (Percival 1943). The sainfoin root system has few main branches and numerous fine laterals (Spedding and Diekmahns 1972) (Plate 7.1). Sainfoin is reported to have twice as many lateral roots as lucerne (Kozyr 1948; Kalugin 1950; Massaudilov 1958).



Plate 7.1: A view of a single sainfoin plant grown in the pot (1.5m tall).

Studies on the effect of water stress on roots are often limited by the difficulty in maintaining specific soil water content throughout an entire soil volume during plant growth. Numerous experiments have been done where plants were grown in soil maintained at arbitrary soil moisture contents such as 10%, 20%, or 30% of the original soil dry weight, or at a percentage of the capillary or field capacity (Kramer 1980). Shantz (1925) and Kramer (1980) have pointed out the difficulty of achieving this goal because of physical forces acting on soil water. They pointed out the addition of a small quantity of water to a mass of dry soil only wets the soil to a given depth. The remainder of the soil mass remains un-wetted. A container filled with dry soil having a field capacity of 30% and enough water added to the surface to wet the whole mass to 15% will only have the upper half wetted to pot capacity, whereas the lower half will remain relatively dry.

The TDR has shown a good ability to measure the volumetric soil water content (Topp and Davis 1985, Dalton and Van Genuchten 1986). Blum *et al.* (1991) have used TDR to measure the volumetric soil water content of the pots and this approach appears to have potential for monitoring soil moisture throughout the pot.

S/R ratio is widely used to describe carbon allocation to the above and below ground plant parts. Kummerow (1980) mentioned two problems with using this ratio. First, the difficulty of complete extraction of the whole root system from the soil causes underestimation of the true value of S/R, and, second, the considerable seasonal and stage of development fluctuations in root and shoot biomass. In this experiment the first problem was minimized by using a long pot to simulate field conditions, and the second by harvesting at three different growth stages.

In the first experiment (Chapter 3) Grasslands G35 sainfoin showed greater root mass than the other sainfoin cultivars (Fakir and Melrose) and lucerne. In a climate room experiment (Chapter 6) Eski showed greater root length than the other three sainfoin cultivars (Fakir, Remont, and Cotswold-Common). In this study the sainfoin cultivars Eski and Grasslands G35, and lucerne were used to compare root responses at different soil moisture levels and stages of growth.

The objectives of this experiment were a) to design a system that maintained

a specific soil moisture through the whole soil mass in a 1 m tall container (b) to investigate the influence of water stress on the root development and distribution of sainfoin and lucerne, and (c) to investigate the osmotic adjustment of the roots and leaves of sainfoin and lucerne during water stress.

7.2. Materials and Methods

7.2.1 Plant materials and culture

Seed of two sainfoin cultivars (Eski and 'Grasslands G35') and lucerne ('Grasslands Oranga') were germinated in water agar on 10 March 1992 and three seedlings were transferred to each pot in the glasshouse on 15 March. Growth conditions were 16 h daylength, day/night temperature 25/15 °C, and average relative humidity 63%. The pot media comprised 50% soil and 50% river sand. The soil was a Haplquept (fine, loamy, mixed, mesic). The media was amended with 300 g dolomite, 50 g iron sulphate, 100 g isobutylidene-diurea, and 500 g "Osmocote plus" 16-4-11 (N-P-K), per 100 l of media (Bunt 1988) to minimize the effect of water stress on nutrient availability. Plants were fertilized with nitrogen (N) rather than applying rhizobium to stimulate nodulation, in order to minimise variability due to the effect of water stress on N fixation (Sprent 1972).

7.2.2 Plant growth container

Pots of 0.0176 m³ capacity were constructed of 1050 mm long and 150 mm diameter polyvinyl chloride pipe (PVC). To minimize temperature effects on root growth, twin-wall white PVC with air between the walls was used (Fig. 7.1). A 20 mm thick circle of wood was fitted inside the base of the pot. Six 30 mm long screws were used to attach the wood 10 mm from the bottom of the pot. Six 10 mm diameter holes, 45° angle to the ground, were drilled through the wood to allow drainage of water. Coarse sand (average 5 mm diam.) was placed to 10 mm depth on the wood to enhance drainage. Control pots were placed on a 50 mm high and 200 mm diameter plastic plate to enable irrigation from the bottom as well as the top of the pot. Pairs of 5 mm diameter holes were drilled in the side of the pot at 200, 500, and 850 mm from the top (Fig. 7.1). These holes were used to insert horizontal Time Domain Reflectometry (TDR) stainless steel probes (150 mm long) to measure the soil moisture at these depths (Topp *et al.* 1984). In addition, two 700 mm probes were placed vertically in the middle of each pipe to measure the average volumetric soil water content (Fig. 7.1).

Pots were filled with 27 kg of dry media. An internal tube (100 mm diam., and 1m) was used for filling the pots. Media were compacted at each 100 mm to ensure homogeneity of soil density through depth. The soil bulk density was approximately 1.5 g/cm^3 . A 20 mm thick layer of plastic chips was placed on the soil surface to prevent evaporation (Plate 7.2 a). The pots were designed to be opened length-wise to enable root and soil samples to be taken for root water status measurements (Plate 7.2 b).

7.2.3 Design and treatments

A randomised complete block design of four replicates was used with each block comprising a factorial arrangement of two moisture levels, three cultivars and three harvests, giving a total of 72 experimental units.

Harvests of both roots and shoots were at monthly intervals, with the first (early) harvest on 27 April 1992, one month after the imposition of the moisture stress. The second (late) harvest was on 27 May and at the same time the remaining plants were cut to 20 mm high. The regrowth of these plants was harvested one month later on 27 June in the third (regrowth) harvest.

Two soil moisture levels were imposed. The control treatment was maintained at pot capacity and the stressed treatment at half of pot capacity. Pot capacity was determined by watering until drainage occurred from the bottom of a pot. After gravitational water drained from the pots (approximately 24 h) the soil moisture content was considered to be pot capacity. The average volumetric soil water content (VSWC), gravimetric soil water content (GSWC) and pot water content at pot capacity were 24.6% (cm^3/cm^3), 16.54% (g/g), and approximately 4.9 kg/pot respectively.

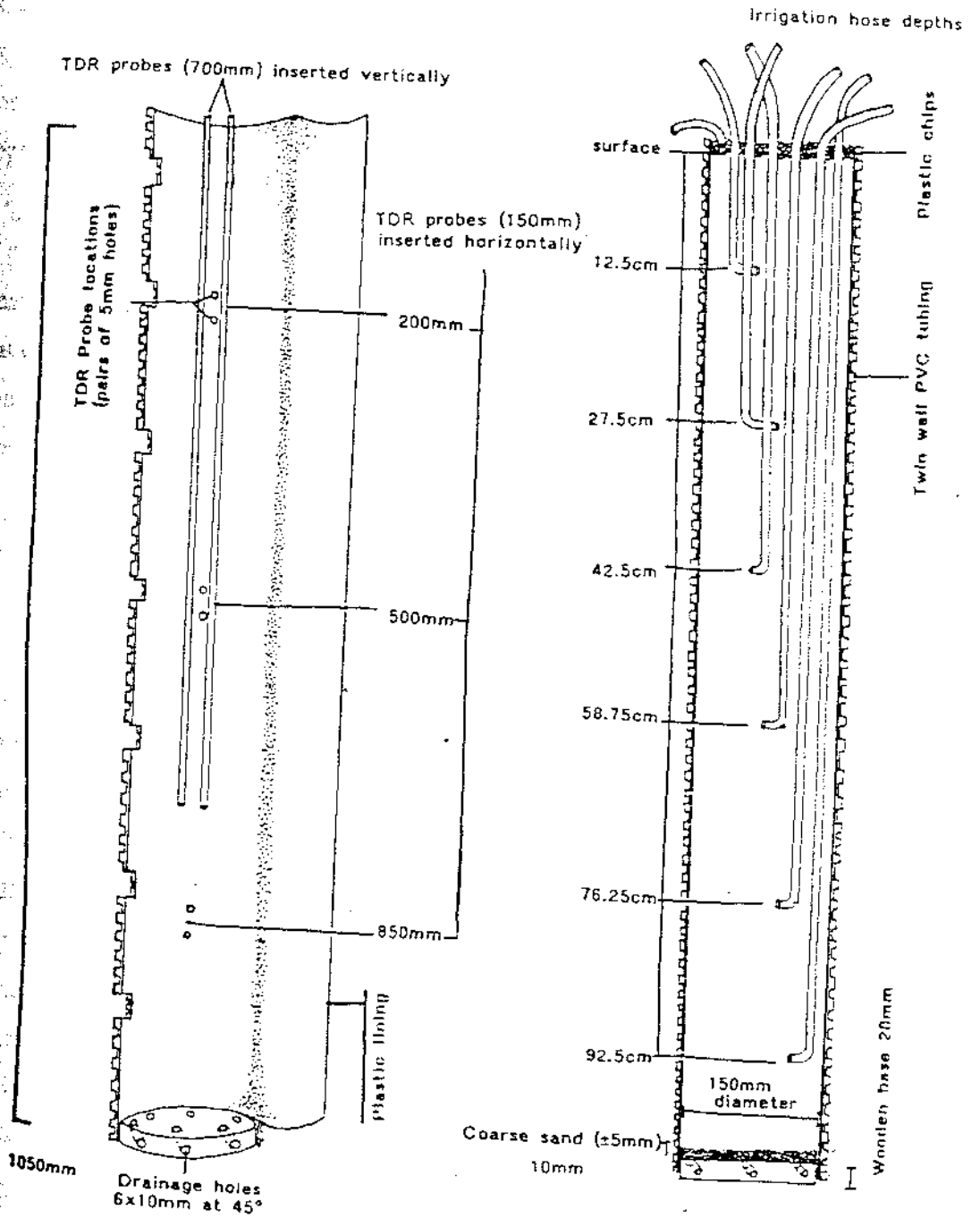


Fig 7.1: Plant growth container (Drawn by Cally McKenzie)

7.2.4 Soil Moisture.

Soil moisture was determined by measuring VSWC (cm^3/cm^3) and GSWC (g/g) and weighing the pots. A Time Domain Reflectometry (Soil moisture Equipment Corp-Trase 1, TDR) system connected to the horizontal permanently-positioned stainless steel probes was used to measure VSWC daily, at different depths (Plate 7.4 a). At the early harvest, GSWC¹ was measured at probe positions at each depth on soil samples of 6 cm diameter and 9 cm length. The average VSWC of pots was determined daily by TDR using the two permanent 700 mm long probes located vertically in the centre of each pot. The total soil water content of each pot was determined following imposition of the soil moisture treatment (27 March 1992), using the following formula: Soil moisture= pot weight- (tube weight + plastic chips + probe weight + dry soil).

Irrigation of the control treatment was by a fully automatic irrigation system from the top and bottom of the pots, to keep the soil moisture at pot capacity. Irrigation of the stressed treatment was done at 0, 35, and 670 mm depths for the first two harvests but at 0, 125, 275, 425, 587.5, 762.5, and 925 mm depths for the third harvest (Plate 7.3). Water was applied at each depth through a 3 mm diameter hose (Plate 7.3). The end of each hose was wrapped in a piece of felt and located in the centre of the pot to ensure the gradual absorption of water and homogenous distribution of water in the soil mass. The stressed pots were irrigated in the evening to allow homogenous distribution of moisture through the soil profile while transpiration was at a minimum. The amount of water for each depth was calculated by the difference between the required moisture level (VSWC= 14%) and the respective TDR reading for that depth (Appendix 1). The average amount of water used in the stressed pots was 231 ml per day per pot.

¹ Soil samples were placed in aluminum cans (Weight=C) and immediately weighed (A), heated at 105 °C for 24 h, re-weighed (B), and GSWC was calculated by:
GSWC= ((A-B)/(B-C))*100.

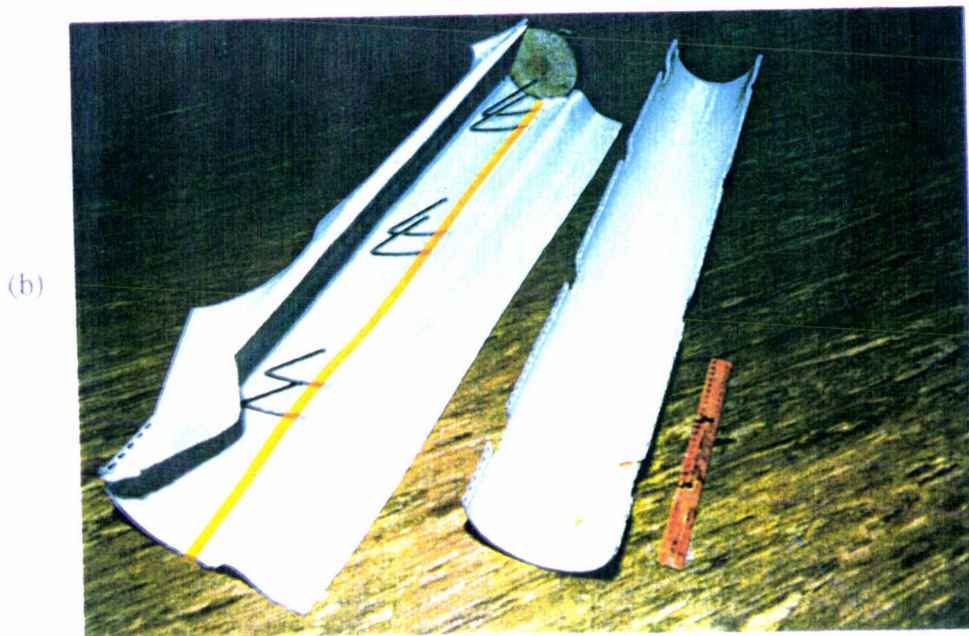


Plate 7.2: (a) Plastic chips used for minimising evaporation, (b) Construction of the plant growth container.



Plate 7.3 Watering by hoses at seven depths (left), and location of hoses (right).

7.2.5 Morphological measurements

At each harvest leaves were separated from stems, and their area measured by planimeter (Li-Cor model 3100). Roots were harvested from seven separate segments of 0-100, 100-200, 200-300, 300-400, 400-600, 600-800, and 800-1000 mm (Plate 7.4 a). Roots were washed free of soil using a 2 mm screen. Root length for each segment was measured using a Comair root length scanner (Voorhees *et al.* 1980). Leaf, stem, and root dry matter were measured after 24 h drying at 80°C.

7.2.6 Physiological measurements

The physiological characters of roots and leaves were measured before each harvest. The leaf water potential (Ψ) was measured at dawn and midday by pressure bomb (Plant Water Consol, Model 3005) and thermocouple psychrometer (Wescor) simultaneously. Osmotic potential (π) of the leaf was determined at dawn and midday using the Wescor as described in Chapter 6. To measure the osmotic potential at full turgor, leaf samples (6-8 leaflets) were taken at dawn and midday and soaked in distilled water (4 °C, dark, 12h), then after removing water from the leaf surface, samples were frozen using liquid air and measured by thermocouple psychrometer (Decagon Model SC-10). Turgor potential (P) was calculated as the difference between Ψ and π for both leaves and roots and was not adjusted for possible dilution by apoplastic water. Root-tip samples were taken at two depths (100-300 mm and 500-700 mm) from each pot in the afternoon and the total and osmotic potentials were determined as for leaves. Plant transpiration rate (ml/day/pot) was measured at the late harvest by weighing the pots for five consecutive nights before harvest, using a balance with accuracy of 50 g. Transpiration rate was considered as the difference between pot weight after irrigation and pot weight of the previous night before irrigation. Because of the 20 mm plastic chips on the soil surface the evaporation rate was ignored (Plate 7.2a).

Stomatal resistance (R_s) of adaxial and abaxial leaf surfaces was measured by porometer, MK3 as described in Chapter 6. Total stomatal resistance of the leaf was calculated as the ratio of $\{(\text{adaxial } R_s * \text{abaxial } R_s)/(\text{adaxial } R_s + \text{abaxial } R_s)\}$ (Monteith 1973).

(a)



(b)

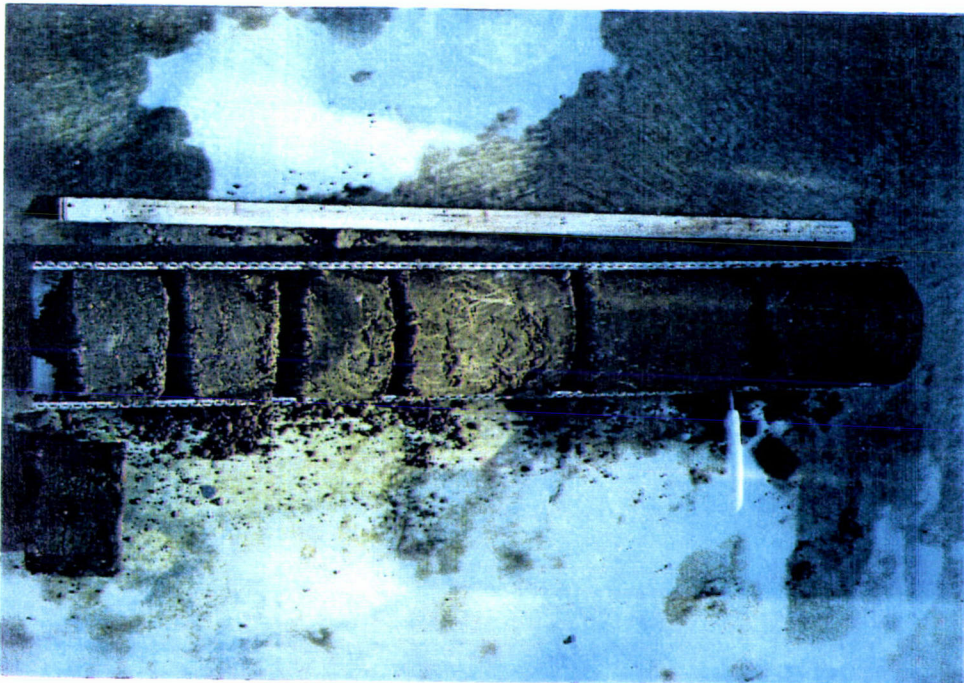


Plate 7.4 (a): Measurement of soil moisture using TDR, (b) Segments of soil for measurement of root length and root mass at seven depths.

7.2.7 Statistical analysis

7.2.7.1 Analysis of morphological measurements

Analysis of variance was undertaken for leaf area, leaf DW, stem and petiole DW, LA, and total root length and root density using the General Linear Model (GLM) procedure of SAS (SAS Institute Inc. 1991). In addition, because of the effect of regrowth on yield components, only the data of early (harvest 1) and late (harvest 2) harvests were pooled. Repeated measures analysis was used for root length and weight density at the seven depths (Rowell and Walters, 1976, Gill 1986)

7.2.7.2 Analysis of physiological measurements

Analysis of variance was used for RWC, R_s of whole leaf, R_s of abaxial and adaxial leaf surfaces, for each harvests, and for Tr at only late harvest. Also, the data for RWC, and R_s for the whole leaf and each leaf surfaces were pooled over three harvests using the General Linear Model (GLM) procedure of SAS (SAS Institute Inc. 1991).

The effects of time of measurement (dawn vs midday) on Ψ , π , P , and π_{100} of the leaf were examined with repeated measures analysis (Rowell and Walters 1976) for each harvest, and pooled over three harvests using the General Linear Model (GLM) procedure of SAS (SAS Institute Inc. 1991).

To compare root Ψ , π , P , and π_{100} at different depths, a split-plot design considering the depth as a sub-plots and pots as a main-plots was used, for each harvests and pooled over the three harvests (Dr. I. Gordon pers comm.)

Root and leaf Ψ , π , P and π_{100} were compared using a split plot design, with root and leaf water status as sub-plot and pots as a main plots for each harvest and pooled over three harvests (Dr. I. Gordon pers comm.).

7.2.7.3 Analysis of soil moisture

Analysis of variance for each harvest and pooled over the three harvests was done for soil moisture measured by the 700 mm probe. Since the soil moisture at each depth was influenced by the VSWC of the next depth repeated measures analysis was used to study the effect of VSWC through the soil depths for each harvest, and pooled over the three harvests.

The VSWC measured by TDR at three depths was regressed against the GSWC at the same depths using Quattro-Pro. Also the regressions between VSWC from the 700 mm probes and average VSWC from the three 150 mm probes, and between soil moisture content using pot weight and soil moisture calculated by VSWC measured at the three depths, by TDR were done using Quattro-Pro.

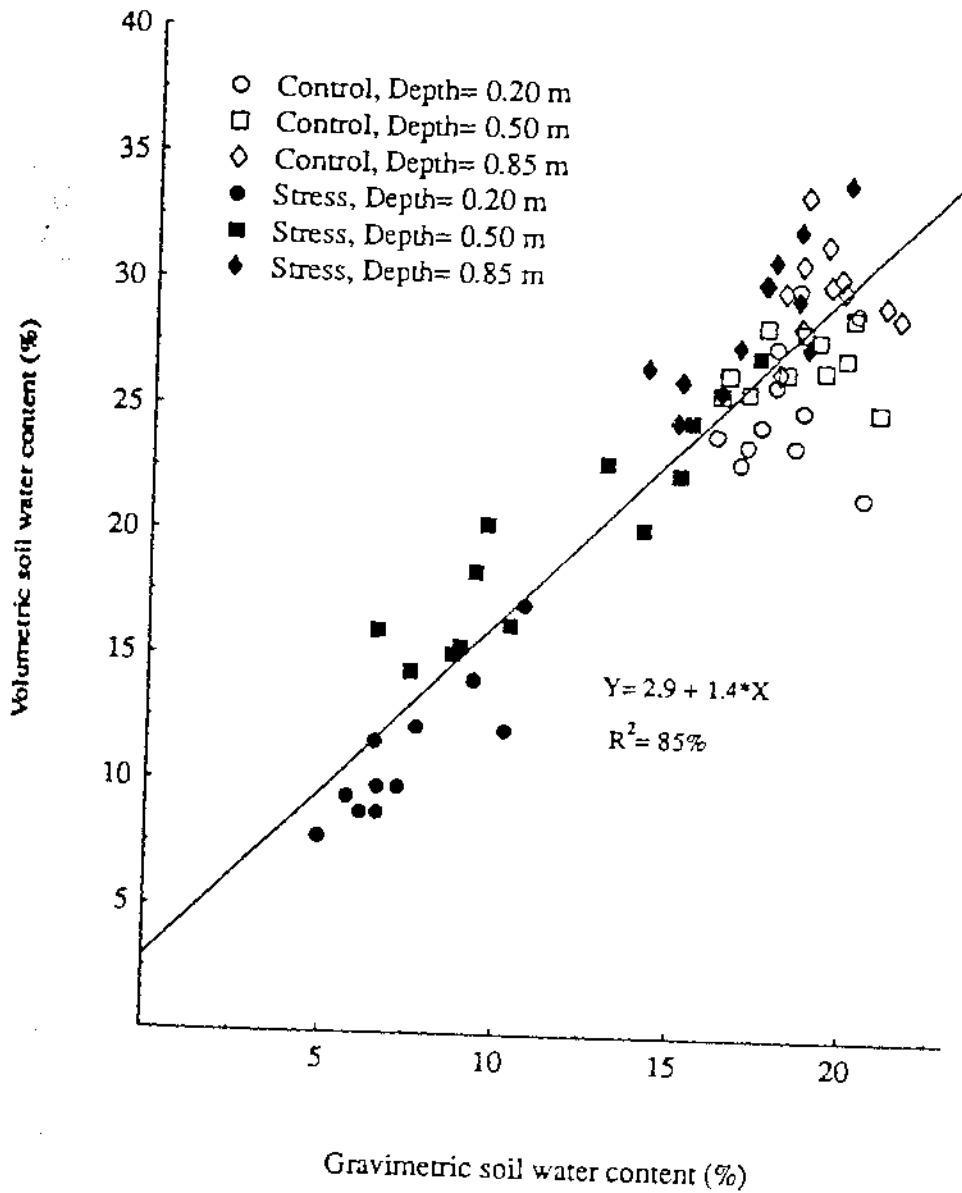


Fig. 7.2: Relationship between gravimetric soil water content determined from sampling and volumetric soil water content determined by TDR.

7.3. Results

7.3.1 Soil moisture

The VSWC measured by TDR at three depths was closely related to GSWC (Fig. 7.2). Analysis of VSWC at three depths over all three harvests showed significant effects for soil moisture ($P < 0.0001$), harvest ($P < 0.0001$), and their interaction ($P < 0.0001$). Neither the cultivar effect nor any other interactions were significant (Fig 7.3).

Over all three harvests, the VSWC of the control treatment (26%) was significantly higher than the VSWC of the stressed treatment (14.1%). Early harvest VSWC (23.6%) was higher than that of the late (17.5%), and regrowth (19.0%) harvests. The VSWC of the stressed treatment was higher at the early harvest (VSWC= 19.7%), than at the late (10.9%), and regrowth (11.6%) harvests (Fig. 7.3).

Repeated measures analysis of the data for VSWC over harvests at three depths showed significant effects for depth ($P < 0.0001$), and the interaction of depth and harvest ($P < 0.0001$), depth and soil moisture ($P < 0.05$), and depth, soil moisture and harvest ($P < 0.0001$) (Appendix 7.3). All other interactions with depth were not significant.

The VSWC at 850 mm depth (23.3%) was higher than at 0.5 m (20.1%), or 0.2 m (16.8%) depth. At the early harvest the differences between VSWC at 85 cm depth and 0.5 m depth (6.0%) were greater than the late (1.9%), or regrowth harvests (1.6%) (Fig. 7.3). The VSWC of both the control and stressed treatments increased with depth (Fig. 7.3).

The interaction of soil moisture by harvest by depth (Fig 7.3) found the control treatment VSWC increased with depth at all three harvests but the slope was different for the late harvest than for the early and regrowth harvests. The change in stress treatment VSWC with depth was greater for the early harvest than for the other two harvests. The interaction of soil moisture by depth was significant for the early and late harvests, but not for the regrowth harvest (Fig. 7.3).

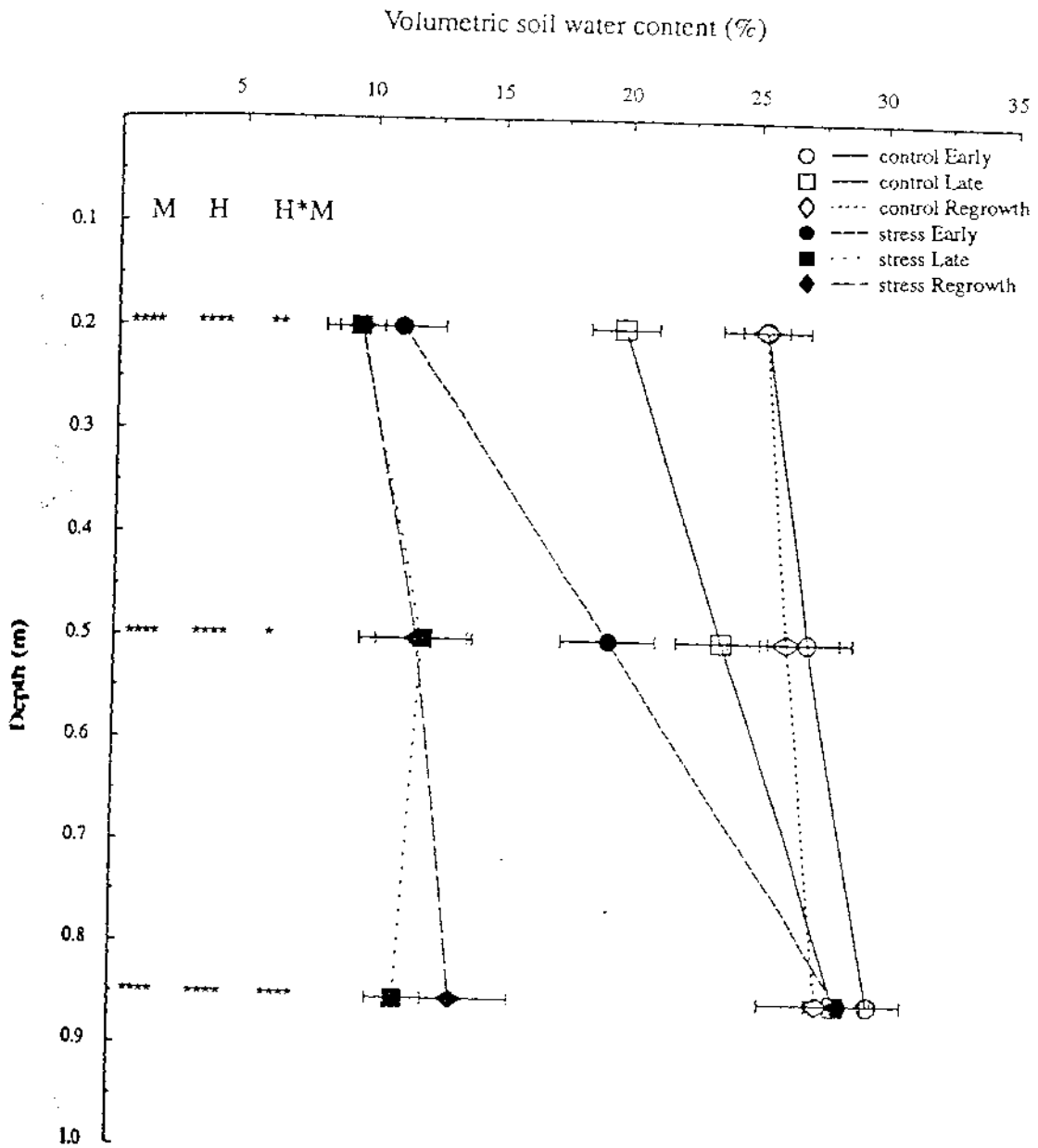


Fig. 7.3 Volumetric soil water content (cm^3/cm^3) of two moisture treatments (M) (control and stressed) at 0.2, 0.5, and 0.85 m depths for three harvests (H) (early, late, and regrowth harvests), and *, **, and *** show significance at the 0.05, 0.01, and 0.0001 levels, respectively.

7.3.1.1 Average soil moisture (0-0.7 m depth)

The VSWC at 0-0.7 m depth measured with a pair of 0-0.7 m vertical probes per pot over all three harvests showed significant effects for soil moisture ($P < 0.001$), harvest ($P < 0.01$), cultivar ($P < 0.05$), and the interaction of harvest by soil moisture ($P < 0.01$), no other interactions were significant (Appendix 7.2). The control treatment had a higher VSWC at 0-0.7 m than the stressed treatment (28.4 vs 15.9%).

The early harvest had a higher VSWC (24.8%) than the late (21%) and regrowth harvests (21.1%). Over three harvests lucerne had a lower VSWC (20.62%) at 0-0.7 m depth than Eski (23.9%) and G35 (22.5%).

Separate analysis of VSWC at each harvest showed a significant difference between the cultivars at only the regrowth harvest with lucerne having lower VSWC (18.5%) than Eski (23.3%) and G35 (21.6%). The difference between the control and the stress treatments at the regrowth harvest (5.9%) was higher than for late (12.3%) and early harvests (9.3%).

The VSWC at 0-0.7 m depth was compared with VSWC measured at different depths (by horizontal probes at 0.2, 0.5, and 0.85 m depth). The following formula was used to integrate VSWC :

$$[(VSWC_{0.2} * 35) + (VSWC_{0.5} * 30) + (VSWC_{0.85} * 5)] / 70$$

where VSWC_{0.2}, 0.5, and 0.85, were the VSWC measured by 0.15 m probes horizontally located at 0.2, 0.5, and 0.85 m depths, respectively. The regression equation between these two measurements was $Y = -1.1 + 0.89X$, $R^2 = 85\%$ where $Y =$ VSWC measured at 0.2, 0.5, and 0.85 m depth, and X was VSWC measured by 0.7 m probes in the middle of the pots.

7.3.1.2 Water content of the pots measured by weighing

Pot water content measured by weighing found a significant difference ($P < 0.01$) between moisture content of the control (4.96 ± 0.08 kg) and stressed pots (2.97 ± 0.08 kg). Cultivars received similar soil moisture, and there were significant

effects for harvest and interactions ($P < 0.05$). The soil moisture content of the pots measured by weighting was compared with soil moisture content calculated by VSWC measured at three different depths² and a positive relationship was found. The regression line between the measurements was $Y = 1.06 + 0.92X$ $R^2 = 82\%$, where Y was soil moisture content of the pot calculated by VSWC at 0.2, 0.5, and 0.85 m depths, and X was pot moisture content measured by weighing.

To calculate the soil moisture content using VSWC at 0.2, 0.5, and 0.85 m depths the following formula was used: $\{[(VSWC_{0.2} \cdot 35) + (VSWC_{0.5} \cdot 32.5) + (VSWC_{0.85} \cdot 32.5)] / 1000\} \cdot V$, where V was pot volume (lit).

7.3.2 Morphological measurements

7.3.2.1 Leaf area

At the late (harvest 2) and the regrowth (harvest 3) harvests cultivar ($P < 0.01$) and soil moisture ($P < 0.001$) treatments and their interaction ($P < 0.01$) were significantly different (Table 7.1). However, at the early harvest (harvest 1) no treatment effects were significantly different. The LA of lucerne was higher than for the sainfoin cultivars, and the control plants had higher LA than the stressed plants at both these harvests. At the late harvest water stress decreased the LA of lucerne by 1455 cm^2 relative to the control plants, whereas this difference was 245 and 79 cm^2 for Eski and G35, respectively (Table 7.1). The reduction in the LA of the stressed plants at the regrowth harvest for lucerne, Eski, and G35 was 68, 41, and 37%, respectively (Table 7.1).

Pooled analysis of data over early and late harvests showed significant differences in LA for cultivar ($P < 0.001$), soil moisture level ($P < 0.01$), harvest ($P < 0.001$), and interactions of harvest by soil moisture ($P < 0.05$), harvest by cultivar, and harvest by soil moisture by cultivar ($P < 0.05$) (Table 7.1). The control plants had a higher LA than stressed plants ($1435 \text{ vs } 1096 \pm 85 \text{ cm}^2$) over the early and late harvests. Lucerne had a greater LA than the sainfoin cultivars ($1623 \text{ vs } 1185 \text{ and } 986 \pm 104 \text{ cm}^2$), and the late harvest showed a higher LA than the early harvest ($1930 \text{ vs } 600 \pm 85$). The effect of water stress on lucerne at the late harvest was greater than the sainfoin cultivars. The reduction in LA due to water stress at the late harvest was 1455, 245, and 79 cm^2 for lucerne, Eski, and G35, respectively.

7.3.2.2 Leaf dry weight (LDW)

At the late and regrowth harvests LDW of the cultivars was significantly different, with lucerne having a higher LDW than the sainfoin cultivars (Table 7.2). However, LDW was not affected by water stress or cultivar at the early harvest. Soil moisture treatment did not affect LDW of the late harvest, but was significantly affected at the regrowth harvest. The interaction of cultivar by soil moisture was significantly different at the late and regrowth harvests (Table 7.2).

Pooled data analysis of LDW from the early and late harvests showed a significant effect on LDW for harvest, cultivar, and the interactions of harvest by cultivar, and soil moisture by cultivar by harvest. The LDW at the late harvest was approximately three times greater than at the early harvest. At the early harvest the LDW of lucerne and sainfoin cultivars were similar but at the late harvest lucerne had a higher LDW than the sainfoin cultivars and Eski had a higher LDW than G35. At the late harvest water stress had a relatively greater effect on the LDW of lucerne than on the LDW of the sainfoin cultivars (Table 7.2).

Table 7.1: Leaf area (cm²/pot) of Eski and Grasslands G35 sainfoin, and Grasslands Oranga lucerne, at the early harvest (45 days after planting (DAP)) late harvest (75 DAP), and regrowth harvest (30 days regrowth, 105 DAP) at two soil moisture levels.

Treatment	Early harvest (45 DAP)	Late harvest (75 DAP)	Regrowth (30 days)	Total ¹
		Control		
Eski	656	1974	830	2652
G35	644	1455	995	2753
Lucerne	641	3278	2737	6353
		Stress		
Eski	436	1703	509	1812
G35	484	1377	582	1726
Lucerne	754	1822	891	2944
Pr>F Cu ²	0.4500	0.0001	0.0001	0.0001
Pr>F W	0.4300	0.0018	0.0001	0.0001
Pr>F Cu*W	0.4200	0.0052	0.0020	0.0105
SEM Cu	91.4	111.1	137.3	286.1
SEM W	74.6	136.1	112.1	233.5
SEM Cu*W	129.4	192.4	194.13	404.6

¹ Total= total leaf area of regrowth treatment only.

² Cu= cultivar, W= soil moisture levels, and Cu*W= interaction of cultivar by soil moisture levels.

Pooled ANOVA of early and late harvest showed: Pr>F harvest= 0.0001, SEM harvest= 84.8, Pr>F W= 0.0079 and SEM W=84.8, Pr>F Cu=0.0005 and SEM= 103.9, Pr>F Harvest*W=0.042 and SEM Harvest*W= 120, Pr>F Harvest*Cu= 0.007 and SEM Harvest*Cu= 147, Pr>F Harvest*Cu*W= 0.014 and SEM Harvest*Cu*W= 104

Table 7.2: Leaf dry weight (g/pot) of Eski and Grasslands G35 sainfoin and Grasslands Oranga lucerne at early harvest (45 days after planting, DAP) late harvest (75 DAP) and regrowth harvest (30 days regrowth and 105 DAP) at two soil moisture levels.

Treatment	Early Harvest (45 DAP)	Late Harvest (75 DAP)	Regrowth (30 days)	Total ¹
Eski	3.22	Control 8.23	4.73	12.85
G35	3.60	6.33	5.29	13.81
Lucerne	2.53	12.05	11.28	25.79
Eski	2.7	Stress 10.14	3.44	9.88
G35	3.17	7.64	3.60	9.23
Lucerne	3.25	9.53	4.38	15.37
Pr>F Cu ²	0.6504	0.0021	0.0007	0.0059
Pr>F W	0.8765	0.7458	0.0002	0.0175
Pr>F Cu*W	0.4973	0.0470	0.0068	0.386
SEM Cu	0.41	0.61	0.59	1.94
SEM W	0.38	0.50	0.48	1.59
SEM Cu*W	0.57	0.87	0.83	2.74

¹ Total= total dry weight of regrowth treatment only.

² Cu= cultivar, W= soil moisture levels, Cu*W= interaction of soil moisture by cultivars H= harvest.

Pooled ANOVA of early and late harvests showed: Pr>F H= 0.0001 SEM harvest= 0.311, Pr>F W=0.86 SEM W=0.31, Pr>F Cu=0.016 SEM Cu= 0.38, Pr>F H*W= 0.7 SEM H*W= 0.4, Pr>F H*Cu= 0.0014 SEM H*Cu= 0.54, Pr>F H*W*Cu= 0.86 SEM H*Cu*W= 1.21

7.3.2.3 Stem dry weight (SDW)

Water stress only affected the SDW of the plant at the late harvest ($P < 0.05$) (Table 7.3). Cultivar SDW was only significantly different at the regrowth harvest ($P < 0.05$). The interaction of soil moisture and cultivar was not significantly different for any harvests. At the regrowth harvest, lucerne had a higher SDW (6.1 g/pot) than Eski (2.9 g/pot) and G35 (3.0 g/pot).

Pooled analysis of SDW of the early and late harvests showed the only significant effects on SDW were harvest and soil moisture levels (Table 7.3). The SDW of the plants at the late harvest was approximately twice the SDW of the plants at the early harvest, and stressed plants had a lower SDW than control plants.

7.3.2.4 Specific leaf area (cm^2/g)

Water stress affected the SLA of the plants over all three harvests ($P < 0.01$), and cultivars had significantly different SLA ($P < 0.01$) (Table 7.4). There were significant interactions over all harvests. The SLA of the control treatment (220 ± 3.2) was higher than for stressed plants (181.5 ± 3.2), and lucerne SLA (236) was higher than that of Eski (185), and G35 (182).

Separate analysis of each harvest showed a significant effect of water stress at only the late harvest ($P < 0.001$), and cultivar SLA was significantly different only at the early harvest ($P < 0.01$) (Table 7.4). At the late harvest SLA of control plants (245 ± 9.63) was higher than for stressed plants (184.5 ± 9.63). At the early harvest lucerne had the highest SLA (235.06 ± 11.8) (Table 7.4).

7.3.2.5 Shoot:Root ratio

The effects of soil moisture ($P < 0.01$), cultivar ($P < 0.05$), and harvest ($P < 0.01$) were significant for S/R ratio over all three harvests. The S/R ratio of the control plants (4.54 ± 0.17) was higher than for the stressed plants (2.7 ± 0.17).

The lucerne S/R ratio (4.46 ± 0.21) was higher than for Eski (3.19 ± 0.21). The shoot:root ratios of the plants at the early and the late harvests were the highest (4.48 ± 0.22) and the lowest (2.72 ± 0.22), respectively.

Separate ANOVA for each harvest showed a significant effect of soil moisture ($P < 0.01$) at each harvest. Cultivars had significantly different S/R ratio at the early and regrowth harvests (Table 7.4). At all three harvests the S/R ratio of the control plants was higher than for stressed plants and lucerne had a higher S/R ratio than the sainfoin cultivars at the early and regrowth harvests (Table 7.4).

Table 7.3: Stem and petiole dry matter (g/pot) of Eski, and Grasslands G35 sainfoin and Grasslands Oranga lucerne at the early harvest (45 days after planting), late harvest (75 DAP) and the regrowth harvest (30 days regrowth, 105 DAP) at two soil moisture levels.

Treatment	Early (45 DAP)	Late (75 DAP)	Regrowth (30 days)	Total ¹
ESKI	3.74	Control 12.24	3.45	11.10
G35	5.22	10.84	4.62	11.69
LUCERNE	3.57	14.64	7.62	20.36
ESKI	2.16	Stress 10.37	2.29	9.77
G35	3.21	8.38	3.01	8.94
LUCERNE	3.78	8.74	4.57	14.96
Pr>F Cu ²	0.1943	0.3751	0.0038	0.0455
Pr>F W	0.0568	0.0153	0.2683	0.0011
Pr>F Cu*W	0.2418	0.3839	0.7101	0.5218
SEM Cu	0.5	1.1	0.4	1.3
SEM W	0.4	0.9	0.5	1.0
SEM Cu*W	0.6	1.5	0.6	1.8

¹ Total= total stem dry matter of regrowth treatment only.

² Cu= cultivar, W= soil moisture levels, Cu*W= interactions of soil moisture by cultivars.

Pooled ANOVA of early and late harvest showed: Pr>F Harvest= 0.0001 and SEM Harvest= 0.5, Pr>F W=0.0024 and SEM W=0.5, Pr>F Cu= 0.64 and SEM Cu= 0.61, Pr>F Harvest*W= 0.111 and SEM Harvest*W= 0.7, Pr>F Harvest*Cu= 0.18 and SEM Harvest*Cu= 0.86, Pr>F Harvest*Cu*W= 0.17 and SEM= 1.2

Table 1.4: The specific leaf area (cm²/g) and shoot:root ratio of two subgen cultivars and lucerne at the early harvest (45 days after planting, 45 DAP), late harvest (75 DAP), and the regrowth harvest (30 days, 105 DAP) at two soil moisture levels.

	Specific leaf area				Shoot:root ratio		
	Early harvest	Late harvest	Regrowth harvest	Early harvest	Late harvest	Regrowth harvest	
		Control			Control		
Eski	199.4	238.7	176.3	4.06	3.45	4.41	
G35	179.0	226.6	186.9	5.09	3.19	3.34	
Lucerne	248.5	272.2	255.9	6.92	3.88	6.53	
		Stress			Stress		
Eski	170.4	169.3	158.2	3.24	1.92	2.09	
G35	157.1	181.7	159.8	3.06	2.10	2.48	
Lucerne	230.5	202.5	204.3	4.46	1.79	3.19	
Pr> F W	0.1110	0.0004	0.2712	0.0010	0.0005	0.0001	
Pr> F Cu	0.0017	0.1031	0.1690	0.0039	0.9036	0.0033	
Pr> F W*Cu	0.9449	0.6989	0.8834	0.3096	0.5263	0.0755	
SEM W	9.593	9.625	19.97	0.307	0.251	0.290	
SEM Cu	11.75	11.79	24.47	0.376	0.307	0.356	
SEM W*Cu	16.62	16.67	34.61	0.532	0.435	0.503	

¹ Data are mean of four replicates.

² W= soil moisture levels, Cu= cultivar, W*Cu= interaction of soil moisture and cultivars.

7.3.2.6 Root length density

At the early harvest, cultivars had a similar root length density at each of the seven measurement depths. Root length was affected by soil moisture level at six of the seven depths (Appendix 7.4). Control plants had a greater root length density than stressed plants at 0-30 cm depth, while the stressed plants of the early harvest had a greater root length density at 40-100 cm (Fig. 7.4). The only significant interaction of cultivar by soil moisture was at 60-80 cm depth. At 60-100 cm depth the ratio of stressed to control root length was higher for sainfoin cultivars than lucerne (Appendix 7.4). The total root length at the early harvest was not significantly affected by cultivar or soil moisture level. Nevertheless, the control plants had a greater root length than the stressed plants ($P < 0.1$) (Table 7.5).

At the late harvest, the root length of the cultivars was significantly different at only 30-40 cm depth with root length of lucerne being almost twice the root length of sainfoin (Appendix 7.5). Soil moisture had significant effects on root length at the seven depths. Control plants had approximately twice the root length of stressed plants at 0-30 cm depth, but stressed plants had higher root length than control plants at 40-100 cm depth. At 30-40 cm depth the reduction of lucerne root length of the control plants relative to stressed plants was significant but that of the sainfoin cultivars was similar (Appendix 7.5).

Total root length at the late harvest was significantly different between cultivars, with lucerne higher than sainfoin. There was no significant effect for interaction of soil moisture levels with cultivar. However, the effect of soil moisture level on total root length of the plants of the late harvest was significant, with stressed plants having larger roots than control plants (Table 7.5).

Root length density of the regrowth harvest showed significant effects for root length of the cultivars at 0-10, and 30-40 cm depth (Appendix 7.6). At 0-10 cm depth G35 had larger root length than Eski and lucerne, but at 30-40 cm depth lucerne root length was larger than sainfoin cultivars (Appendix 7.6). Root length was affected by soil moisture level at six of the seven depths (the exception being 30-40 cm depth). The control plants had larger root length at 0-30 cm depth but smaller root

length density at 40-100 cm depth. The interaction of cultivar by soil moisture was not significant at any depth (Appendix 7.6).

The total root length density for the regrowth harvest showed no significant effects for soil moisture levels, cultivar or their interaction with soil moisture.

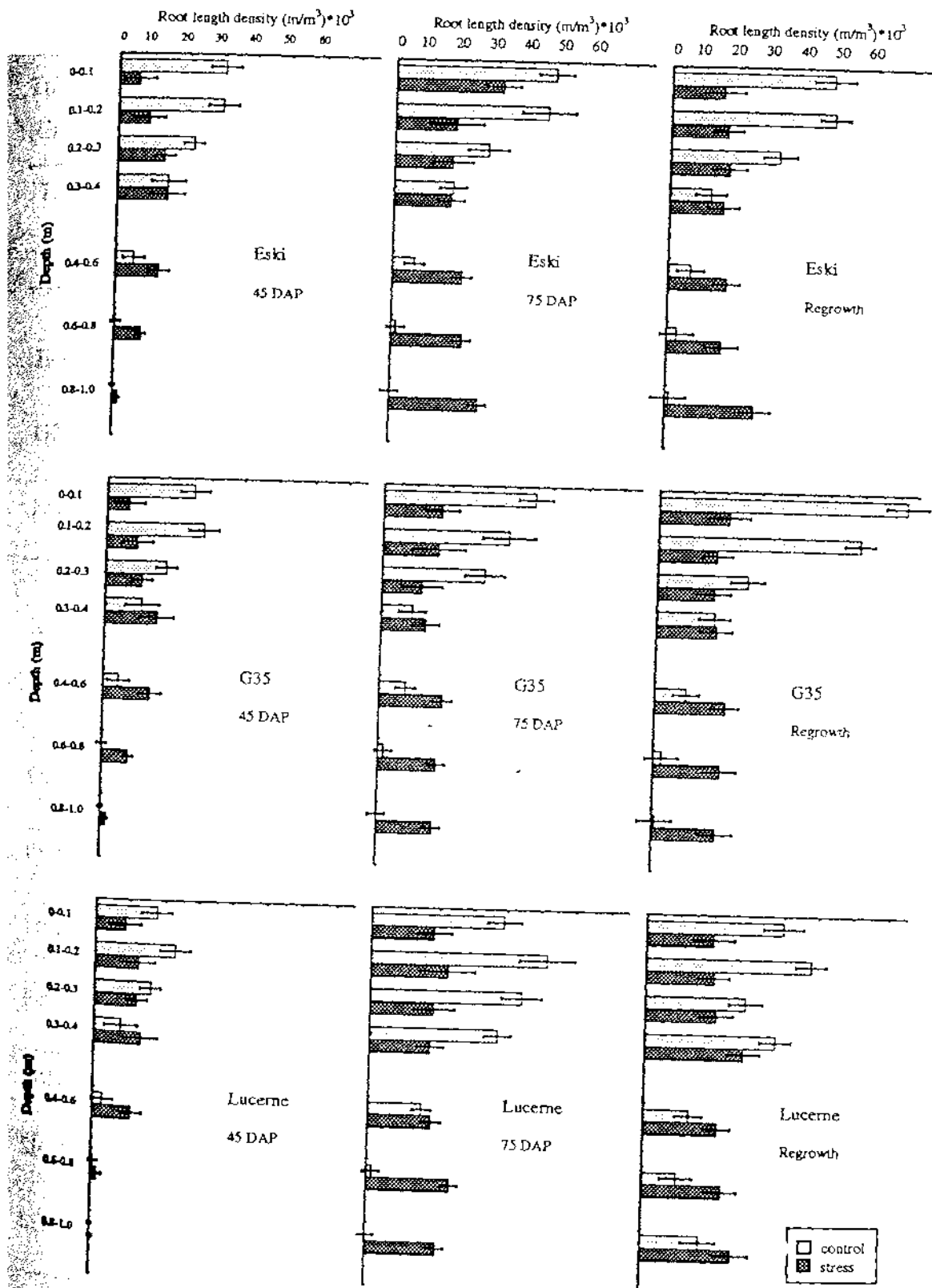


Fig. 7.4: Root length density (m/m^3) * 10^3 of the Eski, Remont, and lucerne at different depths for two soil moisture levels. Bars show \pm SEM.

Table 7.5: Total root length density (m/m^3)* 10^3 of Eski, Grasslands G35, and lucerne (Grasslands Oranga) at two soil moisture levels and three harvests.

Treatment	Early harvest (45 DAP)	Late harvest (75 DAP)	Regrowth (30 days)
		Control	
Eski	11.0 ¹	15.3	16.1
G35	9.0	14.1	20.0
Lucerne	7.1	21	23.7
		Stress	
Eski	8.5	21.9	18.5
G35	8.6	16.0	19.1
Lucerne	7.3	20.6	23.3
P>F Cu ²	0.4012	0.0055	0.1087
P>F W	0.5582	0.0275	0.8845
P>F Cu*W	0.7698	0.08557	0.8138
SEM Cu	1.3	1.0	1.9
SEM W	1.0	0.8	1.6
SEM Cu*W	1.8	1.4	2.8

¹ Numbers are mean of four replicates.

² Cu= Cultivar, W= soil moisture levels, and W*Cu= interactions of soil moisture and cultivar.

7.3.2.7 Root weight density (RWD)

The root weight of all three cultivars at the early harvest was similar at all seven depths, but was significantly different for soil moisture levels at 30-100 cm depth (Appendix 7.7). The interaction of cultivar by soil moisture was generally not significant (Appendix 7.7). The stressed plants had a higher root dry weight than the control plants at 30-100 cm depth. At the early harvest there was no significant effect of cultivar, soil moisture, or their interaction (Appendix 7.7).

At the late harvest, the root dry weight density of the cultivars was significantly different at four of the seven depths. In the shallow depths lucerne had more root dry weight than the sainfoin cultivars, but at the 40-100 cm depth sainfoin root dry weight was higher than for lucerne (Appendix 7.8). Soil moisture effects were significant at 30-100 cm depth with a higher root dry weight for the stressed plants than the control plants (Fig 7.5). The interaction of cultivar and soil moisture was only significant at the 80-100 cm depth, with G35 having highest root dry weight in the control treatment and Eski having higher RWD in the stressed treatment (Fig. 7.5).

The total root dry weights of cultivars in late harvest were significantly different, with G35 being lower than Eski and lucerne (Table 7.6). The total dry weight of stressed plants was significantly higher than that of control plants (Table 7.6).

At the regrowth harvest, although cultivars had similar root dry weight, soil moisture levels had a significant effect at the 40-100 cm depth and the interaction of cultivar and soil moisture was significant at the shallowest and deepest depths. At 10-20 and 80-100 cm depth the RWD of the control plants of G35 were the highest and lowest root dry weight, respectively, whereas stressed plants of G35 were lowest and highest at these depths, respectively. In contrast to the root dry weight of the plants, at the late harvest there were no significant differences in root dry weight for cultivars, soil moisture levels, or their interaction (Table 7.6).

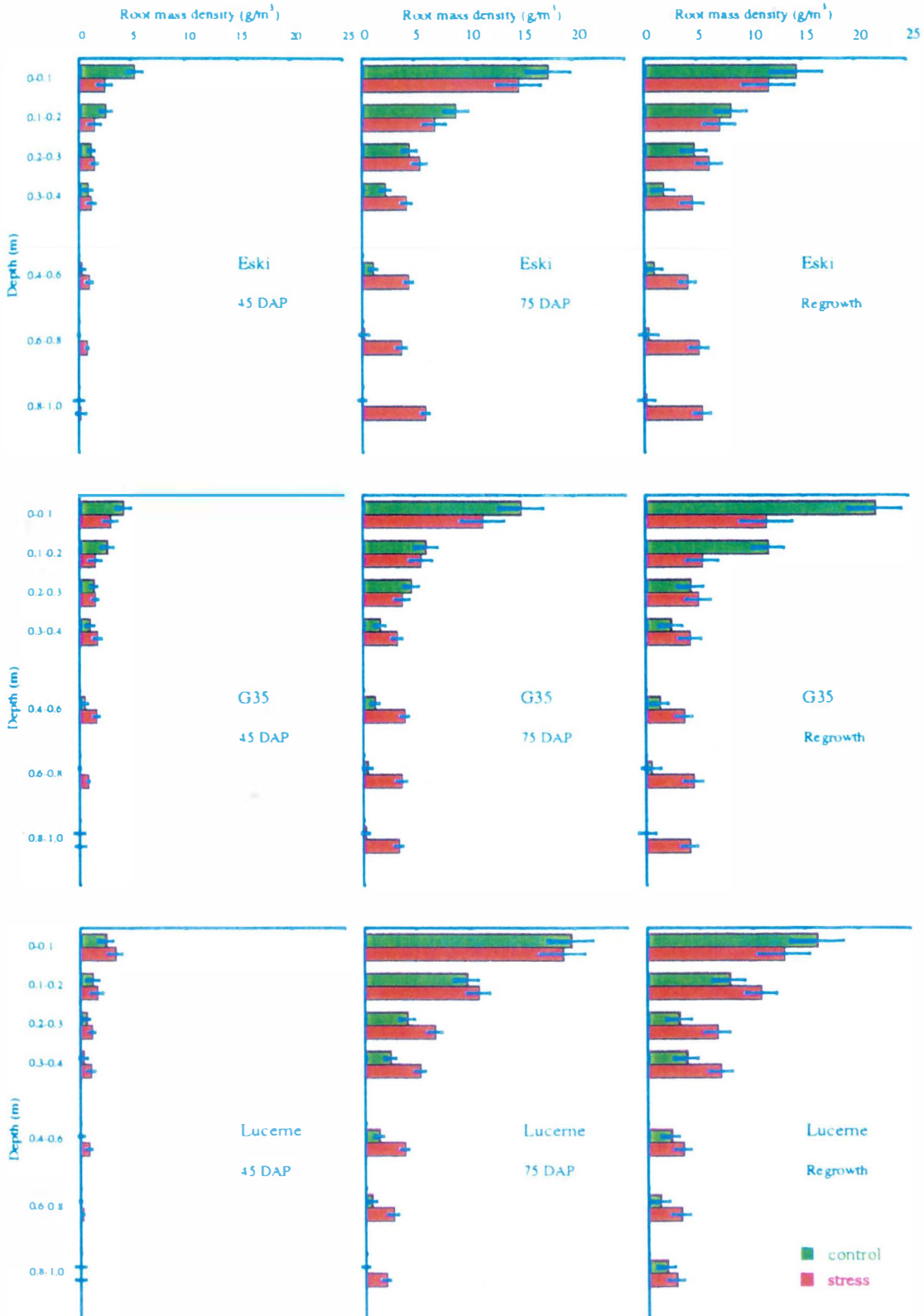


Fig. 7.5: Root mass density (g/m³) of Eski, Remont, and lucerne at different depths for two soil moisture levels. Bars show ±SEM.

Table 7.6: Total root dry weight density (g/m^3) of Eski, Grasslands G35, and lucerne (Grasslands Oranga) at two different soil moisture levels for three harvests.

Treatment	Early harvest (45 DAP)	Late harvest (75 DAP)	Regrowth (30 days)
Eski	108.2 ¹	364.1	325.1
G35	104.2	314.3	443.3
Lucerne	48.7	401.1	409.1
Eski	105.7	609.1	597.7
G35	127.4	464.9	508.4
Lucerne	90.1	587.9	555.3
P>F Cu ²	0.1109	0.0928	0.9744
P>F W	0.2633	0.0002	0.0516
P>F Cu*W	0.6110	0.6373	0.5484
SEM Cu	15.4	34.9	66.1
SEM W	12.6	28.5	53.9
SEM Cu*W	21.8	49.4	93.4

¹ Numbers are means of four replicates.

Cu= cultivar, W= soil moisture, Cu*W= Interaction of soil moisture and cultivar.

7.3.3 Physiological measurements

7.3.3.1 Relative water content (RWC)

Relative water content of the plants was affected by soil moisture ($P < 0.01$) at the three harvests (Table 7.7). Stressed plants had a lower RWC than the controls. The RWC of the cultivars was similar (Fig. 7.6a) There were no significant interactions between soil moisture and cultivar.

Pooled analysis over harvests showed significant effects of soil moisture, harvests, and their interaction on RWC. Greater RWC was found for control plants across harvests. Regrowth plants had a lower RWC than early and late harvested plants. The RWC of stressed and control regrowth plants was lower than that of the early and late harvested plants.

7.3.3.2 Transpiration rate

Transpiration rate at the late harvest, measured by weighing pots, showed significant effects ($P < 0.01$) only for soil moisture treatments. The transpiration rate of the control plants (329.8 ± 21.4 ml/pot/day) was higher than for the stressed plants (240.8 ± 21.4 ml/pot/day).

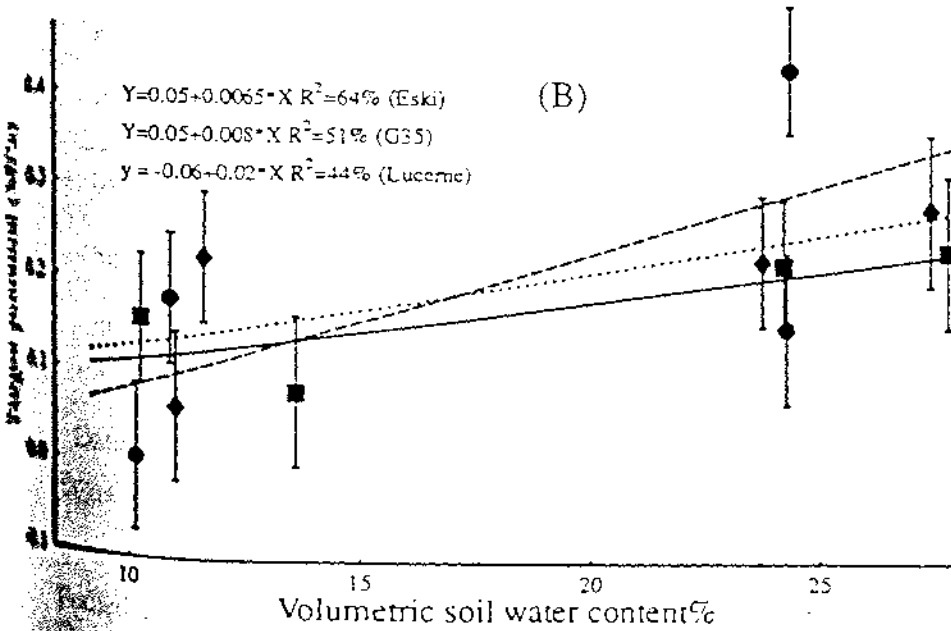
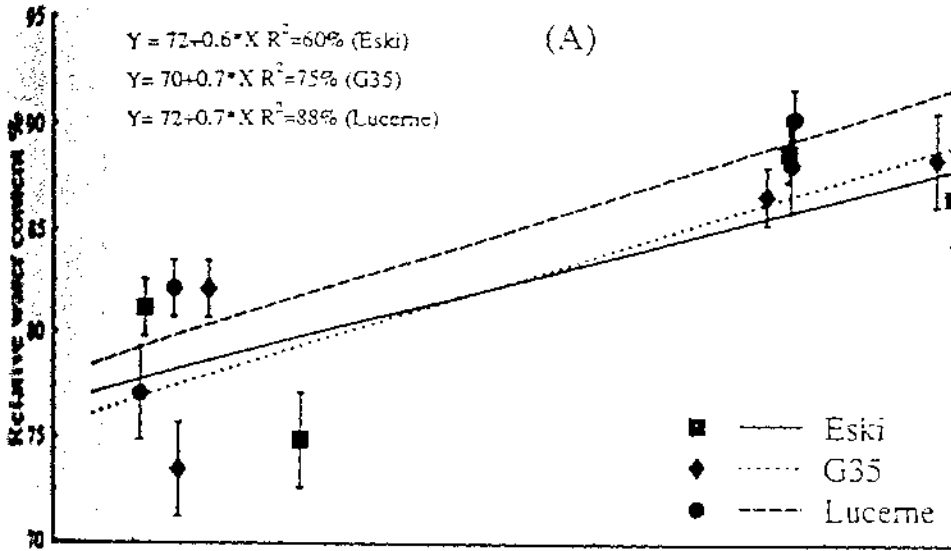


Fig. 7.6: Relationships between volumetric soil water content, and (A) relative water content %, and (B) turgor potential for Eski, Grasslands G35, and lucerne. Symbols are means of four replicates, vertical bars represent \pm SEM.

Table 7.7: Relative water content (%) of two sainfoin cultivars and lucerne at the early (45 days after planting, DAP) late, (75 DAP) and regrowth (30 days after cutting, 105 DAP) harvests at two soil moisture levels.

	Early harvest	Late harvest	Regrowth
Control			
Eski	86.0 ¹	88.4	86.3
G35	87.3	86.4	88.1
Luucerne	88.0	90.0	87.9
Stress			
Eski	79.0	81.1	74.8
G35	83.2	81.9	73.5
Lucerne	84.2	82.0	77.0
Pr>F Cu ²	0.2700	0.4770	0.6572
Pr>F W	0.0100	0.0001	0.0001
Pr>F Cu*W	0.7400	0.3610	0.6608
SEM Cu	1.57	0.97	1.56
SEM W	1.28	0.79	1.27
SEM Cu*W	2.23	1.37	2.21

¹ Data are the mean of four replicates.

² Cu= cultivar, W= soil moisture levels, Cu*W= interaction of W and Cu.

Pooled ANOVA over all harvests showed Pr>F W=0.0001, SEM W=0.72, Pr>F Cu=0.2183 SEM Cu=0.88, Pr>F Harvest (H)= 0.0082 SEM H= 0.88, Pr>F W*Cu=0.9148 SEM W*Cu= 1.25, Pr>F W*H= 0.0132 SEM W*H= 0.88, Pr>F Cu*H=0.8502 SEM Cu*H= 1.5312, Pr>F W*Cu*H= 0.6957 SEM W*Cu*H=2.17

7.3.3.3 Stomatal resistance (Rs)

Pooled analysis over three harvests showed that leaf stomatal resistance was significantly affected by soil moisture level. Stressed plants had a higher stomatal resistance (2.33 ± 0.11 s/cm) than control plants (1.86 ± 0.11 s/cm) (Table 7.8a) (Fig. 7.6b).

Pooled over all three harvests, Rs of the adaxial leaf surface showed significant effects for soil moisture level ($P < 0.01$) and cultivar ($P < 0.05$). The Rs of lucerne (2.06 ± 0.099 s/cm) was higher than for Eski (1.66 ± 0.099 s/cm) and G35 (1.70 ± 0.099) (Fig. 7.6b)

Separate analysis of Rs for each harvest showed significant differences in the Rs due to soil moisture level at only the late and regrowth harvests (Table 7.8a). Analysis of the Rs of the abaxial and adaxial leaf surfaces at each harvest showed soil moisture level affected adaxial Rs at the late and regrowth harvests whereas it affected the abaxial Rs at the regrowth harvest (Table 7.8b). The Rs of the adaxial leaf surface was also significantly different ($P < 0.05$) between cultivars at the regrowth harvest, with lucerne having a higher adaxial Rs (2.53 s/cm) than Eski or G35 (mean=1.63 s/cm) (Fig. 7.7 a,b).

The interaction of soil moisture by cultivar was significant only at the regrowth harvest for the adaxial leaf surface. The difference between the adaxial Rs of control vs stressed lucerne (1.8 s/cm) was higher than for G35 (0.05 s/cm) and Eski (0.04 s/cm) (Table 7.8b).

Table 7.8 (a): Total stomatal resistance (s/cm) of sainfoin cultivars and lucerne at two soil moisture levels for early (45 days after planting), late (75 days after planting), and regrowth (30 days after cutting) harvests.

	Early harvest	Late harvest	Regrowth
	Control		
Eski	1.88 ¹	1.82	2.31
G35	1.90	1.58	2.18
Lucerne	1.80	1.54	1.72
	Stress		
Eski	2.10	2.47	2.05
G35	1.95	2.20	2.23
Lucerne	2.15	2.23	3.62
Pr >F W ²	0.1621	0.0230	0.0417
Pr >F Cu	0.9480	0.6501	0.2402
Pr >F W*Cu	0.6179	0.9936	0.0113
³ SEM W	0.091	0.184	0.188
SEM Cu	0.112	0.225	0.231
SEM W*Cu	0.158	0.318	0.326

¹ Data are mean of four replicates.

² W= soil moisture levels, Cu= Cultivar, and W*Cu = Interaction of soil moisture by cultivar.

³ SEM= Standard error of the mean.

Pooled analysis of data over three harvests showed Pr>F W= 0.0029, SEM W=0.1087, Pr>F Cu= 0.6610, SEM Cu=0.1332, Pr>F Cu*W= 0.0755, SEM Cu*W=0.1883, Pr>F Harvest (H)=0.0812, SEM H= 0.1332, Pr>F H*W=0.4099, SEM H*W=0.18883, Pr>F H*Cu= 0.5339, SEM H*Cu= 0.2906, Pr>F H*W*Cu= 0.1474, SEM H*W*Cu= 0.3262.

Table 7.8 (b): Stomatal resistance (s/cm) of abaxial and adaxial surfaces of leaves of sainfoin cultivars and lucerne in two soil moisture levels at early harvest (45 days after planting), late harvest (75 days after planting), and regrowth.

	Early harvest		Late harvest		Regrowth	
	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial
Control						
Eski	2.24 ¹	1.65	3.23	1.39	3.33	1.59
G35	2.13	1.72	1.92	1.41	2.54	1.41
Lucerne	1.89	1.72	1.61	1.48	1.78	1.66
Stress						
Eski	2.52	1.76	4.14	1.96	3.42	1.63
G35	2.35	1.67	2.41	2.07	4.21	1.91
Lucerne	2.33	2.01	2.44	2.09	3.92	3.39
Pr >F W ²	0.1103	0.3500	0.3495	0.0016	0.0061	0.0028
Pr >F Cu	0.4993	0.4704	0.1817	0.8616	0.4895	0.0045
Pr >F W*Cu	0.8875	0.5265	0.9713	0.9681	0.1350	0.0145
SEM Cu ³	0.129	0.087	0.543	0.113	0.289	0.149
SEM W	0.159	0.107	0.665	0.139	0.354	0.184
SEM Cu*W	0.225	0.151	0.941	0.196	0.499	0.259

¹ Data are the mean of four replicates.

² Cu= Cultivar, W= soil moisture level, and Cu*W= Interaction of soil moisture by cultivar.

³ SEM= Standard error of the mean.

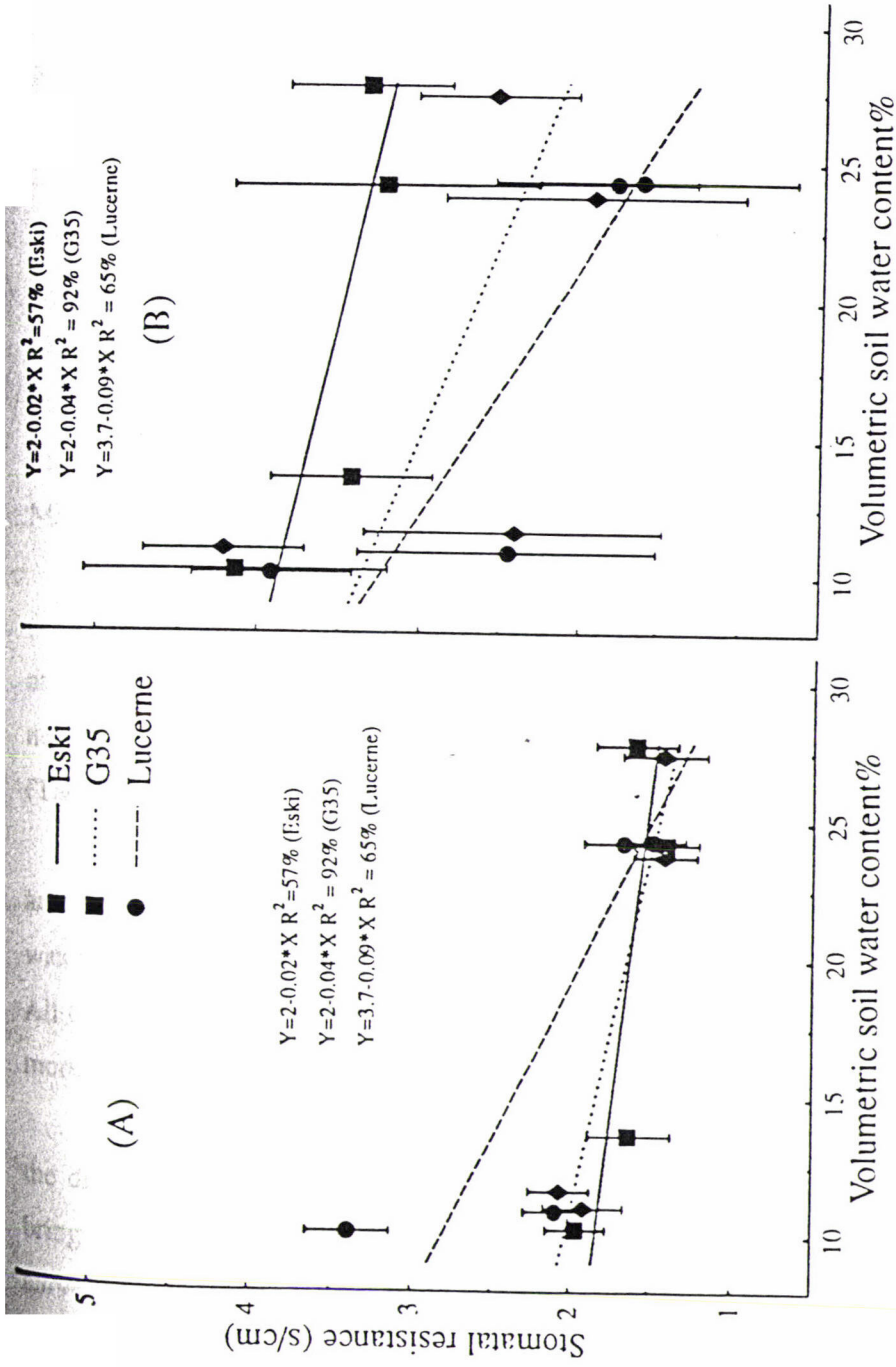


Fig 7.7: Stomatal resistance of (A) adaxial, and (B) abaxial leaf surface of Eski, Grasslands G35, and lucerne at different soil moisture. Symbols are mean of four replicates. Vertical bars are \pm SEM.

7.3.3.4 Leaf water potential (Ψ) by pressure bomb

Leaf water potential was significantly different between harvests, cultivars, soil moisture levels ($P < 0.01$), and all their first order interactions except the interaction between water and cultivar (Appendix 7.10).

The time of measurement (dawn or midday) significantly affected Ψ ($P < 0.001$). There were significant interactions of time by harvest ($P < 0.001$), time by soil moisture ($P < 0.01$), and time by soil moisture by harvest ($P < 0.01$) (Appendix 7.11). The Ψ at the early harvest was higher (less negative) (-0.56 MPa) than at the late (-0.79 MPa), and regrowth harvests (-0.84 MPa) (Table 7.9). The difference between Ψ at dawn and midday at the early harvest was smaller (0.27 MPa) than for late (0.42 MPa) and regrowth (0.30 MPa) harvests.

Control plants had significantly higher Ψ (-0.55 MPa) than stressed plants (-0.91 MPa) (Table 7.9). Water stress affected the Ψ of plants at midday more than at dawn, with the Ψ of stressed plants at dawn being 0.27 MPa lower (more negative) than for control plants, whereas this difference at midday was 0.45 MPa (Table 7.9).

Lucerne had lower (more negative) Ψ (-0.89 MPa) than Eski (-0.61 MPa) and G35 (-0.70 MPa). Furthermore, the difference between dawn and midday Ψ was greater for lucerne (0.415 MPa) than Eski (0.268 MPa) and G35 (0.298 MPa). All three cultivars had their highest Ψ at the early harvest and lucerne Ψ decreased more than for sainfoin cultivars at subsequent harvests.

There was a significant interaction between soil moisture and harvest with the difference between the Ψ of stressed and control plants at the early harvest being smaller (0.176 MPa) than at the late (0.423 MPa) and regrowth (0.483 MPa) harvests ($P < 0.0001$).

The difference between Ψ of control and stressed plants varied between harvests. This difference was smaller for the early harvest (0.265 MPa) than the late (0.42 MPa) or regrowth harvests (0.42 MPa) (Table 7.9).

Table 7.9: Total leaf water potential (MPa) by pressure bomb of early (45 days after planting, DAP) late (75 DAP) and regrowth (30 days after cutting, 105 DAP) harvests of two sainfoin cultivars and lucerne at two levels of soil moisture, at dawn and midday.

	Dawn			Midday		
	Early harvest	Late harvest	Regrowth	Early harvest	Late harvest	Regrowth
Control						
Eski	-0.34	-0.38	-0.39	-0.44	-0.54	-0.55
G35	-0.38	-0.45	-0.44	-0.51	-0.78	-0.62
Lucerne	-0.43	-0.52	-0.55	-0.76	-0.76	-1.00
Stress						
Eski	-0.36	-0.55	-0.77	-0.67	-1.20	-1.00
G35	-0.51	-0.72	-0.80	-0.77	-1.26	-1.15
Lucerne	-0.58	-0.84	-1.16	-1.02	-1.46	-1.57
Pr>F Cu ²	0.0085	0.0347	0.0052	0.0036	0.1732	0.0001
Pr>F W	0.0144	0.0008	0.0001	0.0028	0.0001	0.0001
Pr>F Cu*W	0.3002	0.6036	0.1926	0.9759	0.6325	0.9805
SEM Cu	0.031	0.053	0.054	0.061	0.086	0.052
SEM W	0.025	0.043	0.044	0.049	0.07	0.043
SEM Cu*W	0.044	0.075	0.076	0.087	0.122	0.075

¹ Means are mean of four replicates.

² Cu=cultivar, W= soil moisture level, Cu*W= Interaction of soil moisture by cultivar, and SEM= standard error of the mean.

7.3.3.5 Leaf water potential (Ψ) by Wescor

Over three harvests, total Ψ showed significant effects for harvest ($P<0.001$), cultivar ($P<0.001$), soil moisture level ($P<0.001$), interactions of cultivar by harvest ($P<0.01$), soil moisture by harvest and soil moisture by cultivar ($P<0.001$) (Appendix 7.10) and time of the measurement (dawn vs midday) ($P<0.001$) (Appendix 7.11). There were also significant interactions for time by harvest, time by soil moisture, and time by soil moisture by harvests (Appendix 7.11). The Ψ of the plants at the early harvest (-0.78 MPa) was higher than for the late (-1.03 MPa) and regrowth harvests (-1.11 MPa).

Midday Ψ was lower than the dawn Ψ for all three harvests, but at the early harvest the difference (0.003 MPa) was smaller than late (0.48 MPa) and regrowth (0.35 MPa) harvests (Table 7.10).

Lucerne had a lower Ψ (-1.05 MPa) than for Eski (-0.97 MPa) and G35 (-0.89 MPa) (Table 7.10). Over all three harvests the Ψ of the stressed plants was lower than that of the control plants (-1.02 vs -0.82 MPa). A significant time interaction with soil moisture showed that the difference between Ψ of control plants at dawn and midday (0.17 MPa) was smaller than that of the stressed plants (0.39 MPa) (Table 7.10). Lucerne had a lower (more negative) Ψ than that of the sainfoin cultivars at the late and regrowth harvests (Table 7.10). The Ψ of stressed plants at the regrowth harvest (-1.38 MPa) was lower than that of the early (-0.92 MPa) and late harvest (-1.05 MPa) (Table 7.10).

Table 7.10 : Total leaf water potential by Wescor (MPa) of early (45 days after planting, DAP) and late harvest (75 DAP) and regrowth (30 days after cutting, 105 DAP) harvest of two sainfoin cultivars and lucerne, at two levels of soil moisture, at dawn and midday .

	Dawn			Midday		
	Early harvest	Late harvest	Regrowth harvest	Early harvest	Late harvest	Regrowth harvest
	Control					
Eski	-1.21	-0.68	-0.71	-0.71	-1.24	-0.99
G35	-0.68	-0.66	-0.67	-0.57	-0.96	-0.88
Lucerne	-0.63	-0.77	-0.65	-0.70	-1.01	-1.10
	Stress					
Eski	-0.81	-0.65	-1.01	-0.98	-1.24	-1.42
G35	-0.84	-0.69	-1.18	-0.99	-1.19	-1.41
Lucerne	-0.83	-0.93	-1.39	-1.07	-1.64	-1.89
Pr>F W ²	0.9479	0.2239	0.0001	0.0005	0.0018	0.0002
Pr>F Cu	0.4239	0.0057	0.3180	0.5915	0.0509	0.0617
Pr>F W*Cu	0.3704	0.2189	0.1325	0.7433	0.0132	0.4607
SEM W	0.133	0.030	0.059	0.057	0.050	0.083
SEM Cu	0.163	0.040	0.073	0.070	0.070	0.101
SEM W*Cu	0.230	0.050	0.104	0.099	0.093	0.144

† Data are means of four replicates.

* W= soil moisture level, Cu=cultivar, Cu*W= interaction of soil moisture by cultivar.

7.3.3.6 Osmotic potential (π)

Osmotic potential was significantly different between cultivars ($P < 0.01$), soil moisture levels ($P < 0.001$), and the interaction of soil moisture by harvest ($P < 0.01$) and soil moisture by cultivar ($P < 0.01$)

Dawn and midday π was significantly different over all harvests ($P < 0.001$). The interactions of time with harvest ($P < 0.001$), cultivars ($P < 0.05$), and soil moisture by harvest ($P < 0.05$) were also significant (Appendix 7.11).

Lucerne had a lower π (-1.37 MPa) than Eski (-1.20 MPa) or G35 (-1.184 MPa) (Table 7.11). The π of stressed lucerne plants (-1.60 MPa) was lower than that of the control plants (-1.143 MPa). This difference (0.46 MPa) was greater than for Eski (0.12 MPa) and G35 (0.33 MPa) (Table 7.11).

Eski had a higher π at dawn (-1.06 MPa) than G35 (-1.12 MPa) and lucerne (-1.23 MPa) but at midday G35 had a higher π (-1.29 MPa) than Eski (-1.35 MPa) and lucerne (-1.51 MPa) (Table 7.11).

Over all harvests and times π of the stressed plants (-1.40 MPa) was lower than for control plants (-1.10 MPa). The difference between π of stressed and control plants at the regrowth harvest was lower (0.47 MPa) than that of the early (0.30 MPa) and late (0.14 MPa) harvests (Table 7.11). The difference between π of the plants at dawn and midday varied between harvests, and was lower for the early harvest (0.04 MPa) than for the late (0.285 MPa) and regrowth (0.379 MPa) harvests.

Table 7.11: Osmotic potential by Wescor (MPa) of early (45 days after planting, DAP) late (75 DAP) and regrowth (30 days after cutting, 105 DAP) harvests of two sainfoin cultivars and lucerne, at two levels of soil moisture, at dawn and midday.

	Dawn			Midday		
	Early harvest	Late harvest	Regrowth harvest	Early harvest	Late harvest	Regrowth harvest
Control						
Eski	-1.15	-1.03	-0.84	-1.16	-1.46	-1.22
G35	-1.10	-1.04	-0.82	-0.84	-1.18	-1.16
Lucerne	-1.10	-1.30	-0.68	-1.11	-1.44	-1.25
Stress						
Eski	-1.11	-1.04	-1.20	-1.34	-1.40	-1.50
G35	-1.45	-1.12	-1.20	-1.42	-1.42	-1.48
Lucerne	-1.30	-1.50	-1.53	-1.56	-1.82	-1.90
Pr>F W ²	0.0305	0.3290	0.0001	0.0001	0.0169	0.0004
Pr>F Cu	0.2945	0.0147	0.7017	0.1186	0.0048	0.0817
Pr>F W*Cu	0.1233	0.7233	0.1200	0.1191	0.0555	0.2381
SEM W	0.053	0.068	0.072	0.053	0.049	0.066
SEM Cu	0.064	0.083	0.088	0.065	0.060	0.080
SEM W*Cu	0.091	0.117	0.125	0.091	0.085	0.114

¹ Data are means of four replicates.

² W= soil moisture level, Cu=cultivar, Cu*W= interaction of soil moisture by cultivar

7.3.3.7 Turgor potential of the leaf

The turgor potential of the plants was significantly different at each harvest, however neither cultivar, soil moisture levels nor any interaction were significant ($P < 0.05$) (Appendix 7.11).

Separate analysis for each harvest showed P was significantly affected by soil moisture at midday for the regrowth harvest (Table 7.12). At the early harvest plants had higher P (0.38 MPa) than at the late (0.33 MPa) and regrowth (0.12 MPa) harvests (Table 7.12). The differences between dawn and midday P of the plants at the late harvest plants was higher than for the other two harvests.

To examine cell elasticity, the turgor potential data was plotted against RWC of the leaf at late and regrowth harvests (Fig. 7.8). A strong relationship was found between P and RWC of the leaves for Eski, G35, and lucerne. Comparison of the slopes did not show any significant difference between lucerne and any of the sainfoin cultivars.

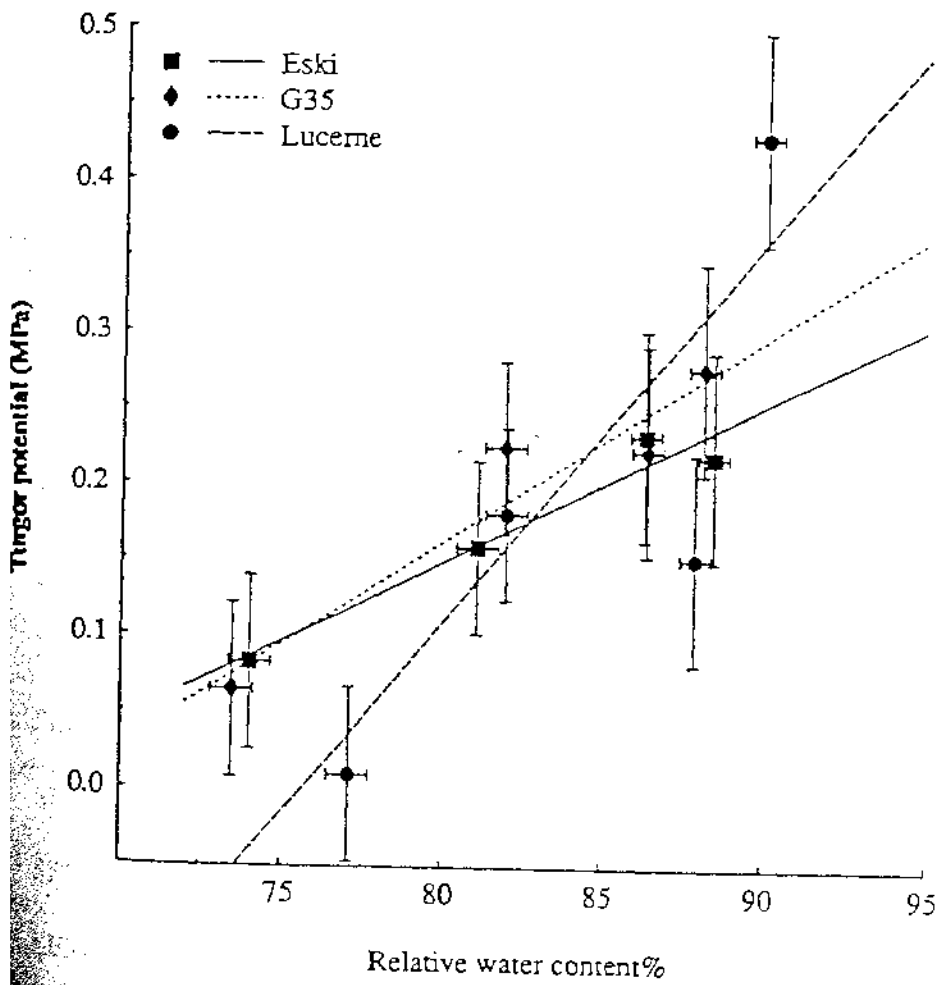


Fig 7.8: Relationship between turgor potential and relative water content of Eski, Grasslands G35, and lucerne. Points are mean of four replicates. Respective regression equations and standard error of slope (SE) are $Y = -0.68 + 0.01X$, $R^2 = 95\%$, $SE = 0.0016$, $Y = -0.9 + 0.01X$, $R^2 = 92\%$, $SE = 0.0029$, $Y = -0.79 + 0.012X$, $R^2 = 89\%$, $SE = 0.1148$.

Table 7.12: Turgor potential (MPa) of early (45 days after planting, DAP) late (75 DAP) and regrowth (30 days after cutting, 105 DAP) harvests of two sainfoin cultivars and lucerne, at two levels of soil moisture, at dawn and midday.

	Dawn			Midday		
	Early harvest	Late harvest	Regrowth harvest	Early harvest	Late harvest	Regrowth harvest
Control						
Eski	0.07	0.35	0.13	0.45	0.22	0.23
G35	0.41	0.38	0.15	0.27	0.22	0.28
Lucerne	0.46	0.53	0.03	0.41	0.43	0.15
Stress						
Eski	0.29	0.38	0.19	0.36	0.16	0.08
G35	0.61	0.43	0.02	0.43	0.22	0.06
Lucerne	0.47	0.55	0.13	0.48	0.18	0.01
Pr>F W ²	0.4024	0.6211	0.7450	0.4641	0.0912	0.0222
Pr>F Cu	0.3028	0.1705	0.2947	0.2859	0.2573	0.5022
Pr>F W*Cu	0.8102	0.9962	0.1144	0.4632	0.2209	0.8585
SEM W	0.156	0.056	0.031	0.044	0.040	0.046
SEM Cu	0.191	0.096	0.038	0.054	0.069	0.057
SEM W*Cu	0.270	0.097	0.054	0.076	0.069	0.080

¹ Means are mean of four replicates.

² W= Soil moisture levels, Cu=Cultivar, Cu*W= Interaction of soil moisture by cultivar, and SEM= Standard error of the mean.

7.3.3.8 Osmotic potential at full turgor (π_{100})

Over all three harvests, the π_{100} was significantly different for soil moisture levels ($P < 0.05$), however neither cultivar, harvest nor their interaction with soil moisture were significantly different (Appendix 7.10). The time of measurement (dawn and midday) significantly interacted with soil moisture level, and cultivar by soil moisture level (Appendix 7.11).

The π_{100} for the plants at the early harvest (-0.96 MPa) was higher than for the late (-1.11 MPa) and regrowth (-1.02 MPa) harvests (Table 7.13). The interaction of time of measurement (dawn and midday) with soil moisture indicated that the difference between π_{100} at dawn and midday was greater for stressed plants. (Table 7.13). At midday, the stressed lucerne plants relative to the control plants had a more negative π_{100} than the sainfoin cultivars. In the other words, the difference between π_{100} of the stressed and control plants at midday was higher (0.41 MPa) for lucerne than for the sainfoin cultivars (0.08 MPa) (Table 7.13).

Table 7.13: The full turgor osmotic potential (MPa) using the Decagon of Eski, Grasslands G35 and lucerne (Grasslands Oranga) at dawn and midday at early harvest (45 days after planting, DAP), late (75 DAP), and regrowth (30 days after cutting, 104 DAP) harvests at two levels of soil moisture.

Treatment	Dawn			Midday		
	Early harvest	Late harvest	Regrowth harvest	Early harvest	Late harvest	Regrowth harvest
Control						
Eski	-0.860	-0.892	-1.078	-1.030	-1.063	-0.978
G35	-0.745	-0.949	-0.995	-0.908	-1.077	-0.870
Lucerne	-0.930	-1.499	-0.963	-0.943	-1.090	-0.745
Stress						
Eski	-0.890	-1.045	-1.038	-0.940	-1.083	-0.978
G35	-1.080	-0.882	-1.085	-1.090	-1.203	-1.117
Lucerne	-0.875	-1.154	-1.023	-1.320	-1.390	-1.303
Pr>F W ²	0.2410	0.6293	0.6080	0.1994	0.0106	0.0047
Pr>F Cu	0.9282	0.1599	0.7395	0.5419	0.0483	0.8946
Pr>F W*Cu	0.1813	0.5231	0.7343	0.2892	0.0980	0.0411
SEM W	0.0603	0.1225	0.0857	0.0001	0.0336	0.0001
SEM Cu	0.0739	0.1501	0.0606	0.1011	0.0411	0.0700
SEM W*Cu	0.1044	0.2123	0.0857	0.1429	0.0581	0.0990

¹ Means are mean of four replicates.

² W= soil moisture levels, Cu=cultivar, Cu*W= interaction of soil moisture by cultivar, and SEM= standard error of the mean.

7.3.3.9 Total, osmotic, and turgor potential of roots

Total, osmotic, and turgor potential of roots over all harvests was significantly affected by soil moisture ($P < 0.05$) (Appendix 7.12). Roots Ψ of control plants was higher than that of stressed plants (-0.63 vs -1.19 ± 0.419 MPa) (Table 7.14). Similarly, the root π of controls was higher than that of stressed plants (-0.78 vs -1.22 MPa ± 0.442) (Table 7.15). Water stress affected P with controls being greater than stressed roots (0.1469 vs 0.0251 MPa).

Over all harvests, the only significant interaction was between cultivar and harvests for root Ψ ($P < 0.05$) (Appendix 7.12). Lucerne roots had a lower Ψ (-1.03 MPa) than Eski (-0.86 MPa) and G35 (-0.98 MPa). For root π over all three harvests there was significant interactions for cultivar and soil moisture and, harvest and soil moisture (Appendix 7.12). The roots of the control plants of lucerne had a higher π (-0.65 MPa) than that of Eski (-0.884 MPa) and G35 (-0.793 MPa), but the stressed plants of lucerne had a lower π (-1.31 MPa) than that of the Eski (-1.17 MPa) and G35 (-1.167 MPa). In stressed plants roots at the early harvest there was a higher π (-1.04 MPa) than roots at the late or regrowth harvest (-1.31 MPa) (Table 7.15).

Separate analysis of each harvest for Ψ , π , and P of the roots showed a significant effect of soil moisture on root water potential at all three harvests (Table 7.14) whereas root π was only significantly affected at the late and regrowth harvests (Table 7.15). There was an interaction between cultivar and location (depth) at the regrowth harvest for P of the roots (Table 7.16). At 50-75 cm depth Eski and G35 had a higher root P than at 20-30 cm depth, whereas the P of lucerne roots at 50-75 cm depth was lower than that at 20-30 cm depth.

Table 7.14: The total potential (MPa) of roots of sainfoin cultivars and lucerne at two depths, and two soil moisture levels at early (45 days after planting, DAP), late (75 DAP), and regrowth (30 days after cutting, 105 DAP) harvests .

Depth (cm)	Early harvest		Late harvest		Regrowth	
	20-30	50-75	20-30	50-75	20-30	50-75
Control						
Eski ¹	-0.537	-0.525	-0.720	-0.621	-0.813	-0.609
G35	-0.849	-0.791	-0.603	-0.539	-0.757	-0.587
Lucerne	-0.384	-0.492	-0.682	-0.505	-0.640	-0.659
Stress						
Eski	-1.087	-1.049	-1.273	-1.541	-1.015	-1.009
G35	-1.237	-1.068	-1.057	-1.188	-1.229	-1.127
Lucerne	-0.979	-0.879	-1.558	-1.392	-1.411	-1.385
Pr >F CU ²		0.0391		0.4238		0.3178
Pr >F W		0.0001		0.0001		0.0001
Pr >F W*CU		0.6150		0.6044		0.1324
Pr >F D		0.5151		0.7356		0.1701
Pr >F W*D		0.4061		0.0799		0.5243
Pr >F CU*D		0.7944		0.1245		0.6182
Pr >F C*W*D		0.8588		0.3829		0.6890
³ SEM CU		0.0618		0.0938		0.0599
SEM W		0.0757		0.1149		0.0734
SEM W*CU		0.1070		0.1625		0.1038
SEM D		0.0476		0.0363		0.0402
SEM W*D		0.0674		0.0513		0.0568
SEM CU*D		0.0825		0.0628		0.0696
SEM W*CU*D		0.1167		0.0888		0.985

¹Data are means of four replicates.

²CU= Cultivar, W= soil moisture level, and D= Depth.

³SEM= Standard error of the mean.

Table 7.15: The osmotic potential (MPa) of roots of sainfoin cultivars and lucerne at two soil moisture levels at early (45 days after planting, DAP), late (75 DAP), and regrowth (30 days after cutting, 105 DAP) harvests .

Depth (cm)	Early harvest		Late harvest		Regrowth	
	20-30	50-75	20-30	50-75	20-30	50-75
Control						
Eski	-1.294 ¹	-0.687	-0.821	-0.825	-0.880	-0.794
G35	-0.883	-0.820	-0.783	-0.641	-0.850	-0.782
Lucerne	-0.482	-0.637	-0.751	-0.671	-0.779	-0.577
Stress						
Eski	-1.088	-1.096	-1.066	-1.392	-1.202	-1.201
G35	-1.207	-1.027	-1.193	-1.181	-1.191	-1.204
Lucerne	-0.979	-0.827	-1.597	-1.423	-1.591	-1.471
Pr >F CU ²		0.2157		0.3127		0.7031
Pr >F W		0.1255		0.0001		0.0001
Pr >F W*CU		0.7903		0.1546		0.1201
Pr >F D		0.3006		0.8219		0.2633
Pr >F W*D		0.8121		0.3121		0.5428
Pr >F CU*D		0.6488		0.1112		0.6784
Pr >F C*W*D		0.3388		0.3527		0.9999
³ SEM CU		0.1032		0.0588		0.0722
SEM W		0.1264		0.0720		0.0383
SEM W*CU		0.1783		0.1019		0.1250
SEM D		0.0927		0.0405		0.0472
SEM W*D		0.1311		0.0573		0.0668
SEM CU*D		0.1605		0.0702		0.0818
SEM W*CU*D		0.2270		0.0992		0.1156

¹ Data are means of four replicates.

² CU= Cultivar, W= soil moisture levels, and D= Depth.

³ SEM= Standard error of the mean.

Table 7.16: The turgor potential (MPa) of roots of sainfoin cultivars and lucerne at different depths at two soil moisture levels at early (45 days after planting, DAP), late (75 DAP), and regrowth (30 days after cutting, 105 DAP) harvests.

Depth (cm)	Early harvest		Late harvest		Regrowth	
	20-30	50-75	20-30	50-75	20-30	50-75
	Control					
Eski ¹	0.7565	0.1620	0.1010	0.2045	0.0678	0.1845
G35	0.0343	0.0298	0.1798	0.1008	0.0920	0.1950
Lucerne	0.0973	0.1450	0.0698	0.1665	0.1395	-0.0815
	Stress					
Eski	0.0003	0.0465	-0.2067	-0.1485	0.1873	0.1913
G35	-0.0293	-0.0413	0.1363	-0.0070	-0.0380	0.0773
Lucerne	0.0005	0.0520	0.0388	0.0313	0.1795	0.0860
Pr >F CU ²		0.2895		0.6314		0.2945
Pr >F W		0.1105		0.1275		0.7504
Pr >F W*CU		0.4810		0.5187		0.1147
Pr >F D		0.4101		0.8833		0.9138
Pr >F W*D		0.4402		0.2829		0.9050
Pr >F CU*D		0.5418		0.0580		0.0207
Pr >F C*W*D		0.3631		0.9299		0.4359
³ SEM CU		0.1106		0.0875		0.0313
SEM W		0.0903		0.0714		0.0383
SEM W*CU		0.1564		0.1238		0.0542
SEM D		0.0796		0.0228		0.0263
SEM W*D		0.1125		0.0322		0.0696
SEM CU*D		0.1378		0.0394		0.0456
SEM W*CU*D		0.1949		0.0557		0.0644

¹ Data are means of four replicates.

² CU= Cultivar, W= soil moisture level, and D= Depth.

³ SEM= Standard error of the mean.

7.3.3.10 Root osmotic potential at full turgor

Over all three harvests there were significant effects for soil moisture level, cultivar, and harvest (Appendix 7.12). Root π_{100} for the stressed plants was lower (-0.964 MPa) than for the control plants (-0.840 MPa). The π_{100} of lucerne was higher (-0.813 MPa) than for the sainfoin cultivars (-0.947 MPa). The late harvest had a lower π_{100} (-1.034 MPa) than that of the early (-0.893 MPa), or regrowth (-0.783 MPa) harvests (Table 7.16).

Separate analysis of data for each harvest showed root π_{100} was affected by soil moisture levels at the early and late harvests, and was lower (more negative) for stressed than control plants. Cultivars had different root π_{100} at only the late harvest, with lucerne and Eski having the highest and lowest (more negative) root π_{100} respectively (Table 7.17).

The root π_{100} for different depths was similar. No interaction effect was found at the early and late harvests, however, at the regrowth harvest the interactions of cultivar by depth, and cultivar by depth by soil moisture level were significant (Table 7.17). Sainfoin cultivars and lucerne had lower (more negative) root π_{100} at the deep and shallow depths respectively. Stressed plants of lucerne and sainfoin had the lowest (most negative) and highest root π_{100} at the shallow and deep depths, respectively, whereas for control plants this effect was reversed.

Table 7.17: The root osmotic potential at full turgor (MPa) of two sainfoin cultivars and lucerne at two depths and two soil moisture levels at early (45 DAP), late (75 DAP), and regrowth (30 days after cutting, 105 DAP) harvests.

Depth (cm)	Early harvest		Late harvest		Regrowth	
	20-30	50-75	20-30	50-75	20-30	50-75
	Control					
Eski ¹	-0.820	-0.820	-0.998	-1.049	-0.715	-0.820
G35	-0.948	-0.817	-0.933	-0.983	-0.863	-0.813
Lucerne	-0.775	-0.745	-0.770	-0.863	-0.718	-0.715
	Stress					
Eski	-1.060	-0.982	-1.213	-1.227	-0.855	-0.923
G35	-0.907	-1.010	-1.268	-1.235	-0.665	-0.843
Lucerne	-0.883	-0.945	-0.843	-1.023	-0.795	-0.673
Pr >F CU ²		0.4620		0.0496		0.5515
Pr >F W		0.0350		0.0262		0.8159
Pr >F W*CU		0.7108		0.6924		0.5692
Pr >F D		0.7959		0.0921		0.1960
Pr >F W*D		0.4025		0.9030		0.5977
Pr >F CU*D		0.8908		0.2712		0.0253
Pr >F C*W*D		0.4240		0.5594		0.0112
³ SEM CU		0.0538		0.0714		0.0547
SEM W		0.0439		0.0583		0.0669
SEM W*CU		0.0760		0.1009		0.0947
SEM D		0.0337		0.0238		0.0154
SEM W*D		0.0476		0.0337		0.0217
SEM CU*D		0.0583		0.0413		0.0266
SEM W*CU*D		0.0824		0.0584		0.0376

¹ Data are means of four replicates.

² CU= Cultivar, W= soil moisture level, and D= Depth.

³ SEM= Standard error of the mean.

7.3.3.11 Comparison of total, osmotic and turgor potential of root and leaf

7.3.3.11.1 Total potential of leaf-root

Analysis of root and leaf water potential at midday over harvests showed a significant effect of soil moisture level, harvest, location of sample [midday leaf or root (average of two depths)] on plant water potential. There were also interactions between soil moisture level and cultivar, cultivar and location of sample, and harvest and location of sample (Appendix 7.13). The total potential of stressed plants was lower (-1.26 MPa) than that of the control plants (-0.77 MPa) (Table 7.18). The plants at the early harvest had a higher (-0.83 MPa) water potential than the plants at the late (-1.10 MPa) or regrowth (-1.11 MPa) harvests.

The leaf water potential (-1.11 MPa) was lower than root water potential (-0.91 MPa) (Table 7.18). The difference between leaf water potential and root water potential of control plants was greater (0.28 MPa) than that of the stressed plants (0.12 MPa) (Table 7.18). The differences between leaf and root water potential at the late (0.23 MPa) and regrowth (0.36 MPa) harvests were greater than that at the early harvest (0.01 MPa).

At the early harvest the leaf and root water potential interacted with cultivar. The difference between leaf and root water potential of lucerne (0.203 MPa) was greater than for the sainfoin cultivars (0.07 MPa) (Table 7.18). At the late harvest, the interaction of leaf and root water potential with soil moisture was significant. The difference between leaf and root water potential of stressed plants (-0.02 MPa) was less than that of the control plants (-0.5 MPa) (Table 7.17).

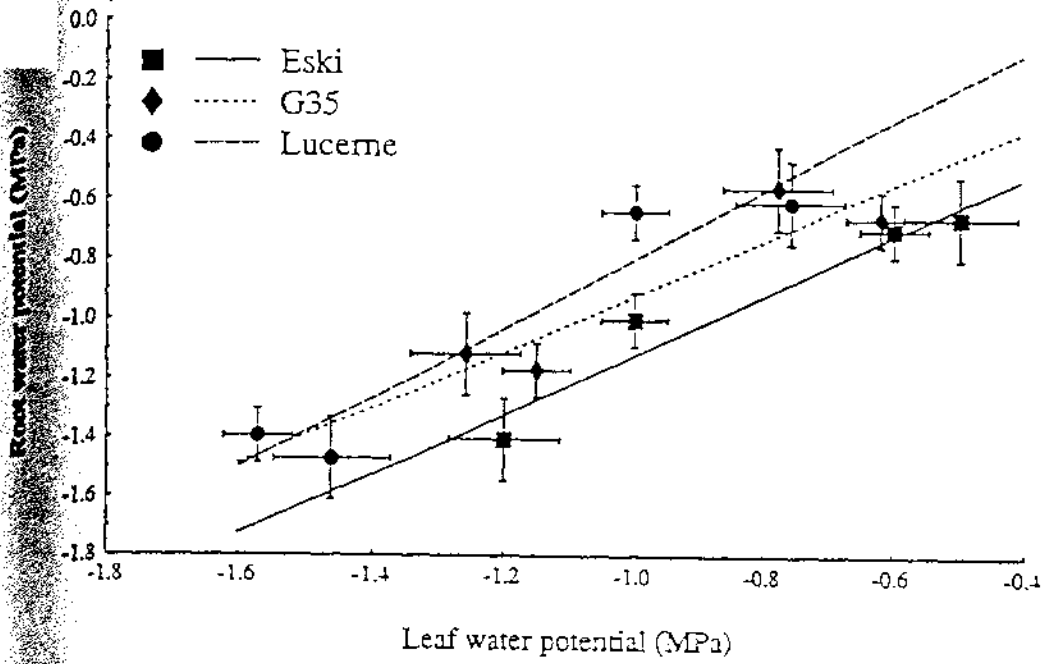


Fig. 7.9(a): Relationship between leaf and root water potential of lucerne, Eski, and Grasslands G35. Respective regression equations are $Y=0.28+1.1X$, $R^2=92.3\%$, $Y=0.47+1.5X$ $R^2=80\%$, $Y=-0.031+0.99X$ $R^2=99\%$.

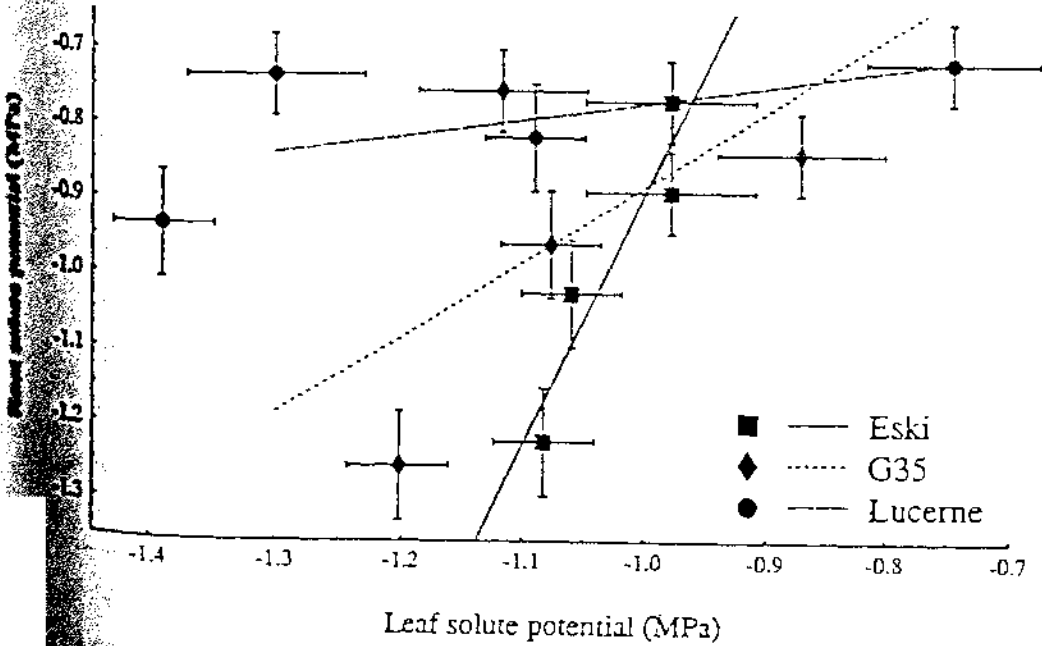


Fig. 7.9(b): Relationship between leaf and root osmotic potential at full turgor for lucerne, Eski, and Grasslands G35. Respective regression equations are $Y=-0.56+0.23X$ $R^2=42\%$, $Y=2.4+3.3X$ $R^2=87\%$, $Y=0.11+0.99X$ $R^2=33\%$.

Table 7.18 : The total water potential (MPa) of roots and leaves of sainfoin cultivars and lucerne at different depths for two soil moisture levels at early (45 days after planting), late (75 days after planting), and regrowth harvests.

Depth (cm)	Early harvest		Late harvest		Regrowth	
	Leaf	Root	Leaf	Root	Leaf	Root
Control						
Eski ¹	-0.711	-0.531	-1.240	-0.670	-0.994	-0.711
G35	-0.572	-0.819	-0.961	-0.571	-0.877	-0.673
lucerne	-0.698	-0.439	-1.011	-0.619	-1.103	-0.649
Stress						
Eski	-0.983	-1.069	-1.240	-1.407	-1.422	-1.013
G35	-0.996	-1.152	-1.196	-1.123	-1.413	-1.178
lucerne	-1.075	-0.929	-1.639	-1.475	-1.888	-1.398
Pr >F CU ²		0.5293	0.1382		0.0908	
Pr >F W		0.0001	0.0001		0.0001	
Pr >F W*CU		0.9510	0.2035		0.2122	
Pr >F L		0.7099	0.0073		0.0001	
Pr >F W*L		0.2693	0.0139		0.5008	
Pr >F CU*L		0.0035	0.1234		0.1172	
Pr >F C*W*L		0.2472	0.3865		0.8968	
³ SEM CU		0.0618	0.0784		0.0772	
SEM W		0.0504	0.0639		0.0630	
SEM W*CU		0.0874	0.1108		0.1091	
SEM L		0.0297	0.0554		0.0331	
SEM W*L		0.0419	0.0784		0.0468	
SEM CU*L		0.0514	0.0959		0.0573	
SEM W*CU*L		0.0727	0.1357		0.0811	

¹ Numbers in cultivars rows are mean of four replicates.

² CU=Cultivar, W= soil moisture levels, and L= Location of sample (Leaf or Root)

³ SEM= Standard error of the means.

7.3.3.11.2 Osmotic potential of leaf-root

Analysis of leaf and root data over all three harvests for osmotic potential showed significant effects for harvest ($P < 0.01$), soil moisture level ($P < 0.0001$), cultivar ($P < 0.05$) and location of sample (leaf or root) ($P < 0.0001$). Also the interactions of cultivar by soil moisture level ($P < 0.01$), cultivar by location of sample ($P < 0.01$), and cultivar by harvest by location of sample ($P < 0.01$) were significant (Table 7.18) (Appendix 7.13).

The osmotic potential of stressed plants (-1.37 MPa) was lower than that of control plants (-0.99 MPa). The osmotic potential of Grassland G35 was higher (-1.1 MPa) than that of Eski (-1.19 MPa) and lucerne (-1.25 MPa). The early harvest (-1.07 MPa) had a higher π than the late (-1.24 MPa) and regrowth (-1.23 MPa) harvests. Lucerne π of stressed plants was lower (-1.54 MPa) than that of the sainfoin cultivars (-1.28 MPa). Leaf π (-0.39 MPa) was greater than root π (-0.98 MPa). The difference between π of leaves and roots of lucerne (0.53 MPa) was greater than that for the sainfoin cultivars (0.31 MPa). The differences between π of leaves and roots for lucerne at the early harvest (0.6 MPa) was greater than that for the late (0.52 MPa) and regrowth (0.46 MPa) harvests. Similarly, the difference between leaf and root π for Grasslands G35 (-0.91 MPa) at the early harvest was greater than for the late and regrowth harvests (-0.36 and -0.31 MPa), respectively (Table 7.19).

Separate analysis at each harvest of leaf and root π found significant effects for soil moisture levels at the late and regrowth harvests with a more negative π for stressed than control plants (Table 7.19). Also, leaf and root π were significantly different at all three harvests, with more negative π for leaves than roots. The only interaction found was between soil moisture and location of sample, at the late harvest. The difference between π of the leaf and the root of the control plants (0.61 MPa) was greater than that for the stressed plants (0.23 MPa) (Table 7.19).

Table 7.19: The osmotic potential (MPa) of the roots and leaves of sainfoin cultivars and lucerne at two soil moisture levels at the early (45 days after planting), late (75 days after planting), and regrowth harvests.

Depth (cm)	Early harvest		Late harvest		Regrowth	
	Leaf	Root	Leaf	Root	Leaf	Root
Control						
Eski ¹	-1.157	-0.991	-1.459	-0.823	-1.223	-0.837
G35	-0.842	-0.851	-1.183	-0.712	-1.157	-0.816
Lucerne	-1.107	-0.559	-1.439	-0.711	-1.252	-0.678
Stress						
Eski	-1.339	-1.092	-1.400	-1.229	-1.503	-1.202
G35	-1.421	-0.967	-1.415	-1.187	-1.474	-1.197
Lucerne	-1.559	-0.904	-1.821	-1.511	-1.900	-1.531
Pt >F CU ²		0.4763		0.0200		0.2577
Pt >F W		0.0048		0.0001		0.0001
Pt >F W*CU		0.4822		0.0501		0.1329
Pt >F L		0.0005		0.0001		0.0001
Pt >F W*L		0.1025		0.0005		0.1468
Pt >F CU*L		0.1036		0.3043		0.2249
Pt >F C*W*L		0.5673		0.5715		0.7315
SEM CU		0.0775		0.0545		0.0786
SEM W		0.0633		0.0445		0.0642
SEM W*CU		0.1096		0.0771		0.1113
SEM L		0.0568		0.0313		0.0274
SEM W*L		0.0803		0.0443		0.0387
SEM CU*L		0.0984		0.0543		0.0475
SEM W*CU*L		0.1391		0.0767		0.0672

¹ Numbers in cultivars rows are mean of four replicates.

² CU= Cultivar, W= soil moisture levels, and L= Location of sample (Leaf or Root)

³ SEM= Standard error of the mean.

7.3.3.11.3 Osmotic potential of leaf and root at full turgor (π_{100})

Significant effects were found for π_{100} of leaves and roots for soil moisture level ($P < 0.0001$), harvest ($P < 0.0001$), and location of sample ($P < 0.05$) (Table 7.20). Also, there was an interaction between cultivar and sample location (Appendix 7.13). The π_{100} of stressed plants (-1.05 MPa) was lower than that of the control plants (-0.89 MPa) (Table 7.20). At the regrowth, harvest π_{100} was higher (-0.892 MPa) than that at the early (-0.96 MPa) and late (-1.06 MPa) harvest (Table 7.20). Over all three harvests leaf the π_{100} (-0.99 MPa) was lower than for roots (-0.832 MPa). The difference between full turgor π of lucerne was greater (0.3 MPa) than for sainfoin (-0.07 MPa) (Table 7.20).

Table 7.20: The osmotic potential (MPa) of roots and leaves of sainfoin cultivars and lucerne at full turgor at different depths at two soil moisture levels at the early (45 days after planting), late (75 days after planting), and regrowth harvests.

Depth (cm)	Early harvest		Late harvest		Regrowth	
	Leaf	Root	Leaf	Root	Leaf	Root
Control						
Eski ¹	-1.030	-0.823	-1.028	-1.020	-0.978	-0.770
G35	-0.908	-0.780	-1.010	-0.955	-0.870	-0.843
lucerne	-0.943	-0.763	-1.060	-0.818	-0.745	-0.715
Stress						
Eski	-0.940	-1.020	-1.105	-1.220	-0.978	-0.890
G35	-1.090	-0.955	-1.077	-1.253	-1.118	-0.755
lucerne	-1.320	-0.915	-1.293	-0.935	-1.303	-0.735
Pr >F CU ²		0.6554		0.5032		0.8336
Pr >F W		0.0025		0.0030		0.0033
Pr >F W*CU		0.2006		0.9207		0.0709
Pr >F L		0.0585		0.3612		0.0036
Pr >F W*L		0.9105		0.5577		0.0651
Pr >F CU*L		0.5041		0.0636		0.06210
Pr >F C*W*L		0.4430		0.5639		0.1271
³ SEM CU		0.0396		0.0405		0.0356
SEM W		0.0001		0.0001		0.0290
SEM W*CU		0.5611		0.0574		0.5030
SEM L		0.0568		0.0468		0.0451
SEM W*L		0.0804		0.0663		0.0638
SEM CU*L		0.0985		0.0811		0.0782
SEM W*CU*L		0.1393		0.1148		0.1106

¹ Numbers in cultivars rows are mean of four replicates.

² CU=Cultivar, W= soil moisture levels, and L= Location of sample (Leaf or Root)

³ SEM= Standard error of the mean.

7.5. Discussion

7.5.1 Soil moisture

Measurement of soil moisture level by different methods gave similar results. A positive relationship ($R^2=85\%$) between VSWC and GSWC showed that TDR was a useful method for measuring pot soil moisture (Fig 7.2). The slope of this relationship (1.4) was similar to actual soil bulk density (1.5g/cm^3) (Fig. 7.2). Since the VSWC in this comparison was from three depths through the profile it suggests a homogeneous soil bulk density. The accuracy of TDR measurements was also supported by the high correlation with total water content of the pots, measured directly by weighing ($R=94\%$).

The non-significant interaction of soil moisture treatments with soil depth during the regrowth harvest indicated soil moisture at the three different depths showed a similar pattern down the profile for the stressed and control pots, although the water content of the stressed pots was almost half that of the control pots. Pennypacker *et al.* (1990) developed a container using two tensiometer placed at depths of 30 and 67 cm, in which they watered the stressed pots when the lower tensiometer read -0.08 MPa. The disadvantage of their technique was that when they irrigated the stressed pots from the top some parts of the pot wetted to levels equal to the control pots, but other parts remained dry (Shantz 1925, Kramer 1980). Similar problems occurred for Carter *et al.* (1982), Jodari-Karimi *et al.* (1983), Blum *et al.* (1990, 1993).

Irrigation at seven depths during the regrowth period maintained a constant soil moisture through out the stressed pots. The constant root water potential and osmotic potential at different depths also demonstrated the soil moisture content through the pot was unchanged.

7.5.2 Morphology

Water stress decreased leaf area, leaf dry weight, and stem dry weight of lucerne proportionally more than sainfoin. The total reduction by water stress over all three harvests of lucerne dry weight was 34% whereas for Eski and G35 it was

12 and 13% respectively. Cowett and Sprague (1962) found a 67% reduction in the yield of stressed (soil moisture potential=1 MPa) lucerne plants. Relative to non-stressed plants, Peterson *et al.* (1992) found reduced yield of stressed plants (irrigation occurred when 75% of extractable soil moisture depleted) for lucerne, birdsfoot trefoil (*Lotus corniculatus* L.), cicer milkvetch (*Astragalus cicer* L.), and red clover (*Trifolium pratense* L.), 43.2, 54.3, 23.5, and 68.5 %, respectively.

Over three harvests water stress decreased the LA of lucerne 48% whereas the decrease for Eski and G35 was only 24, and 21% respectively. Acosta-Gallegos and Adams (1991) imposed water stress 40 to 57 days after emergence in bean (*Phaseolus vulgaris*) and found a 32% reduction in the LA of the stressed treatment. In this study water stress decreased LA and LDW of lucerne 48 and 34 %, respectively but only 22 and 2.5 % for the sainfoin cultivars respectively (Tables 7.1, 7.2).

Although water stress greatly decreased the herbage mass of the lucerne, the total dry weight of lucerne was still higher than that for sainfoin. Over all three harvests the control and water stressed treatments of lucerne had more herbage mass than those of sainfoin. Peterson *et al.* (1992) found the average yield of stressed and non-stressed lucerne was 2.1 and 3.8 * 10³ kg DW/ha whereas for birdsfoot trefoil it was 1.1, and 2.5*10³ kg DW/ha. The regrowth of lucerne was also higher than for sainfoin in agreement with Mir-Hosseini-Dehabadi *et al.* (1993a) (chapter 3) who found lucerne responded to water stress earlier than sainfoin.

Overall, the SLA of lucerne was greater than for the sainfoin cultivars. This was in agreement with Mir-Hosseini-Dehabadi *et al.* (1993a) (Chapter 3) and Sheehy and Pople (1981).

The greater effect of water stress on LA than on LDW resulted in a lower SLA for the stressed plants than for the control plants of both sainfoin and lucerne. The decrease in SLA due to water stress probably resulted from water stress affecting cell expansion more than cell division. Kriedeman (1986) pointed out that water stress affected cell enlargement earlier and to a greater extent than cell division. However, as leaf growth diminishes, photo-assimilates accumulate and

osmotic adjustment ensues. Plants adapted to water stress by decreasing SLA, decreasing the transpiration surface and increasing the photosynthesis efficiency.

7.5.2.1. Root growth

Initially (early harvest) roots were concentrated at the top of the soil profile, but as moisture stress developed at the top of the soil profile, roots grew to greater depth (Fig. 7.4, 7.5). Similar results were observed for lucerne in the field by Bennett and Doss (1960) and by Blum *et al.* (1991) in pots, but, in contrast, Carter *et al.* (1982) found no significant difference for root length and weight density distribution within the soil profile at different soil moisture regimes. The soil moisture regime and the duration of their experiment might have caused this contrast. Carter *et al.* (1982) irrigated the pots according to the average soil moisture at 45 cm and 90 cm depth. Since soil moisture at 45 cm depth was much higher than soil moisture at 90 cm depth the adjustment, however, might have been influenced by the soil moisture of 45 cm depth, rather than at 90 cm depth thereby resulting in a similar pattern of soil moisture as in the control treatments through the depths (0.04 MPa).

In dry conditions the surface layers of the soil often dry rapidly. As a result, a desirable plant needs the ability to quickly develop a root system to depth in order to ensure a continuing water supply for transpiration (Hurd 1968, Gregory 1989). Although the root length and mass of sainfoin and lucerne were similar at the early growth stages, sainfoin root length below 60 cm depth was significantly greater than for lucerne. At the early harvest sainfoin showed its adaptation to dry conditions by having a similar total root mass, stem dry weight and leaf dry weight to lucerne, but less leaf area and deeper roots relative to lucerne. Lucerne had a greater root length than sainfoin at the late harvest but this mostly was due to greater root mass at depths less than 40 cm. This suggests lucerne has a higher potential for the extraction of soil moisture during non-limiting soil water conditions.

G35 root distribution was less suited to dry conditions than Eski, since water stressed G35 had a higher root mass at 10-20 cm depth and a lower root

mass at 80-100 cm depth than Eski. However, Mir-Hosseini-Dehabadi *et al.* (1993a) (Chapter 3) found a higher root mass for G35 than for lucerne one month after imposing water stress at the flowering stage. This result possibly reflects the breeding of G35 for a temperate climate, whereas Eski originated under dry conditions (Turkey) and has outyielded lucerne under dryland conditions (Eslick *et al.* 1967).

The total root mass of the control plants of sainfoin and lucerne was less than that of the stressed plants at the late harvest. Bennett and Doss (1960) and Jordari-Karimi *et al.* (1983) also found a greater root mass for stressed than for control lucerne. The greater shoot:root ratio of lucerne (4.5) compared with sainfoin (3.2) implied a greater allocation of carbohydrate to the roots than the shoots of sainfoin relative to lucerne. Similarly, other investigators found that water stress decreased the shoot:root ratio of lucerne (Jodari-Karimi *et al.* 1983, and Mir-Hosseini-Dehabadi *et al.* 1993b (Chapter 4). The lower shoot:root ratio is a useful character under dry conditions since plant adjust the water demand (by leaf) and water supply (by roots).

7.5.3 Physiology

7.5.3.1 Relative water content

Water stress decreased RWC at all harvests without any significant differences between species or cultivars (Fig 7.6a). Stressed plants at the regrowth harvest had a lower RWC than at the other harvests. The lower RWC of stressed plants at the regrowth harvest compared with prior harvests could have been due to higher soil moisture of the stressed treatment below 50 cm soil depth at the early harvest (Fig. 7.3). The soil moisture of the stressed treatment of the last two harvests was similar (Fig. 7.3).

The greater response of RWC to water stress than Rs at all three harvests, indicated that RWC might be a better indicator of leaf water status than Rs. This was in agreement with Sinclair and Ludlow (1985) who noted that relative water content might provide an integrated response to the balance of water supply and transpiration.

7.3.2 Stomatal resistance.

Stomatal resistance was insensitive to initial changes in soil moisture. This was in contrast to results of other studies that found R_s responded to water stress independent of water status of the leaves (Bates and Hall 1981; Black *et al.* 1985; Goumbi 1985; Jensen *et al.* 1989). In the present study, R_s was affected by soil moisture only at the late and regrowth harvests, i.e. during severe water stress. This observation was supported by other investigators who found R_s increased only after a threshold Ψ or P was attained (Hsiao and Acevedo 1974; Turner 1974; Boardwell and Cohen 1975; Jordan *et al.* 1975; Brown and Jordan 1976; Radin and Jackson 1981; Teare *et al.* 1982; Bennett *et al.* 1984; Baldocchi *et al.* 1985; Markhart 1985).

The adaxial and abaxial stomatal resistance of the leaves were different between sainfoin and lucerne (Fig. 7.7). Carter *et al.* (1982) and Mir-Hosseini-Dehabadi (1993b) (Chapter 4) have noted the equal stomatal resistance of the abaxial and the adaxial leaf surfaces of lucerne. In sainfoin, relative to R_s of adaxial surface the R_s of the abaxial surface of the leaves was less affected by water stress. The adaxial R_s of sainfoin leaves was affected by soil moisture at the late and regrowth harvest whereas abaxial R_s was only affected at the regrowth harvest. A lesser response to water stress of the abaxial stomata of leaves to environmental conditions has been reported by Pallardy and Kozlowski (1979). The higher sensitivity of the adaxial R_s of sainfoin leaves than of lucerne, showed that relative to lucerne total R_s of sainfoin was influenced by adaxial R_s more than abaxial R_s , and suggested at extreme stress conditions sainfoin would be able to control transpiration better than lucerne.

7.3.3 Leaf water potential

Lucerne had lower (more negative) Ψ than sainfoin, and the difference in Ψ between midday and dawn was greater for lucerne than for sainfoin (Table 7.9 and 7.16). Castongvay and Markhart (1992) found that the leaf water potential of water stressed bean (*P. acutifolius*) was -0.36 MPa lower than the controls. The Ψ

of water stressed lucerne, Eski, and G35 at the late harvest was about 0.7, 0.66, and 0.48 MPa lower than the control, respectively (Table 7.9). The water stressed bean plants of Castongvay and Markhart (1992) had a soil water potential of -0.5 MPa compared with -0.4 MPa in this experiment.

Higher (less negative) Ψ would contribute to higher P at a given π and moderate the effects of water stress. The greater difference between the Ψ of the control and the water stressed lucerne than for sainfoin might have been caused by the higher leaf area of lucerne and higher transpiration demand. The root length differences of sainfoin and lucerne were not as great as for the LA which would have contributed to the higher water loss by lucerne relative to its water uptake compare to sainfoin. The difference between leaf Ψ and root Ψ of lucerne was higher than for sainfoin (Fig. 7.8). Since root Ψ of both species was similar, this difference was the result of higher leaf Ψ of lucerne (Fig. 7.8a). Since the Rs of sainfoin and lucerne was similar, (Table 7.8a) the lower leaf Ψ of lucerne indicated a greater flow rate of water to the air.

The midday leaf water potential of sainfoin and lucerne was lower (more negative) than at dawn. Brown and Tanner (1981) found that the midday leaf water potential of lucerne was lower than at dawn in non-stress conditions. The difference in the Ψ between dawn and midday at the early harvest was less than for the late and regrowth harvests, indicating the lesser ability of stressed plants to buffer diurnal changes in water supply.

A positive linear relationship was found between Ψ measured by Pressure bomb and Wescor ($R^2=84\%$). The Wescor measurement over-estimated Ψ relative to the Pressure bomb. Similar results were found in lucerne by Brown and Tanner (1981), and in cowpea by Petrie and Hall (1992).

7.5.3.4 Osmotic potential (π)

The osmotic potential of lucerne was usually lower than for sainfoin. Water stressed lucerne π at midday was 0.5 MPa lower (more negative) than for the control plants (Table 7.11), whereas this difference in sainfoin was 0.2 MPa (Table 7.11). These differences between species could be attributed to dehydration

of the leaves or to accumulation of solute in the cells (Turner and Jones 1980). The similar RWC of the species suggested that the contribution of cell dehydration to this effect was minor, rather, solute accumulation was probably the cause of the more negative lucerne π (Table 7.7). This was confirmed by π_{100} measured by Decagon (Table 7.13). Over three harvests π_{100} of the leaves of stressed lucerne at midday was 0.41 MPa whereas for sainfoin it was 0.089 MPa (Table 7.13).

Pennypacker *et al.* (1990) found osmotic adjustment of 0.12 MPa for lucerne leaves. The smaller osmotic adjustment of lucerne in the study of Pennypacker *et al.* (1990) may have been due to a higher soil moisture in their water stress treatment. They irrigated the stressed pots after four weeks of growth at soil water potential of -0.08 MPa, and 30 cm depth while in this study the stressed pots had a soil water potential of about -0.4 MPa throughout the soil profile. The higher osmotic adjustment enabled the plant to increase P at a given Ψ , and thereby maintain the growth and development of the plants (Turner and Burch 1983). Plants with a higher osmotic adjustment are more likely to tolerate the effects of water stress (Turner and Burch 1983).

Sainfoin, and lucerne P was similar, and although lucerne had a lower leaf Ψ than sainfoin, its greater osmotic adjustment moderated the effect of water stress to result in similar P.

Sainfoin had a higher leaf water potential and osmotic potential at the same RWC as lucerne. A higher elasticity of cell walls in sainfoin, than lucerne, might have caused this higher leaf water potential and osmotic potential at the same RWC (Fig.7.8). Water stressed lupin leaves showed no change in leaf elasticity (Turner *et al.* 1987), but Barker *et al.* (1993) found that the more elastic cell wall (low modulus of cell wall elasticity) of C3 grasses maintained turgor, despite loss of water (Fig. 7.6 a, b, and 7.8).

7.5.3.5 Root water status

Water stress had a greater effect on reducing lucerne root Ψ than sainfoin, the root water status of watered plants was similar. Root water potential followed the Ψ as it was lower for stressed than for control plants and also was lower for lucerne than sainfoin.

Solute accumulation of lucerne roots over all three harvests was less than for sainfoin, and did not follow the leaf solute accumulation (Fig 7.9 b). The osmotic potential at full turgor of sainfoin roots induced an osmotic potential of 0.136 MPa lower than that for lucerne. The lower π of sainfoin roots than lucerne enabled the water stressed sainfoin plants to maintain relatively higher root P at a given root water potential than lucerne, and induced root development (Green 1968).

At the regrowth harvest, lucerne and sainfoin had different root solute accumulation with depth. Lucerne and sainfoin roots accumulated more solute at higher and lower depths, respectively. The higher solute accumulation of sainfoin at depth could help the plant to uptake water and maintain the growth of roots at greater depth than lucerne.

Although root water potential was not affected by the water stress treatment at the early harvest, the leaf water potential of water stressed and control plants was different, suggesting the leaves were more responsive to water stress than the roots. The higher sensitivity of leaves than roots to water stress could have been due to environmental conditions they encountered. At midday, the air (above ground) was drier and warmer than the air around the roots.

The effect of water stress on root osmotic potential at full turgor at the early and the late harvests but not at regrowth harvest, might be related to higher non-structural carbohydrate storage in the roots at the first two harvests relative to the regrowth harvest (Thomas 1991). Smith (1962) found that lucerne and red clover attained high levels of total available carbohydrate reserves in roots at maturity (include non-structural carbohydrate). This accumulation reached a peak

at flowering (Reynolds *et al.* 1962; Smith 1962). Cooper and Watson (1968) found a similar increase in total available carbohydrate of lucerne roots up to 10% bloom, and then a decline with new growth. However, they found a different pattern in sainfoin, where total available carbohydrate in the roots increased until the seed had matured. This evidence shows that the higher non-structural carbohydrate of the roots at late harvest than at the regrowth harvests, and supports the lack of osmotic response to water stress on regrowth compared to early and late harvests.

The leaves had a lower (more negative) total water and osmotic potential and greater solute accumulation than roots (Fig. 7.9 a,b). This creates a water gradient in the plant along which water can flow.

In considering the whole plant water status of both species over all three harvests it was found that the differences in total water and osmotic potential of the shoot and root were greater for lucerne than sainfoin. This supports a greater water gradient in lucerne and possibly greater water use.

7.5. Conclusion

A similar distribution of soil moisture through depth for the water stressed and control treatments was achieved by application of water at seven depths, in a long tube based on TDR measurements at three depths. The suitability of TDR for measurement of volumetric soil water content was confirmed by gravimetric soil water content, and pot weight.

The total root mass and density of the water stressed sainfoin and lucerne were higher than the controls at 75 days after imposing water stress. Water stress stimulated the root growth of sainfoin and lucerne at depths below 0.6 m. Although root mass and length of both species were similar, sainfoin developed roots below 0.6 m earlier than did lucerne, although Lucerne had higher root mass at 0-0.4 m than sainfoin suggesting lucerne roots are less suited to water deficits than sainfoin.

Lucerne was affected by soil moisture more than sainfoin. Osmotic adjustment occurred in both species. Lucerne had greater osmotic adjustment than sainfoin in its leaves, but in the roots sainfoin had the greater osmotic adjustment.

Sainfoin showed a lower S/R ratio and specific leaf area than lucerne, and the stomatal resistance of the adaxial and abaxial leaf surfaces was different in sainfoin but similar in lucerne.

Chapter 8

Water relationships of lucerne and sainfoin cultivars in the field.

8. Water relationships of lucerne and sainfoin cultivars in the field.

8.1 Abstract

The physiological and morphological characteristics of the sainfoin cultivars Eski, and Remont, and lucerne were examined in the field under progressive drought stress.

A rain-out shelter was used to impose water stress for four months. Relative water content (RWC), leaf water potential (Ψ), osmotic potential (π), and osmotic potential at full turgor (π_{100}) were measured at pre-dawn and midday. Stomatal resistance and photosynthetic rate were measured only at midday .

Water stress decreased leaf dry weight (LDW), stem dry weight, and leaf area (LA), but increased specific leaf area (SLA) relative to the control. Relative to control plants the yields of water stressed sainfoin and lucerne were decreased 50, and 42% respectively. Lucerne out yielded sainfoin in both water stressed and non-stressed conditions, through higher LA, stem dry weight, and stem density.

Eski and Remont had different growth patterns during the season. Eski grew slower than Remont early in the season, but the total yield of both cultivars over the season was similar. Lucerne showed lower (more negative) Ψ and π than sainfoin at pre-dawn and midday. By the end of the experiment, lucerne Ψ , π , RWC reached -2.52, -2.78 MPa, and 66.3% respectively.

Stomatal resistance and photosynthetic rate were equal for sainfoin and lucerne. Water stress increased the stomatal resistance of both species. The abaxial leaf surface of sainfoin had a higher stomatal resistance than the adaxial surface in sainfoin, but the two surfaces were similar for lucerne. This difference was partly related to differences in the stomatal density of the two species.

Over three harvests, sainfoin showed an adaptation to water stress by maintaining high Ψ , osmotic adjustment, overnight recovery of Ψ producing a high proportion of its yield early in the season, and was thus affected by water stress less than lucerne.

8.2 Introduction

In the previous experiments the responses of sainfoin cultivars and lucerne were studied in controlled environments and showed the physiological and morphological adaptation of sainfoin to water stress. The ability of sainfoin cultivars for adaptation to water deficit was not similar. Since the variability of temperature, relative humidity, and light intensity was less in the controlled environment than outdoors, plants might respond to water stress differently in the outdoors environment. Therefore, a field experiment was carried out to investigate the responses of two sainfoin types (one-cut, and two-cut) to water stress in a more natural environment.

Sainfoin is a forage legume adapted to dry conditions. It is being used for forage production under the dry conditions of the western United States (Bolger 1988), and Iran. Sainfoin provides earlier spring grazing or hay production than lucerne (Melton, 1973). Cooper and Roath (1965) and Murray and Slinkard (1968) reported that sainfoin consistently out yielded lucerne in areas where production was limited to one harvest. Two different growth types have been found in sainfoin. Eski (Eslick *et al.* 1967) is a one-cut type and produces most of its yield in a single harvest. Remont (Carleton and Delaney 1972) is a two-cut type and has a more uniform yield distribution, and it recovers more rapidly following defoliation than Eski (Krall *et al.* 1971). Cooper (1972b) found Remont yield was significantly higher than for Eski in late spring (May 12), but, because of a greater relative growth rate (g/g/wk), Eski surpassed Remont yield in early summer (June 21).

Despite numerous studies on water relationships of lucerne, very little information on the water relationships of sainfoin is available. Koch *et al.* (1972) showed that water use efficiency (WUE) of sainfoin was high at the first cutting, but low for the second cutting due to the slow regrowth, and Bolger *et al.* (1990) found that sainfoin had maximum WUE and yield at the first two harvests.

The main objective of this study was to compare the response of sainfoin and lucerne to progressive development of water stress in the field during the growing season. The morphological and physiological responses to water stress of the two species were regularly monitored.

8.3 Materials and Methods

8.3.1 Site and treatments

Two sainfoin cultivars (Remont and Eski) and lucerne (Grasslands Oranga) were established on 3 April 1992 at the Massey University Pasture and Crop Unit (Moginie) (175° 37' longitude, 40° 21' latitude, and 30 m altitude). The soil was Tokamaru silt loam classified as an aeric fragiaqualf (gleyed yellow-grey earth) (Cowie 1978, Scotter et al 1979a) and has been characterised in detail by Pollock (1975). Moisture characteristics of the soil are presented in Appendix 8.1. Seed was inoculated with the appropriate rhizobium (NZDS454) and sown in four 1 m rows 25 cm apart at seeding rates adjusted to achieve one viable seed per cm of row (40 kg/ha pure live seed for sainfoin and 3 kg/ha pure live seed for lucerne).

Two experiments were conducted, one under (stressed) and one out side (non-stressed) a rain-out shelter (Plates 8.2). The treatments were three cultivars/species and three harvests (at monthly intervals). The area of each plot was 2 m² (1.25m x 1.6 m). Water stress was imposed using a fully automatic rain-out shelter that moved over the stressed experiment within 30 s of the onset of rain (Plate 8.1,2). Plastic sheet was buried to 1 m which prevented lateral flow of soil moisture into the stressed experiment. Soil moisture in the non-stressed experiment was maintained near field capacity (Scotter *et al.* 1979b, Scotter 1976) by natural rainfall (Appendix 8.2) which was supplemented by approximately 20 mm irrigation on each of eight occasions. The experimental area was surrounded by pasture (ryegrass and white clover) to minimize variation in microclimate within the experimental area (Plate 8.2). On 1 November 1992 plants were harvested to 3 cm height and the water stress treatment was imposed. Plots for the three main harvests were harvested after 1, 2, or 3 months growth, with the first harvest on 1 December 1992. Plots of the first and second harvest were again harvested after 35 days regrowth (plate 8.3).

The air temperature and relative humidity was measured at the experimental area using a thermohydrograph, and monthly rainfall and soil temperature was measured at Ag-Research Grasslands (500 m from experimental area) (Appendix 8.2).



(a)



(b)

Plate 8.1: a) Rain out shelter for imposing water stress in the field b) a view of field experiment during establishment.

Water relationships of sainfoin and lucerne.....



(a)



(b)

Plate 8.2: A view of a) non-stressed plants and b) stressed plants in the field just after harvest one.

8.3.2 Measurements

8.3.2.1 Soil water content

The volumetric soil water content was measured by TDR (Topp and Davis 1985; Dasberg and Dalton 1985) to 0.15, 0.30, 0.50, and 0.70 m depth at two sites located at the centre of the plots within each replicate for both experiments.

8.3.2.2. Morphological measurement

The middle (1 m) of the two centre rows within each plot was harvested for yield and yield components. The leaves of harvested plants were separated from the stem, and leaf area (LA) measured by planimeter (Li-Cor Inc. Model 3100). The stem density was determined from the harvested area. Stem and leaf dry weight (LDW) were determined after 24 h at 70 °C.

8.3.2.3 Physiological measurements

The total leaf water potential (Ψ) was measured by psychrometer (Wescor HR33T Dew Point micro voltmeter) and pressure chamber (Model 3005 Soil Moisture Equipment Corp) (Turner 1981), osmotic potential (π), stomatal resistance (R_s), relative water content (RWC), and photosynthetic rate (P_n) were measured as described in Chapter 6. The leaf osmotic potential at full turgor (π_{100}) was measured by Decagon as described in Chapter 7. Turgor potential was calculated from differences between total potential and osmotic potential, and was not adjusted for possible dilution by apoplastic water. All physiological measurements were made at weekly intervals at dawn and midday during the month before harvesting for the three main harvests and at two 15 intervals prior to the regrowth harvests, with the exception of P_n which were only measured at midday.

The stomatal frequency of the abaxial and adaxial surfaces of sainfoin and lucerne leaves was estimated using fully expanded leaves at the top of the canopy. The epidermis was coated by a cellulose acetate film which was then removed from the leaf surfaces (Professor R.G. Thomas pers comm). A grid (10 x 10 mm) was used

to count the stomatal frequency per unit area (cm^2) of the cellulose acetate peel using a magnification of 400 times. A stage micrometer was used for calibration of the grid. The stomatal frequency of the adaxial and abaxial surfaces of nine leaflets from each of Eski, Remont, and lucerne was determined.

8.3.3 Statistical analysis

A randomised complete block design of four replicates was used, each block comprised a factorial arrangement of three cultivar and species and three harvests for the two separate experiments, inside (stressed), and outside (non-stressed) the shelter.

Pooled analysis of variance was done over three harvests and two experiments for morphological and physiological measurements, at dawn and midday.

Additional analyses of variance pooled over the two experiments were done for each of the three harvests and two regrowth treatments. The General Linear Models (GLM) procedure of SAS (SAS institute Inc. 1991) was used for analysis of variance.

Repeated measures analysis (Rowell and Walters 1976) was used to compare the first or second harvest with its related regrowth harvest.

Changes in soil moisture at four depths from both experiments were plotted against time, also the depletion of soil moisture over time was drawn by linear regression. The soil moisture of the stressed and non-stressed experiment at each depth for all three harvests and the regrowth from the first and second harvest were compared by t-test (Steel and Torrie 1981).

8.4 Results

8.4.1 Soil moisture

The volumetric soil water content (VSWC) of all depths decreased over time for the stressed experiment (Fig 8.1 a,b). The depletion was greatest at 0-0.15 m depth (from 34.5 to 11.4%), and smallest at 0.50-0.70 m depth (from 36.6 to 32.5%) (Appendix 8.3).

In the non-stressed experiment, initially the high rainfall (Appendix 8.2) caused higher VSWC at 0-15 cm depth than 50-70 cm depth (Appendix 8.3). In general the VSWC of the non-stressed experiment was higher than 34% at all depths.

At all harvests VSWC of 0-15 cm depth was significantly different ($P < 0.05$) between stressed and non-stressed experiments. Soil moisture of the other depths for stressed and non-stressed experiments were not significantly different at the first harvest, except 50-70 cm depth while the other depths had different ($P < 0.05$) soil moisture for the rest of the experiment (Appendix 8.3). The average VSWC of 0-70 cm depth showed significantly different ($P < 0.05$) soil moisture for stressed and non-stressed experiments at late and regrowth harvests (Table 8.1).

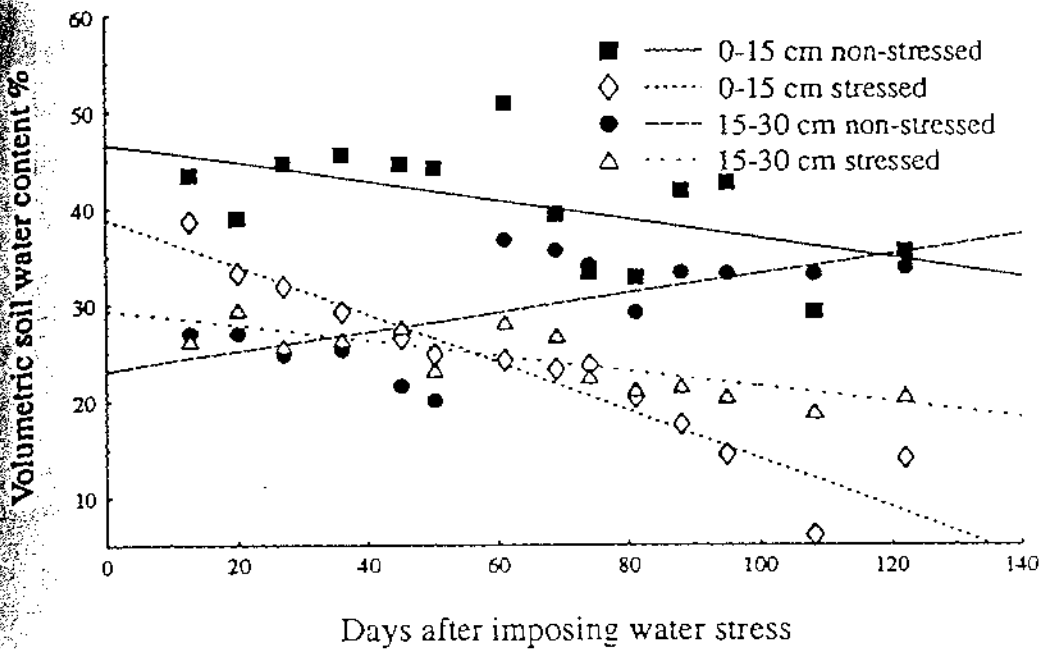


Fig 8.1a: The volumetric soil water content of stressed and non-stressed experiments at 0-15, and 15-30 cm depths during 140 days water stress. The regression equations for 0-15 cm depth of non-stressed and stressed are $Y=47-0.1*X$ $R^2=30\%$, $Y=39-0.25*X$ $R^2=40\%$, $Y=29.4-0.1*X$ $R^2=67\%$ respectively. Markers are means of eight replicates.

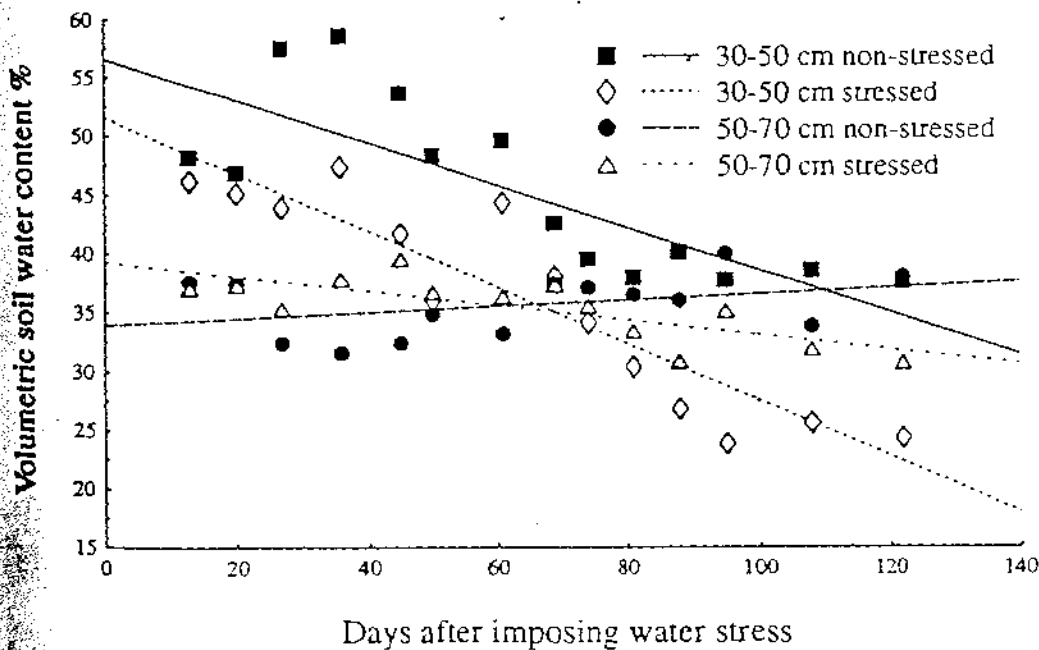


Fig 8.1b: VSWC of the stressed and non-stressed experiment at 30-50cm and 50-70 cm depth during 140 days water stress. The regression equations for 30-50 and 50-70 cm depths for non-stressed and stressed experiment are $Y=57-0.2*X$, $R^2=63\%$, $Y=52-0.24*X$ $R^2=855$, $y=34+0.03*X$, $R^2=11.3\%$, $Y=39-0.06*X$ $R^2=60\%$. Markers are means of eight replicates.

Table 8.1: The average volumetric soil moisture (cm^3/cm^3 %) to 0-70 cm depth for the stressed and non-stressed experiments for three harvests, and regrowth following the second harvest.

	Non-stressed		Stressed		Pr>t
	Mean	SEM ¹	Mean	SEM	
Harvest 1	38.7	0.67	35.8	0.84	0.0529
Harvest 2	38.3	0.99	33.0	1.52	0.0435
Harvest 3	37.8	1.45	28.8	1.64	0.0033
Regrowth 2	36.0	1.36	22.1	0.81	0.0009

¹ SEM= Standard error of the mean.

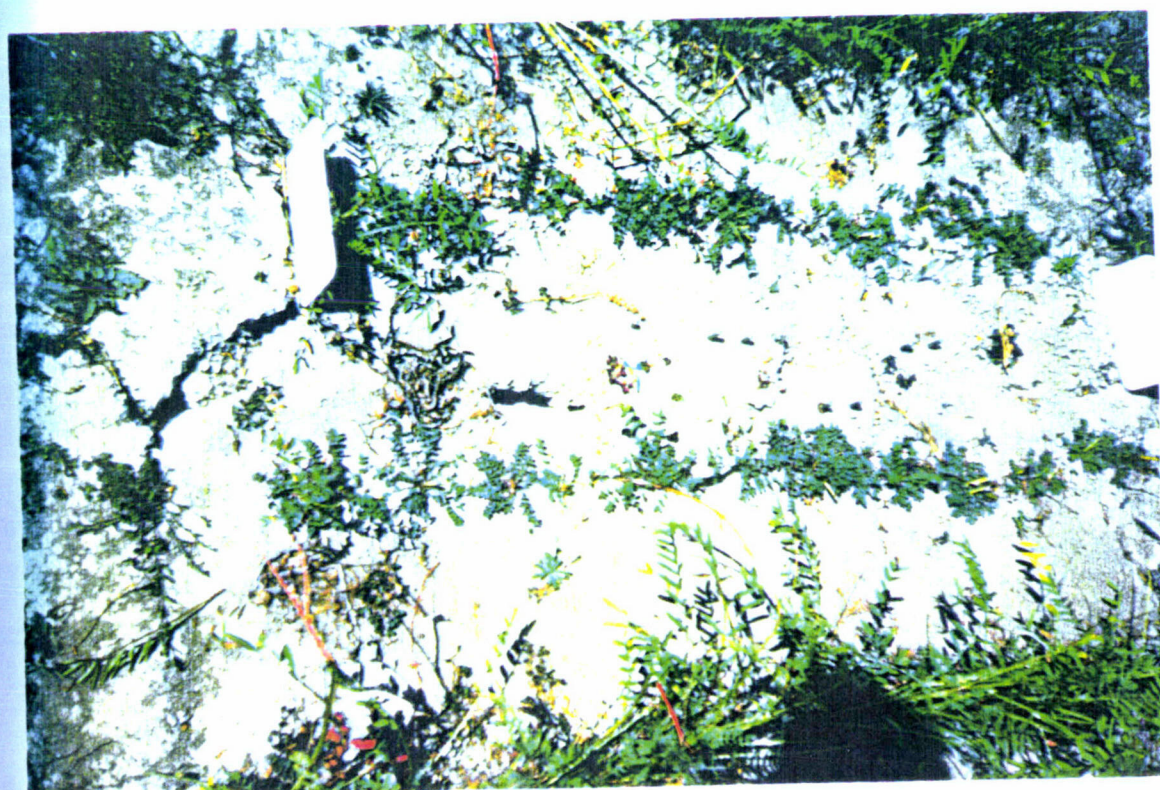
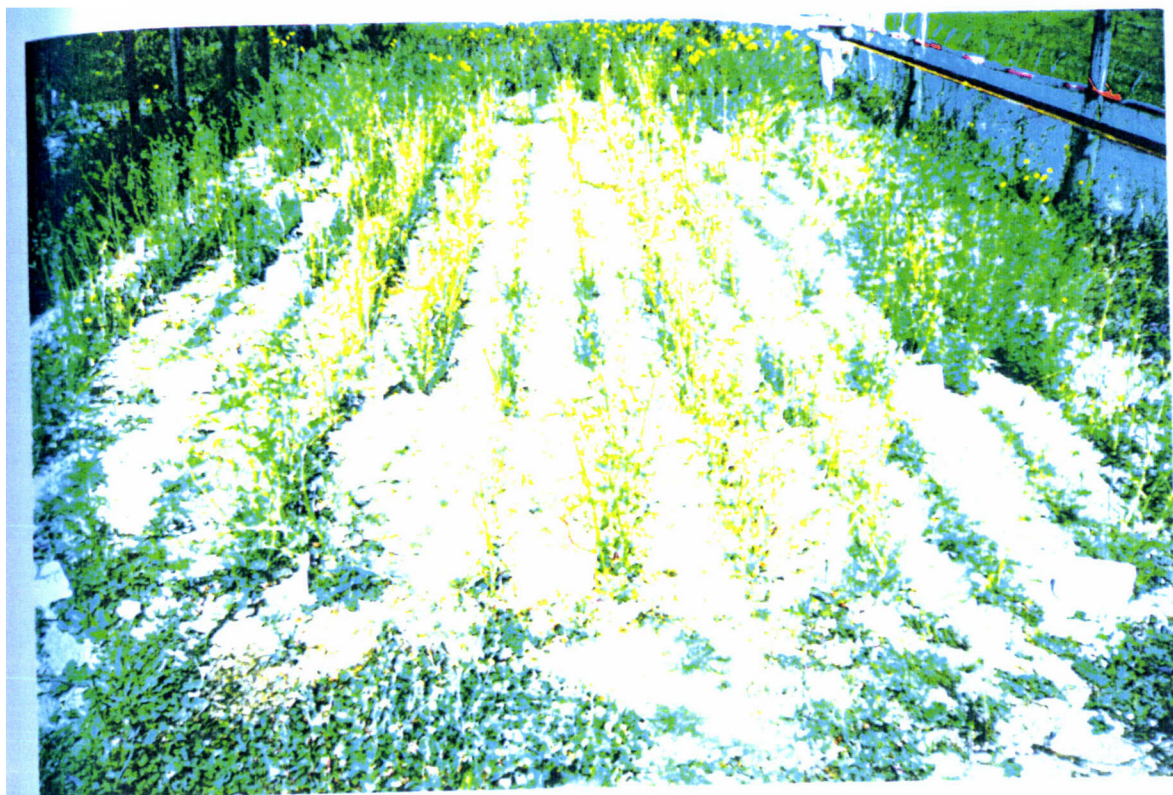


Plate 8.3: Stressed plants under rain-out shelter 140 days after imposing water stress (top) and, soil cracking and impaired sainfoin growth (bottom).

8.4.2 Morphological measurement

8.4.2.1 Leaf area

Over three growth harvests, leaf area was significantly affected by harvest and soil moisture. The interaction of cultivar by harvest was also significant (Table 8.2). The stressed plants had a lower LA than the non-stressed plants (Table 8.2). Remont had a higher LA than Eski at the first and third harvests, but at the second harvest the LA of Eski was higher than for Remont (Table 8.2). The lucerne LA was approximately twice that of Eski and Remont (Table 8.2). The second harvest (60 days after stress imposed) had a higher LA than the first and third harvests.

Repeated measures analysis at the first and second harvests showed that their LA was significantly greater than for their respective regrowth harvests. All interactions were significant except for the interaction of harvest time by soil moisture (Appendix 8.4). The difference between LA of growth and regrowth plants for lucerne was higher than for Eski and Remont. The difference between the LA of lucerne at the Growth 1 and Regrowth harvests was lower than the difference between the LA of the Growth 2 and Regrowth 2 harvests.

8.4.2.2 Leaf dry weight (LDW)

Over the three growth harvests LDW was significantly affected by harvest, cultivar, and soil moisture. None of the interactions were significant (Table 8.3). The third and second harvests had the lowest and the highest LDW respectively, and lucerne had a higher LDW than the sainfoin cultivars. Sainfoin cultivars had similar LDW. The LDW of the water stressed plants was lower than that of the non-stressed plants (Table 8.3).

Repeated measures analysis showed LDW of the growth harvests was less than the regrowth harvests ($P < 0.0001$) (Appendix 8.4). The interaction of time by cultivar was significant ($P < 0.01$) (Appendix 8.4). The difference in LDW between lucerne growth and regrowth was higher than for the sainfoin cultivars.

Table 8.2: Leaf area index (m^2/m^2) of Eski, Remont, and lucerne at five harvests under non-stressed and stressed experiments.

	Growth 1 (30 DS ¹)	Growth 2 (60 DS)	Growth 3 (90 DS)	Regrowth 1 (65 DS)	Regrowth 2 (95 DS)
	Non-stressed				
Eski	0.4645	1.2203	0.7942	0.8500	1.0968
Remont	0.7520	1.0289	0.7606	0.4422	1.5591
Lucerne	1.4723	2.8007	1.9275	1.4072	1.8334
	Stressed				
Eski	0.3917	0.9784	0.4321	0.8639	0.0852
Remont	0.6518	0.7975	0.4145	0.6187	0.1730
Lucerne	0.8930	2.0989	0.8952	0.7576	0.5300
Pr>F W ²	0.0169	0.0231	0.0005	0.1777	0.0001
Pr>F C ³	0.0015	0.0001	0.0006	0.0106	0.1007
Pr>F W*C	0.2317	0.4498	0.0831	0.0966	0.7479
SEM ⁴ W	0.0851	0.1287	0.0963	0.1107	0.1384
SEM C	0.1051	0.1557	0.1165	0.1308	0.1687
SEM W*C	0.1481	0.2194	0.1640	0.1822	0.2718

¹ DS= Days after imposing soil moisture stress.

² W= Soil moisture level.

³ C= Cultivar

⁴ SEM= Standard error of the mean

Pooled analysis over three harvests showed Pr>F H =0.0001, SEM H=0.0858, Pr>F C=0.0001, SEM C =0.0858, Pr>F C*H=0.0120, SEM C*H=0.1484, Pr>F W=0.0001, SEM W=0.0713, Pr>F W*H=0.3996, SEM W*H=0.1218, Pr>F W*C=0.0651, SEM W*C=0.1218, Pr>F W*C*H=0.9602, SEM W*C*H= 0.2095

Table 8.3: Leaf dry weight (g/m²) of Eski, Remont, and lucerne at five harvests under non-stressed and stressed experiments (see text for details).

	Growth 1 (30 DS ¹)	Growth 2 (60 DS)	Growth 3 (90 DS)	Regrowth 1 (65 DS)	Regrowth 2 (95 DS)
Non-stressed					
Eski	29.63	90.50	15.53	42.85	65.86
Remont	43.54	71.48	9.82	22.62	106.05
Lucerne	76.06	148.38	9.82	54.22	111.74
Stressed					
Eski	23.55	73.18	12.03	56.27	17.41
Remont	36.11	58.67	9.82	35.65	22.75
Lucerne	48.42	95.55	9.82	39.34	45.33
Pr>F W ²	0.0208	0.0023	0.0081	0.9635	0.0003
Pr>F C ³	0.0026	0.0005	0.0030	0.0174	0.1119
Pr>F W*C	0.2790	0.1202	0.5315	0.1271	0.5578
SEM ⁴ W	4.25	6.05	6.53	4.47	8.78
SEM C	5.25	7.30	7.90	5.29	10.66
SEM W*C	7.40	10.32	11.14	7.28	15.66

¹ DS= Days after imposing soil moisture stress.² W= Soil moisture level.³ C= Cultivar⁴ SEM= Standard error of the mean

Pooled analysis over three harvests showed Pr>F H =0.0001, SEM H=4.8, Pr>F C=0.0001, SEM C =4.85, Pr>F C*H=0.3238, SEM C*H=8.30, Pr>F W=0.0001, SEM W=3.98, Pr>F W*H=0.4832, SEM W*H=6.76, Pr>F W*C=0.1081, SEM W*C=6.76, Pr>F W*C*H=0.9077, SEM W*C*H=11.77.

8.4.2.3 Stem dry weight (Stem DW)

The stem DW was significantly affected by harvest, cultivar, and soil moisture over the first three harvests (Table 8.4). The interaction of cultivar by harvest ($P < 0.0001$), and soil moisture by harvest were also significant (Table 8.4). The stem DW was lowest and highest for the first and third harvests, respectively. Lucerne had a higher stem DW than the sainfoin cultivars. The stem DW of the sainfoin cultivars was similar. Water stress decreased the stem DW of the stressed plants to approximately half that of the non-stressed plants. The difference between the stem DW of the stressed and non-stressed plants was least at the first harvest and greatest at the third harvest (Table 8.4).

Repeated measures analysis showed the stem DW of the regrowth plants was lower than for the growth plants (Appendix 8.4). The interaction of time by harvest was also significant. At the first regrowth harvest, the stem DW of the plants was higher than that of the growth plants, whereas at the second harvest the stem DW of the regrowth plants was approximately half that of the growth plants (Table 8.4).

8.4.2.4 Stem density

Over the first three harvests, stem density was significantly affected by harvest, cultivar, and soil moisture treatments. The interactions of cultivar by harvest and soil moisture by cultivar were also significant (Table 8.5). The first and third harvests had the lowest and highest stem density, respectively (Table 8.5). Among cultivar/species lucerne had the highest and Eski the lowest stem density. The stressed plants had a lower stem density than the non-stressed plants. Relative to the first harvest, lucerne stem density increased by about 100% at the third harvest whereas Remont increased by only 18% (Table 8.5). Soil moisture stress decreased the stem density of lucerne and Eski by up to 40%, while it decreased Remont by only 7%.

Table 8.4: Stem dry weight (g/m^2) of Eski, Remont, and lucerne at five harvests under stressed and non-stressed experiments (see text for details).

	Growth 1 (30 DS ¹)	Growth 2 (60 DS)	Growth 3 (90 DS)	Regrowth 1 (65 DS)	Regrowth 2 (95 DS)
	Non-stressed				
Eski	10.44	192.42	254.77	57.24	37.91
Remont	36.99	192.98	165.4	32.77	110.93
Lucerne	75.92	259.55	446.4	88.03	214.43
	Stressed				
Eski	10.78	105.95	90.04	49.70	1.72
Remont	27.31	108.17	118.37	47.41	11.60
Lucerne	38.98	119.75	231.48	66.94	51.24
Pr>F W ²	0.0227	0.0001	0.0037	0.7972	0.0001
Pr>F C ³	0.0017	0.1213	0.0023	0.0408	0.0002
Pr>F W*C	0.1367	0.3130	0.1704	0.5282	0.0131
SEM ⁴ W	5.12	12.19	27.26	9.31	9.61
SEM C	6.32	14.71	32.97	10.99	11.72
SEM W*C	8.921	20.78	46.47	14.83	16.57

¹ DS= Days after imposing soil moisture stress² W= Soil moisture level.³ C= Cultivar⁴ SEM= Standard error of the mean

Pooled analysis over three harvests showed Pr>F H =0.0001, SEM H=12.4, Pr>F C=0.0001, SEM C =12.5, Pr>F C*H=0.0005, SEM C*H=21.42, Pr>F W=0.0001, SEM W=10.29, Pr>F W*H=0.0026, SEM W*H=17.58, Pr>F W*C=0.0508, SEM W*C=17.58, Pr>F W*C*H=0.3240, SEM W*C*H= 30.12

Table 8.5: The stem density (stems/m²) of Eski, Remont, and lucerne at five harvests under non-stressed and stressed experiments (see text for details).

	Growth 1 (30 DS ¹)	Growth 2 (60 DS)	Growth 3 (90 DS)	Regrowth 1 (65 DS)	Regrowth 2 (95 DS)
	Non-stressed				
Eski	91	155	230	142	27
Remont	156	183	219	114	135
Lucerne	440	585	899	450	516
	Stressed				
Eski	33	137	120	139	4
Remont	222	145	170	166	13
Lucerne	205	370	575	410	603
Pr>F W ²	0.1068	0.0016	0.0001	0.7898	0.3865
Pr>F C ³	0.0003	0.0001	0.0001	0.0003	0.0001
Pr>F W*C	0.0899	0.0087	0.0039	0.6509	0.0190
SEM ⁴ W	24.62	16.18	20.18	29.18	13.8
SEM C	31.03	16.06	24.4	34.63	16.9
SEM W*C	43.54	29.78	34.40	48.25	23.91

¹ DS= Days after imposing soil moisture stress

² W= Soil moisture level.

³ C= Cultivar

⁴ SEM= Standard error of the mean

Pooled analysis over three harvests showed Pr>F H =0.0001, SEM H=16.3, Pr>F C=0.0001, SEM C =16.3, Pr>F C*H=0.0001, SEM C*H =28, Pr>F W=0.0001, SEM W=13.5, Pr>F W*H=0.0600, SEM W*H=22.5, Pr>F W*C=0.0001 SEM W*C=22.5, Pr>F W*C*H=0.7867, SEM W*C*H=39.5

Repeated measures analysis showed no significant difference in the stem density of the growth and regrowth plants, but the interactions of time by harvest, time by cultivar, and time by soil moisture were significant (Appendix 8.4). At the first regrowth harvest the stem density was higher than the growth harvest, while for the second regrowth harvest the stem density was lower than the growth harvest. Lucerne regrowth had a higher stem density than the growth harvest, but sainfoin cultivars had a lower stem density for the regrowth than growth harvests. Regrowth harvests had a lower stem density in the non-stressed treatment, than the water stressed treatment (Table 8.5).

8.4.2.5 Yield

Yield was significantly affected by harvest, cultivar, and soil moisture (Table 8.6). The interactions of cultivar by harvest ($P < 0.01$), soil moisture by harvest ($P < 0.01$), and soil moisture by cultivar ($P < 0.01$) were also significant.

The yield of lucerne was higher than for sainfoin, but was not significantly different between sainfoin cultivars ($P < 0.05$). The yield of the stressed plants was lower than the non-stressed plants (Table 8.6). The yield differences between the stressed and non-stressed plants were highest at the third harvest and lowest at the first harvest. Lucerne had the highest yield at all three harvests. At the first harvest the lowest yield was for Eski which was about 1/2, and 1/3 of the Remont and lucerne yield, respectively (Fig. 8.2). The lucerne yield was affected by soil moisture more than the sainfoin cultivars.

Repeated measures analysis of the yield of the growth and regrowth plants showed a significant effect of time. The interactions of time by harvest, time by cultivar, and time by cultivar by harvest, were also significant (Appendix 8.4).

The first and second regrowth harvests had a lower yield than the growth harvests, but the lower yield of regrowth was mostly due to the second regrowth harvest. At the second regrowth harvest the yield of the plants decreased to approximately half that of the growth harvests.

8.4.2.6 Specific leaf area (SLA)

The SLA over the first three harvests was significantly different for harvest and cultivar (Table 8.7). The SLA at the third harvest was lower than at the first and second harvests. Lucerne had a higher SLA than the sainfoin cultivars (Table 8.7).

Repeated measures analysis showed the only significant effects were for the interactions of time by harvest, and time by soil moisture. At the first regrowth harvest plants had a higher SLA than the growth harvest, while SLA at the second regrowth harvest was lower than for the growth harvest. The SLA at the first and second harvests was not affected by soil moisture whereas the regrowth stressed plants had lower SLA than that of non-stressed plants.

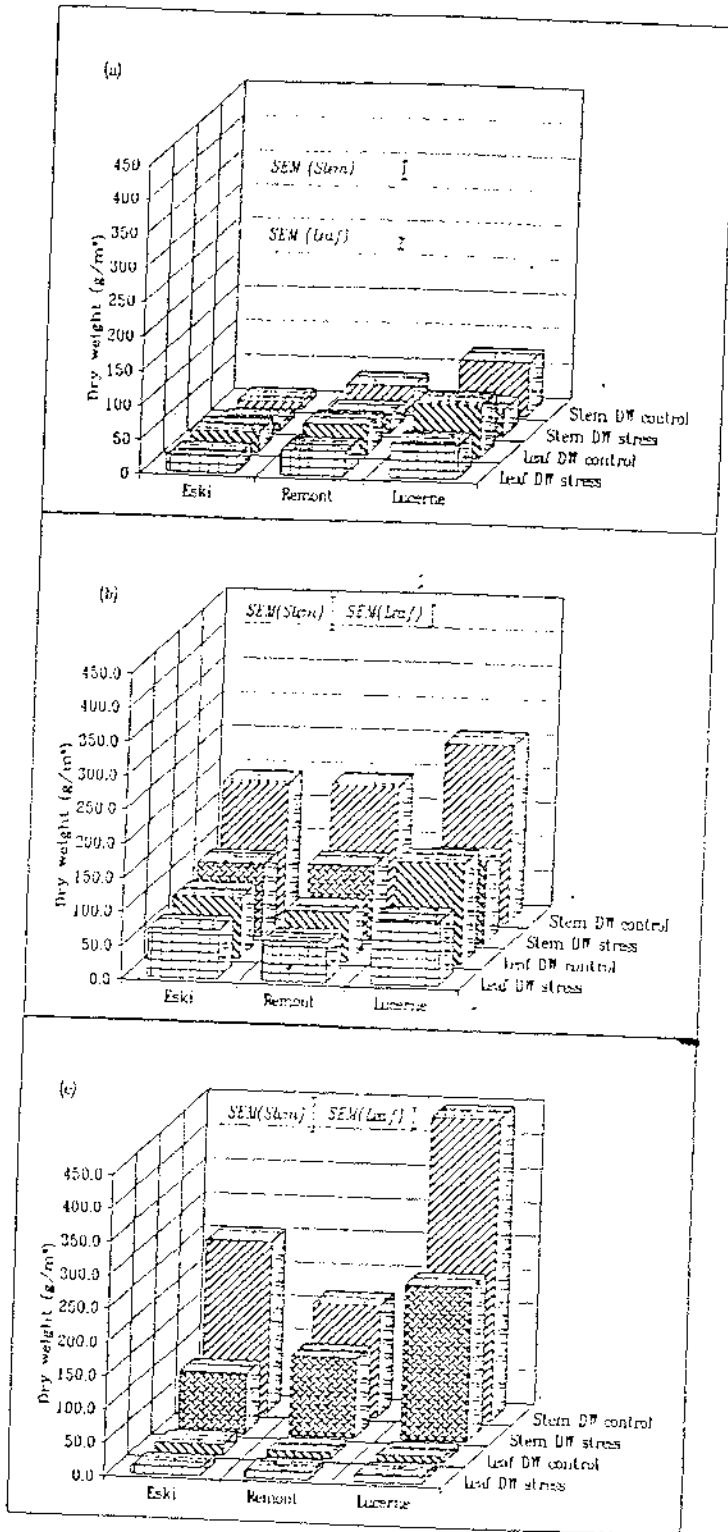


Fig 8.2: Leaf and stem dry weight of Eski, Grasslands G35, and lucerne in the stressed and non-stressed (control) experiments for (a) first harvest (30 days after imposing water stress, DS), (b) second harvest (60 DS), and (c), third harvest (90 DS). Vertical bars present \pm SEM.

Table 8.6: Yield (g/m^2) of Eski, Remont, and lucerne at five harvests under non-stressed and stressed experiments (see text for details).

	Growth1 (30 DS)	Growth2 (60 DS)	Growth3 (90 DS)	Regrowth1 (65 DS)	Regrowth2 (95 DS)
	Non-stressed				
Eski	40.10	282.93	319.90	100.10	103.78
Remont	80.53	264.47	216.4	55.4	216.99
Lucerne	151.98	407.94	554.80	142.26	326.18
	Stressed				
Eski	34.34	179.15	128.50	105.98	19.135
Remont	63.42	166.85	153.74	83.06	34.36
Lucerne	87.39	215.30	301.32	106.28	96.5
Pr>F W ¹	0.0201	0.0001	0.0014	0.8486	0.0001
Pr>F C ²	0.0018	0.0129	0.0007	0.0372	0.0027
Pr>F W*C	0.1834	0.2000	0.1284	0.3789	0.1279
SEM ³ W	9.2	17.3	28	13.5	18.00
SEM C	11.2	20.88	34	15.5	21.93
SEM W*C	15.5	29.3	48	16.47	30.40

¹ W= Soil moisture level.² C= Cultivar³ SEM= Standard error of the mean

Pooled analysis over three harvests showed Pr>F H = 0.0001, SEM = 14.4, Pr>F C=0.0001, SEM C = 14.4, Pr>F C*H=0.0020, SEM C*H=25, Pr>F W=0.0001, SEM W=12, Pr>F W*H=0.0039, SEM W*H=20, Pr>F W*C=0.0224, SEM W*C= 20, Pr>F W*C*H=0.4269, SEM W*C*H=35.

Table 8.7: The specific leaf area (cm²/g) of Eski, Remont, and lucerne at five harvests under non-stressed and stressed experiments (see text for details).

	Growth 1 (30 DS ¹)	Growth 2 (60 DS)	Growth 3 (90 DS)	Regrowth 1 (65 DS)	Regrowth 2 (95 DS)
	Non-stressed				
Eski	163.89	141.7	131.7	199.5	170.9
Remont	169.87	142.6	148.4	197.6	151.6
Lucerne	187.65	192.1	176.6	258.9	164.6
	Stressed				
Eski	168.13	142.0	113.8	147.3	60.9
Remont	157.64	142.3	116.3	173.8	77.1
Lucerne	181.000	225.9	126.9	187.3	114.6
Pr>F W ²	0.3851	0.6134	0.0001	0.0037	0.0001
Pr>F C ³	0.0965	0.1188	0.0067	0.0082	0.0066
Pr>F W*C	0.7237	0.8448	0.1196	0.2980	0.0078
SEM ⁴ W	5.45	21.09	3.86	8.76	4.12
SEM C	6.72	25.52	4.6	10.35	4.99
SEM W*C	9.48	35.97	6.58	14.33	7.11

¹ DS= Days after imposing soil moisture stress.² W= Soil moisture level.³ C= Cultivar⁴ SEM= Standard error of the mean

Pooled analysis over three harvests showed Pr>F H = 0.0099, SEM = 8.45, Pr>F C=0.0033, SEM C =8.46, Pr>F C*H=0.2679, SEM C*H=14.6, Pr>F W=0.2778, SEM W=7.03, Pr>F W*H=0.0842, SEM W*H=12.1, Pr>F W*C=0.9072 SEM W*C=12.1, Pr>F W*C*H=0.8965, SEM W*C*H=20.63

8.4.3 Physiological measurements

8.4.3.1 Relative water content (RWC)

Over the first three harvests RWC was significantly affected by soil moisture at dawn and midday (Table 8.8, 8.9). Harvest time only significantly affected RWC at dawn ($P < 0.01$). At dawn, only the interaction of soil moisture by harvest was significant ($P < 0.05$). The only significant interaction at midday was between cultivar and harvest ($P < 0.05$).

Over the first three harvests at dawn and midday the water stressed plants had lower RWC than the non-stressed plants. The difference between the RWC of the stressed and non-stressed plants was 4.9 and 4.6% at dawn and midday, respectively. At dawn, the RWC of the plants at the second and third harvest was less than that of the first harvest (Table 8.8), whereas at midday only the RWC of plants at the third harvest was less than the other two harvests (Table 8.9). Relative to the first and second harvests, the lucerne RWC decreased dramatically at the third harvest while that of the sainfoin cultivars was similar for all three harvests (Table 8.8, 8.9). The difference between the RWC of the stressed and non-stressed plants at dawn for the first harvest (1.0%) was lower than for the second (7.44%), and the third harvests (6.1%).

Repeated measures analysis at dawn and midday for the growth and regrowth harvests showed significant ($P < 0.01$) effects for time (Appendices 8.3.8.4). The interactions of time by harvest and time by soil moisture were also significant ($P < 0.01$) at dawn and midday. The RWC of the regrowth plants at dawn (84.3%), and midday (83.4), was less than for the growth plants at dawn (92.6%), and midday (86.7%).

To understand the effects of RWC on Ψ and π , RWC was plotted against these two factors at dawn and midday. A linear relationship was found between RWC and Ψ at dawn and midday. Although there was strong relationship between RWC and π at midday this relationship was poor at dawn (Fig. 8.3)

Table 8.8 : Relative water content (%) of Eski, Remont, and lucerne at dawn for five harvests under stressed and non-stressed experiments.

	Growth 1 (30 DS ¹)	Growth 2 (60 DS)	Growth 3 (90 DS)	Regrowth 1 (65 DS)	Regrowth 2 (95 DS)
Non-stressed					
Eski	94.1	94.8	93.1	93.0	90.8
Remont	93.4	94.7	92.4	90.5	89.6
Lucerne	96.7	94.6	94.4	90.9	90.5
Stressed					
Eski	94.3	90.2	85.8	87.6	65.2
Remont	93.2	88.3	89.6	86.7	66.3
Lucerne	93.6	83.4	86.2	88.5	71.6
Pr>F W ²	0.1274	0.0161	0.0002	0.0139	0.0001
Pr>F C ³	0.1000	0.5547	0.5711	0.6770	0.4227
Pr>F W*C	0.1139	0.5939	0.1748	0.5880	0.4682
SEM ⁴ W	0.4440	1.8811	0.8290	0.9540	1.5320
SEM C	0.544	2.3038	1.0160	1.1690	1.8773
SEM W*C	0.7690	3.250	1.4370	0.0355	2.6550

¹ DS= Days after imposing soil moisture stress.

² W= Soil moisture level.

³ C= Cultivar

⁴ SEM= Standard error of the mean

Pooled analysis of data over three harvests showed Pr>F H = 0.0034, SEM H= 0.08319, Pr>F C= 0.8794, SEM C = 0.8319, Pr>F C*H= 0.3803, SEM C*H= 1.4409, Pr>F W= 0.0001, SEM W= 0.6792, Pr>F W*H= 0.0223, SEM W*H= 1.1765, Pr>F W*C= 0.1560, SEM W*C= 1.1765, Pr>F W*C*H= 0.7936, SEM W*C*H= 2.0378.

Table 8.9: Relative water content (%) of Eski, Remont, and lucerne at midday for five harvests under stressed and non-stressed experiments.

	Growth 1 (30 DS ¹)	Growth 2 (60 DS)	Growth 3 (90 DS)	Regrowth 1 (65 DS)	Regrowth 2 (95 DS)
Non-stressed					
Eski	87.2	88.9	90.2	91.0	91.8
Remont	90.3	89.8	80.8	93.1	90.5
Lucerne	89.0	88.0	90.4	88.1	89.6
Stressed					
Eski	81.3	84.9	82.9	85.2	65.2
Remont	86.6	86.7	82.7	81.8	71.6
Lucerne	81.3	85.9	81.6	85.6	66.3
Pr>F W ²	0.0047	0.0015	0.0003	0.0001	0.0001
Pr>F C ³	0.0237	0.3557	0.0001	0.6387	0.4225
Pr>F W*C	0.3174	0.7229	0.0442	0.0102	0.4684
SEM ⁴ W	0.7822	0.5500	1.8127	0.6194	1.5325
SEM C	0.958	0.6566	2.2201	0.8470	1.8769
SEM W*C	1.355	0.9286	3.1397	1.1560	2.6540

¹ DS= Days after imposing soil moisture stress.

² W= Soil moisture level.

³ C= Cultivar

⁴ SEM= Standard error of the mean

Pooled analysis over three harvests showed Pr>F H =0.1362, SEM H=0.887, Pr>F C=0.9753, SEM C =0.887, Pr>F C*H=0.0487, SEM C*H=1.53, Pr>F W=0.0001, SEM W=0.73, Pr>F W*H=0.6127, SEM W*H=1.25, Pr>F W*C=0.1283, SEM W*C=1.25, Pr>F W*C*H=0.4484, SEM W*C*H=2.17.

Water relationships of sainfoin and lucerne.....

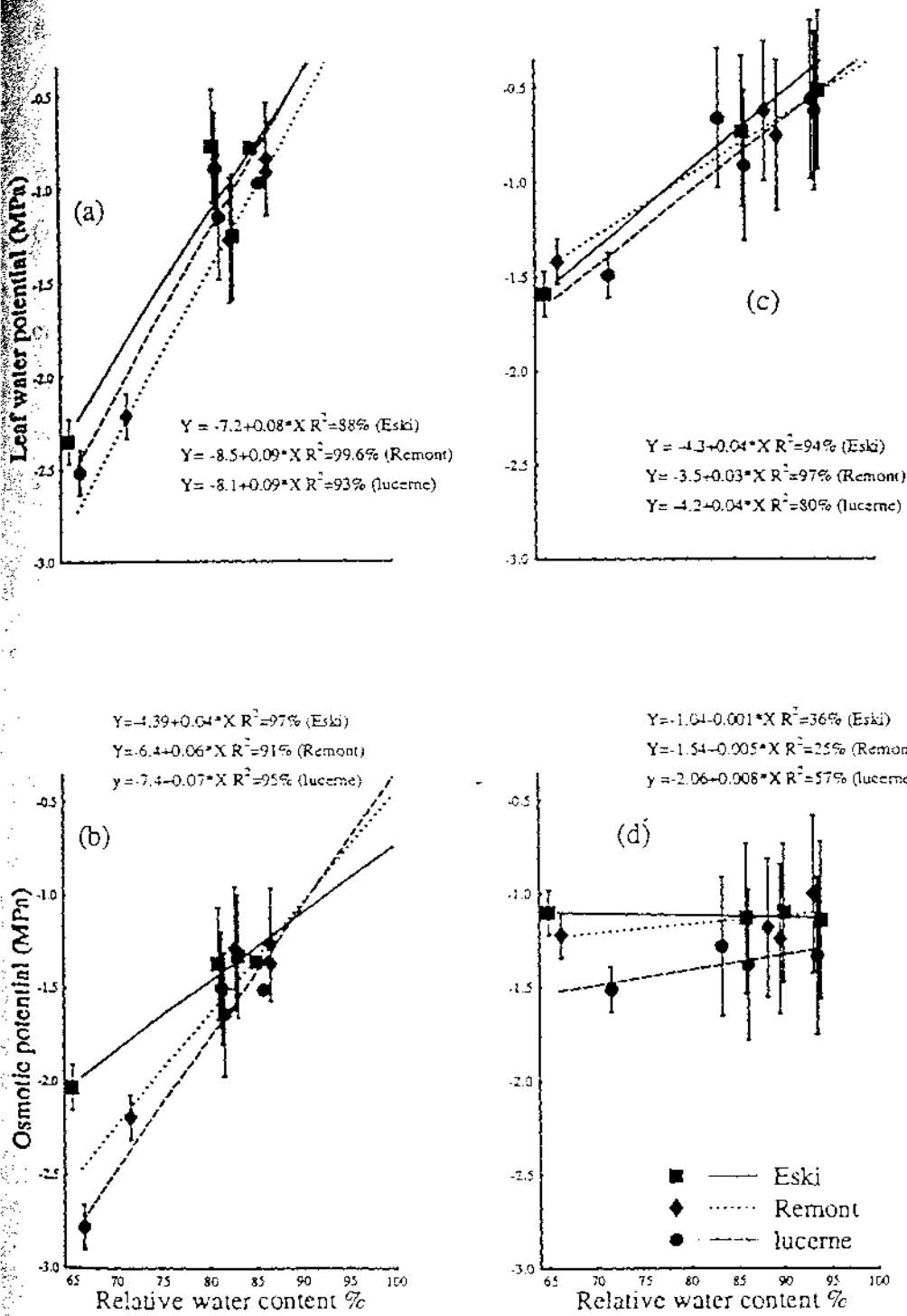


Fig 8.3: Relationships between relative water content, and (a) leaf water potential at midday, (b) osmotic potential at midday, and (c) leaf water potential at dawn, and (d) osmotic potential at dawn. Points are means of four replicates. Bars represent \pm SEM.

8.4.3.2 Total leaf water potential (Ψ)

The pooled analysis of Ψ , measured by Pressure Bomb and Wescor over the first three harvests showed significant effects for harvests, and cultivar ($P < 0.01$) (Tables 8.10-8.13). Plants Ψ at the third harvest was more negative than at the first and second harvests (Table 8.13). Lucerne had a more negative Ψ than the sainfoin cultivars (Tables 8.10 to 8.12) (Fig. 8.3a). Soil moisture significantly affected Ψ at dawn and midday (Table 8.10, 8.11). Over the three growth harvests, the water stressed plants had a lower (more negative) Ψ than the non-stressed plants at dawn and midday.

The Ψ for all harvests, except the first harvest, was greater for the stressed than the non-stressed plants (Tables 8.10-13). At most harvests, the Ψ of lucerne was more negative than sainfoin at both dawn and midday.

Repeated measures analysis showed Ψ between regrowth and respective growth harvests was significantly different at both the first and second harvests at dawn and midday. (Appendices 8.5, 8.6). The interactions of time by harvest, time by cultivar, and time by soil moisture, were also significant.

The Ψ of regrowth plants was more negative than that of the growth plants (Tables 8.11, 8.12). The difference between Ψ of the growth 2 and regrowth 2 harvests was higher than growth 1 and regrowth 1 harvests (Tables 8.10-13).

The Ψ was higher for the regrowth and growth plants of lucerne than for the sainfoin cultivars (Tables 8.10-11). The Ψ of the stressed regrowth and growth harvests differed more than for the non-stressed experiment (Table 8.10 to 8.13)(Appendices 8.5,8.6).

Table 8.10: Leaf water potential (MPa) measured by Pressure Bomb for Eski, Remont, and lucerne at five harvests at dawn under non-stressed and stressed experiments

	Growth 1 (30 DS ¹)	Growth 2 (60 DS)	Growth 3 (90 DS)	Regrowth 1 (65 DS)	Regrowth 2 (95 DS)
Non-stressed					
Eski	-0.34	-0.26	-0.25	-0.26	-0.22
Remont	-0.35	-0.30	-0.29	-0.29	-0.27
Lucerne	-0.55	-0.53	-0.41	-0.54	-0.51
Stressed					
Eski	-0.40	-0.30	-0.38	-0.31	-0.34
Remont	-0.43	-0.35	-0.35	-0.29	-0.44
Lucerne	-0.59	-0.62	-0.65	-0.67	-1.80
Pr>F W ²	0.0900	0.1300	0.0001	0.0711	0.0378
Pr>F C ³	0.0001	0.0001	0.0001	0.0001	0.0141
Pr>F W*C	0.8546	0.4721	0.0460	0.1898	0.0913
SEM ⁴ W	0.0180	0.0143	0.0180	0.0205	0.1638
SEM C	0.0220	0.0174	0.0220	0.0251	0.2006
SEM W*C	0.0310	0.0247	0.0312	0.0355	0.2837

¹ DS= Days after imposing soil moisture stress.² W= Soil moisture level.³ C= Cultivar⁴ SEM= Standard error of the mean

Pooled analysis over three harvests showed Pr>F H = 0.0013, SEM H= 0.0113, Pr>F C= 0.0001, SEM C = 0.0113, Pr>F C*H= 0.1142, SEM C*F= 0.0195, Pr>F W= 0.0001, SEM W= 0.0009, Pr>F W*H= 0.0143, SEM W*H= 0.0159, Pr>F W*C= 0.1270, SEM W*C= 0.0159, Pr>F W*C*H= 0.1243, SEM W*C*H= 0.0276

Table 8.11: Leaf water potential (MPa) measured by Pressure Bomb for Eski, Remont, and lucerne at five harvests at midday under non-stressed and stressed experiments

	Growth 1 (30 DS ¹)	Growth 2 (60 DS)	Growth 3 (90 DS)	Regrowth 1 (65 DS)	Regrowth 2 (95 DS)
Non-stressed					
Eski	-0.64	-0.47	-0.45	-0.35	-0.45
Remont	-0.61	-0.51	-0.50	-0.37	-0.52
Lucerne	-0.95	-0.85	-0.82	-0.88	-0.93
Stressed					
Eski	-0.92	-0.54	-0.67	-0.42	-0.79
Remont	-0.94	-0.63	-0.63	-0.63	-0.79
Lucerne	-1.24	-1.14	-1.44	-1.70	-1.95
Pr>F W ²	0.0030	0.0003	0.0003	0.0002	0.0001
Pr>F C ³	0.0001	0.0001	0.0442	0.0001	0.0001
Pr>F W*C	0.7906	0.0402	0.7929	0.0030	0.0060
SEM ⁴ W	0.02	0.02	0.05	0.05	0.60
SEM C	0.03	0.03	0.06	0.06	0.07
SEM W*C	0.04	0.04	0.09	0.09	0.10

¹ DS= Days after imposing soil moisture stress.

² W= Soil moisture level.

³ C= Cultivar

⁴ SEM= Standard error of the mean

Pooled analysis over three harvests showed Pr>F H =0.0001, SEM H=0.2456, Pr>F C=0.0001, SEM C =0.2456, Pr>F C*H=0.0360, SEM C*F=0.4254, Pr>F W=0.0001, SEM W=0.2005, Pr>F W*H=0.0178, SEM W*H=0.3473, Pr>F W*C=0.0088, SEM W*C=0.3473, Pr>F W*C*H=0.0398, SEM W*C*H=0.6016

Table 8.12: Leaf water potential (MPa) measured by Wescor for Eski, Remont, and lucerne at dawn from five harvests under non-stressed and stressed experiments .

	Growth 1 (30 DS ¹)	Growth 2 (60 DS)	Growth 3 (90 DS)	Regrowth 1 (65 DS)	Regrowth 2 (95 DS)
Non-stressed					
Eski	-0.41	-0.44	-0.65	-0.35	-0.77
Remont	-0.56	-0.46	-0.57	-0.53	-0.59
Lucerne	-0.70	-0.60	-0.82	-0.54	-0.99
Stressed					
Eski	-0.51	-0.34	-0.73	-0.73	-1.59
Remont	-0.56	-0.62	-0.75	-0.82	-1.42
Lucerne	-0.62	-0.66	-0.91	-1.10	-1.49
Pr>F W ²	0.8765	0.3166	0.8344	0.0001	0.0002
Pr>F C ³	0.1265	0.0025	0.1567	0.0059	0.3551
Pr>F W*C	0.6151	0.0797	0.1314	0.2051	0.5190
SEM ⁴ W	0.0519	0.0298	0.0538	0.0400	0.0938
SEM C	0.064	0.0366	0.0659	0.049	0.1149
SEM W*C	0.0900	0.0517	0.0932	0.0700	0.1626

¹ DS= Days after imposing soil moisture stress.

² W= Soil moisture level.

³ C= Cultivar

⁴ SEM= Standard error of the mean

Pooled analysis over three harvests showed Pr>F H = 0.0002, SEM = 0.0382, Pr>F C = 0.0015, SEM C = 0.0382, Pr>F C*H = 0.6941, SEM C*H = 0.0642, Pr>F W = 0.1895, SEM W = 0.0309, Pr>F W*H = 0.5942, SEM W*H = 0.0556, Pr>F W*C = 0.6361, SEM W*C = 0.0556, Pr>F W*C*H = 0.6275, SEM W*C*H = 0.1065

Table 8.13: Leaf water potential (MPa) measured by Wescor for Eski, Remont, and lucerne at midday from five harvests under non-stressed and stressed experiments .

	Growth 1 (30 DS ¹)	Growth 2 (60 DS)	Growth 3 (90 DS)	Regrowth 1 (65 DS)	Regrowth 2 (95 DS)
Non-stressed					
Eski	-0.72	-0.72	-0.85	-0.66	-1.14
Remont	-0.87	-0.74	-0.93	-0.90	-1.13
Lucerne	-1.02	-0.85	-0.95	-1.23	-1.83
Stressed					
Eski	-0.79	-0.80	-1.27	-1.19	-2.35
Remont	-0.86	-0.93	-1.29	-1.27	-2.22
Lucerne	-0.91	-0.99	-1.17	-1.86	-2.52
Pr>F W ²	0.9211	0.0444	0.0050	0.0013	0.0001
Pr>F C ³	0.1230	0.1429	0.9001	0.0040	0.0256
Pr>F W*C	0.6253	0.7744	0.7158	0.6917	0.3073
SEM ⁴ W	0.05	0.4277	0.07	0.09	0.06
SEM C	0.06	0.5280	0.09	0.11	0.07
SEM W*C	0.09	0.7408	0.12	0.15	0.17

¹ DS= Days after imposing soil moisture stress.² W= Soil moisture level.³ C= Cultivar⁴ SEM= Standard error of the mean

Pooled analysis over three harvests showed Pr>F H =0.0006, SEM =0.4471, Pr>F C=0.4479, SEM C =0.0330, Pr>F C*H=0.6488, SEM C*H=0.7114, Pr>F W=0.1451, SEM W=0.3353, Pr>F W*H=0.0942, SEM W*H=0.5808, Pr>F W*C=0.6806, SEM W*C=0.5808, Pr>F W*C*H=0.9853, SEM W*C*H= 0.0061.

8.4.3.3 Osmotic potential (π)

Pooled analysis over the first three harvests at dawn and midday showed π was only significantly affected by cultivar (Figs. 8.3 b,d). Lucerne had a lower (more negative) π than sainfoin cultivars at both dawn and midday (Tables 8.14,8.15).

Separate ANOVA of each harvest showed π was more negative in water stressed plants at the third harvest and for both regrowth harvests (Table 8.15).

Repeated measures analysis showed π of the regrowth harvests was significantly different than the growth harvests. The interactions of time by harvest, time by cultivar, and time by soil moisture were also significant (Appendices 8.3,8.4). The π of the regrowth plants was more negative than that of the growth plants (Tables 8.14,8.15).

Lucerne regrowth plants had a lower π than the growth harvests. Also the differences between π of the growth and regrowth plants of lucerne was greater than the sainfoin cultivars (Table 8.15). At dawn this difference was higher for Eski, than Remont, and lucerne (Table 8.14). At dawn and midday the soil moisture stress decreased the π of the regrowth plants more than that of the growth plants (Tables 8.14,15).

Table 8.14: Osmotic potential (MPa) of Eski, Remont, and lucerne at dawn from five harvests for non-stressed and stressed experiments.

	Growth 1 (30 DS ¹)	Growth 2 (60 DS)	Growth 3 (90 DS)	Regrowth 1 (65 DS)	Regrowth 2 (95 DS)
Non-stressed					
Eski	-1.04	-1.13	-0.99	-1.10	-1.10
Remont	-1.01	-1.10	-0.86	-1.10	-1.15
Lucerne	-1.14	-1.25	-1.24	-1.20	-1.25
Stressed					
Eski	-1.14	-1.10	-1.13	-1.10	-1.10
Remont	-1.00	-1.18	-1.24	-1.20	-1.22
Lucerne	-1.33	-1.28	-1.38	-1.51	-1.51
Pr>F W ²	0.3507	0.7881	0.1400	0.1683	0.2325
Pr>F C ³	0.2834	0.0062	0.0180	0.0455	0.0451
Pr>F W*C	0.8075	0.3050	0.3214	0.4849	0.4884
SEM ⁴ W	0.073	0.034	0.051	0.0632	0.063
SEM C	0.089	0.041	0.619	0.0774	0.078
SEM W*C	0.127	0.058	0.088	0.1094	0.109

¹ DS= Days after imposing soil moisture stress.² W= Soil moisture level.³ C= Cultivar⁴ SEM= Standard error of the mean

Pooled analysis over three harvests showed Pr>F H = 0.5219, SEM H= 0.4150, Pr>F C= 0.0007, SEM C = 0.0416, Pr>F C*H= 0.9005, SEM C*H= 0.0720, Pr>F W= 0.1473, SEM W= 0.0339, Pr>F W*H= 0.1494, SEM W*H= 0.0588, Pr>F W*C= 0.6432, SEM W*C= 0.0588, Pr>F W*C*H= 0.6429, SEM W*C*H= 0.1018

Table 8.15: Osmotic potential (MPa) of Eski, Remont, and lucerne at midday from five harvests for non-stressed and stressed experiments.

	Growth 1 (30 DS ¹)	Growth 2 (60 DS)	Growth 3 (90 DS)	Regrowth 1 (65 DS)	Regrowth 2 (95 DS)
Non-stressed					
Eski	-1.42	-1.37	-1.16	-1.02	-1.65
Remont	-1.39	-1.30	-1.10	-1.33	-1.48
Lucerne	-1.45	-1.44	-1.37	-1.48	-1.97
Stressed					
Eski	-1.37	-1.36	-1.33	-1.65	-2.03
Remont	-1.27	-1.37	-1.29	-1.57	-2.19
Lucerne	-1.50	-1.51	-1.64	-1.69	-2.78
Pr>F W ²	0.6814	0.6761	0.0106	0.0334	0.0006
Pr>F C ³	0.2690	0.4835	0.0075	0.4294	0.0096
Pr>F W*C	0.6554	0.9332	0.8288	0.4687	0.4177
SEM ⁴ W	0.05	0.69	0.05	0.09	0.10
SEM C	0.06	0.84	0.06	0.01	0.12
SEM W*C	0.09	1.19	0.09	0.18	0.17

¹ DS= Days after imposing soil moisture stress.² W= Soil moisture level.³ C= Cultivar⁴ SEM= Standard error of the mean

Pooled analysis over three harvests showed Pr>F H = 0.2848, SEM H= 0.4107, Pr>F C= 0.033, SEM C = 0.4107, Pr>F C*H= 0.6488, SEM C*F= 0.7114, Pr>F W= 0.1451, SEM W= 0.3353, Pr>F W*H= 0.0942, SEM W*H= 0.5808, Pr>F W*C= 0.6806, SEM W*C= 0.5808, Pr>F W*C*H= 0.9853, SEM W*C*H= 1.0061

8.4.3.4 Osmotic potential at full turgor (π_{100})

Pooled analysis of π_{100} over the first three harvests showed a significant effect for harvest at dawn and midday ($P < 0.05$). The π_{100} at midday ($P < 0.05$) was affected by soil moisture (Tables 8.16, 8.17). The π_{100} of cultivars was significantly different at dawn (Table 8.16). Plants at the first harvest had a higher (less negative) π_{100} than the plants at the second and third harvests at dawn and midday (Table 8.16,17). At midday the stressed plants had a lower (more negative) π_{100} than that of the non-stressed plants. At dawn (Table 8.16) lucerne had a more negative π_{100} than that of the sainfoin cultivars.

Repeated measures analysis of π_{100} for regrowth and growth plants showed significant effects for time ($P < 0.05$), the interactions between time and harvest ($P < 0.0001$), and time by soil moisture (Appendices 8.3, 8.4). At midday the π_{100} of the regrowth plants was lower (more negative) than that of the growth plants (Table 8.17).

At dawn the difference between π_{100} of the growth and regrowth plants at the second harvest (60 days after imposing water stress) was lower than that of the first harvest (30 days after imposing water stress) and the growth plants of the first harvest had higher (less negative) π_{100} than those of the second harvest (Table 8.16).

At midday π_{100} of the stressed regrowth plants was lower than that of the growth plants, while non-stressed growth plants had lower π_{100} than that of the regrowth plants.

Table 8.16: The osmotic potential at full turgor π_{100} (MPa) of Eski, Remont, and lucerne at dawn from five harvests for non-stressed and stressed experiments.

	Growth 1 (30 DS ¹)	Growth 2 (60 DS)	Growth 3 (90 DS)	Regrowth 1 (65 DS)	Regrowth 2 (90 DS)
Non-stressed					
Eski	-0.76	-1.01	-0.96	-0.80	-0.91
Remont	-0.77	-0.95	-0.93	-0.82	-0.89
Lucerne	-0.87	-1.10	-0.99	-0.87	-0.99
Stressed					
Eski	-0.65	-1.08	-0.99	-0.99	-1.06
Remont	-0.69	-1.10	-1.04	-1.00	-0.87
Lucerne	-0.74	-1.18	-1.15	-1.07	-1.03
Pr>F W ²	0.0900	0.3800	0.2900	0.0001	0.3995
Pr>F C ³	0.0158	0.2654	0.0087	0.1498	0.1935
Pr>F W*C	0.6572	0.8404	0.1821	0.9700	0.4935
SEM ⁴ W	0.0179	0.0353	0.0168	0.0221	0.0418
SEM C	0.0219	0.0432	0.0205	0.0271	0.0512
SEM W*C	0.0311	0.0611	0.0291	0.0383	0.0724

¹ DS= Days after imposing soil moisture stress.² W= Soil moisture level.³ C= Cultivar⁴ SEM= Standard error of the mean

Pooled analysis of data over three harvests showed Pr>F H = 0.0001, SEM H= 0.0256, Pr>F C= 0.0194, SEM C = 0.0256, Pr>F C*H= 0.0884, SEM C*H= 0.0443, Pr>F W= 0.2771, SEM W= 0.0209, Pr>F W*H= 0.0072, SEM W*H= 0.0362, Pr>F W*C= 0.7105, SEM W*C= 0.0362, Pr>F W*C*H= 0.9490, SEM W*C*H= 0.0627.

Table 8.17: The leaf osmotic potential at full turgor π_{100} (MPa) of Eski, Remont, and Lucerne at midday from five harvests for non-stressed and stressed experiments.

	Growth 1 (30 DS ¹)	Growth 2 (60 DS)	Growth 3 (90 DS)	Regrowth 1 (65 DS)	Regrowth 2 (90 DS)
Non-stressed					
Eski	-0.86	-1.04	-0.97	-0.83	-0.84
Remont	-0.89	-0.93	-1.05	-0.83	-0.86
Lucerne	-0.88	-1.06	-0.91	-0.79	-0.91
Stressed					
Eski	-0.96	-1.00	-1.06	-1.01	-1.03
Remont	-0.94	-1.01	-1.07	-1.12	-0.91
Lucerne	-1.03	-1.07	-1.06	-1.03	-0.95
Pr>F W ²	0.0339	0.6721	0.1165	0.0017	0.0279
Pr>F C ³	0.3429	0.2479	0.5204	0.5476	0.5477
Pr>F W*C	0.3494	0.4953	0.5857	0.7424	0.2064
SEM ⁴ W	0.02	0.03	0.04	0.04	0.03
SEM C	0.02	0.04	0.04	0.05	0.03
SEM W*C	0.03	0.05	0.06	0.07	0.04

¹ DS= Days after imposing soil moisture stress.² W= Soil moisture level.³ C= Cultivar⁴ SEM= Standard error of the mean

Pooled analysis of data over three harvests showed Pr>F H =0.0116, SEM H=0.2378,

Pr>F C=0.8186, SEM C =0.2378, Pr>F C*H=0.3942, SEM C*F=0.4119, Pr>F

W=0.0169, SEM W=0.1942, Pr>F W*H=0.4309, SEM W*H=0.3363, Pr>F

W*C=0.6364, SEM W*C=0.3363, Pr>F W*C*H=0.7081, SEM W*C*H=0.5826

8.4.3.5 Turgor potential (P)

Pooled analysis of variance over the first three harvests showed only significant effects for harvests at dawn and midday (Tables 8.18 and 8.19). At dawn the P of the third harvest was lower than that of the first and second harvest (Table 8.18). At midday, although there was no significant difference between P of plants at the second and third harvests, P was highest at the first harvest (Table 8.19).

Repeated measures analysis of P of the regrowth and growth harvests showed that at midday there was a significant effect of time (Appendix 8.6). The P of the regrowth plants was lower than that of the growth plants (Table 8.19).

Table 8.18: Turgor potential (MPa) of Eski, Remont, and lucerne at dawn from five harvests for non-stressed and stressed experiments.

	Growth 1 (30 DS ¹)	Growth 2 (60 DS)	Growth 3 (90 DS)	Regrowth 1 (65 DS)	Regrowth 2 (95 DS)
Non-stressed					
Eski	0.63	0.69	0.34	0.75	0.33
Remont	0.46	0.63	0.29	0.57	0.56
Lucerne	0.44	0.65	0.41	0.66	0.25
Stressed					
Eski	0.63	0.76	0.40	0.37	-0.19
Remont	0.44	0.57	0.49	0.38	-0.20
Lucerne	0.71	0.62	0.47	0.40	0.02
Pr>F W ²	0.4555	0.1388	0.1469	0.0013	0.5013
Pr>F C ³	0.4096	0.0628	0.6449	0.8772	0.0387
Pr>F W*C	0.4534	0.1721	0.6229	0.6730	0.1070
SEM ⁴ W	0.0827	0.0255	0.0478	0.5295	0.0939
SEM C	0.1000	0.0313	0.0586	0.6170	0.1152
SEM W*C	0.1624	0.0442	0.0829	0.0873	0.1628

¹ DS= Days after imposing soil moisture stress

² W= Soil moisture level.

³ C= Cultivar

⁴ SEM= Standard error of the mean

Pooled analysis of data over three harvests showed Pr>F H = 0.0001, SEM H = 0.0403, Pr>F C = 0.2202, SEM C = 0.0403, Pr>F C*H = 0.6620, SEM C*H = 0.0737, Pr>F W = 0.3365, SEM W = 0.0325, Pr>F W*H = 0.2925, SEM W*H = 0.0586, Pr>F W*C = 0.9418, SEM W*C = 0.0586, Pr>F W*C*H = 0.3824, SEM W*C*H = 0.1123

Table 8.19: Turgor potential (MPa) of Eski, Remont, and lucerne at midday from five harvests for non-stressed and stressed experiments.

	Growth 1 (30 DS ¹)	Growth 2 (60 DS)	Growth 3 (90 DS)	Regrowth 1 (65 DS)	Regrowth 2 (95 DS)
Non-stressed					
Eski	0.70	0.65	0.31	0.36	0.51
Remont	0.51	0.56	0.18	0.43	0.34
Lucerne	0.44	0.59	0.42	0.25	0.15
Stressed					
Eski	0.58	0.56	0.05	0.46	-0.32
Remont	0.41	0.44	0.00	0.30	-0.03
Lucerne	0.59	0.52	0.47	0.00	0.26
Pr>F W ²	0.8071	0.2971	0.4177	0.1506	0.0121
Pr>F C ³	0.1664	0.6100	0.1766	0.3488	0.7717
Pr>F W*C	0.2661	0.9690	0.7156	0.4173	0.0268
SEM ⁴ W	0.05	0.61	0.11	0.11	0.09
SEM C	0.06	0.75	0.13	0.14	0.11
SEM W*C	0.09	1.06	0.18	0.19	0.15

¹ DS= Days after imposing soil moisture stress

² W= Soil moisture level.

³ C= Cultivar

⁴ SEM= Standard error of the mean

Pooled analysis over three harvests showed Pr>F H = 0.0004, SEM H= 0.5794, Pr>F C= 0.1460, SEM C = 0.5794, Pr>F C*H= 0.3012, SEM C*H= 1.0036, Pr>F W= 0.2229, SEM W= 0.4731, Pr>F W*H= 0.8276, SEM W*H= 0.8194, Pr>F W*C= 0.4273, SEM W*C= 0.8194, Pr>F W*C*H= 0.9572, SEM W*C*H=1.4193

8.4.3.6 Photosynthetic rate (P_n)

Pooled ANOVA over three growth harvests showed that P_n was different between harvests ($P < 0.05$) (Table 8.20). Also, the interaction of cultivar by harvest was significant (Table 8.20), with the third harvest having a higher P_n than the other two harvests. Eski had the highest P_n at the first and third harvests (Table 8.20).

Separate ANOVA for each harvest did not show consistent trends for either soil moisture or cultivar (Table 8.20).

Repeated measures analysis of P_n at the first and second harvests and their subsequent regrowth harvest, showed a non-significant effect for time, however, the interaction of time by soil moisture was significant (Appendix 8.6). The regrowth plants had higher and lower P_n than the growth plants under non-stressed and stressed conditions, respectively.

8.4.3.7 Stomatal resistance (R_s)

Pooled ANOVA over three growth harvests showed, stomatal resistance (R_s) measured by a Li-Cor 6200 was not significantly different between soil moisture treatments, cultivars, and harvests. None of interactions was significant over three harvests (Table 8.21). Separate analysis of harvests showed that stressed plant R_s was higher than that of the non-stressed plants at all growth and regrowth harvests (Table 8.21).

A repeated measures analysis of R_s at the first and second harvests and their subsequent regrowth harvests, showed higher R_s for the growth plants than for the regrowth plants ($P < 0.0001$) (Appendix 8.7). The interactions of time by soil moisture by harvest ($P < 0.001$), and time by cultivar by harvest were also significant. The regrowth plants had higher R_s than growth plants under stressed conditions, and the R_s of the regrowth plants of the sainfoin cultivars at the second harvest was lower than that of the growth plants, but it was similar for lucerne.

Table 8.20: Photosynthesis ($\mu\text{mol CO}_2/\text{m}^2/\text{s}$) of Eski, Remont, and lucerne at midday from five harvests for non-stressed and stressed experiments.

	Growth 1 (30 DS ¹)	Growth 2 (60 DS)	Growth 3 (90 DS)	Regrowth 1 (65 DS)	Regrowth 2 (95 DS)
Non-stressed					
Eski	18.8	14.7	16.9	18.9	18.5
Remont	16.8	16.9	16.2	15.2	18.1
Lucerne	14.1	14.4	19.5	17.9	21.3
Stressed					
Eski	15.9	13.2	19.5	16.7	12.8
Remont	13.8	16.4	16.6	16.4	10.5
Lucerne	12.4	16.8	15.1	15.6	14.0
Pr>F W ²	0.0049	0.9343	0.7533	0.2564	0.0003
Pr>F C ³	0.0021	0.4076	0.5885	0.2540	0.1595
Pr>F W*C	0.7380	0.6035	0.1413	0.2435	0.8271
SEM ⁴ W	0.5112	1.1550	0.9453	0.6679	0.8711
SEM C	0.6261	1.4150	1.1577	0.8181	1.0669
SEM W*C	0.8855	2.0010	1.6373	1.1569	1.6561

¹ DS= Days after imposing soil moisture stress.

² W= Soil moisture level.

³ C= Cultivar

⁴ SEM= Standard error of the mean

Pooled analysis of data over three harvests showed Pr>F H =0.0394, SEM H=0.599, Pr>F C=0.6168, SEM C =0.599, Pr>F C*H=0.0290, SEM C*H=1.038, Pr>F W=0.4474, SEM W=0.489, Pr>F W*H=0.2698, SEM W*H=0.848, Pr>F W*C=0.4358, SEM W*C=0.848, Pr>F W*C*H=0.2001, SEM W*C*H=1.468

Table 8.21: Stomatal resistance (s/cm) of Eski, Remont, and lucerne at midday from five harvests for non-stressed and stressed experiments, measured by a Li-Cor 6200.

	Growth 1 (30 DS ¹)	Growth 2 (60 DS)	Growth 3 (90 DS)	Regrowth 1 (65 DS)	Regrowth 2 (95 DS)
Non-stressed					
Eski	0.351	0.361	0.257	0.255	0.155
Remont	0.352	0.289	0.237	0.335	0.143
Lucerne	0.412	0.235	0.227	0.307	0.117
Stressed					
Eski	0.447	1.026	0.370	0.557	0.525
Remont	0.475	0.430	0.400	0.462	0.673
Lucerne	0.558	0.610	0.425	0.573	0.975
Pr>F W ²	0.0420	0.2534	0.0116	0.0008	0.0001
Pr>F C ³	0.0644	0.2781	0.9814	0.7952	0.1602
Pr>F W*C	0.7238	0.4808	0.8082	0.3876	0.0884
SEM ⁴ W	0.0196	0.121	0.0374	0.0371	0.0586
SEM C	0.0239	0.148	0.0458	0.0454	0.0719
SEM W*C	0.0339	0.209	0.0648	0.0643	0.1016

¹ DS= Days after imposing soil moisture stress.

² W= Soil moisture level.

³ C= Cultivar

⁴ SEM= Standard error of the mean

Pooled analysis of data over three harvests showed Pr>F H =0.1636, SEM H=0.0642, Pr>F C=0.5822, SEM C =0.0642, Pr>F C*H=0.3784, SEM C*F=0.1111, Pr>F W=0.0860, SEM W=0.052, Pr>F W*H=0.2705, SEM W*H=0.0907, Pr>F W*C=0.7824, SEM W*C=0.0907, Pr>F W*C*H=0.06680, SEM W*C*H= 0.1572

8.4.3.8 Stomatal resistance measured by Porometer (Rs)

Total Rs was calculated from Rs of the adaxial and abaxial leaf surfaces, and over the first three harvests showed significant differences between Rs of the stressed and non-stressed plants ($P < 0.01$), harvests ($P < 0.001$), and interaction of soil moisture by cultivar ($P < 0.05$). The stressed plants had higher Rs than non-stressed plants, and due to high Rs of the abaxial surface of Eski at the second harvest, plants had higher Rs at this harvest than for the third harvest.

The adaxial and abaxial leaf surfaces had different responses to harvest, soil moisture, and the interaction of the cultivar by soil moisture. Relative to the adaxial surface, the Rs of the abaxial surface was less affected by soil moisture, harvests, and cultivar. The total Rs of the leaf was mostly influenced by Rs of the adaxial surface (Tables 8.22 a,b). At the third harvest a lower total Rs than that at the second harvest was caused by a lower Rs of the adaxial surface (Table 8.22 a).

The higher total Rs of the stressed than the non-stressed plants was caused by higher Rs of the adaxial surfaces. The abaxial Rs was similar for both soil moisture conditions (Table 8.22 a,b).

Repeated measures analysis of Rs showed no significant treatment effects or interactions for the second harvest and its subsequent regrowth (Appendix 8.7).

Table 8.22 (a): Stomatal resistance (s/cm) of adaxial and abaxial leaf surfaces measured by Delta T porometer for Eski, Remont, and lucerne for stressed and non-stressed experiments.

	Harvest 2 (60 DS ¹)		Harvest 3 (90 DS)		Regrowth 2 (105 DS)	
	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
	Non-stressed					
Eski	1.21	4.13	0.92	1.42	1.10	1.31
Remont	1.29	3.26	1.08	1.55	1.21	1.30
Lucerne	1.59	1.93	1.25	2.78	1.69	2.81
	Stressed					
Eski	1.72	2.61	1.68	2.76	2.46	3.69
Remont	1.89	2.64	1.35	3.12	2.22	3.74
Lucerne	2.08	2.21	1.24	1.81	1.10	1.32
Pr>F W ²	0.0001	0.2758	0.0065	0.0660	0.0028	0.0007
Pr>F C ³	0.0001	0.1832	0.8178	0.7966	0.1472	0.2590
Pr>F W*C	0.6518	0.4319	0.0278	0.0127	0.0006	0.0001
SEM ⁴ W	0.0341	0.3875	0.0726	0.2258	0.1111	0.1725
SEM C	0.4180	0.4746	0.0889	0.2764	0.1361	0.2113
SEM W*C	0.0592	0.6713	0.1258	0.3910	0.1926	0.2988

¹ DS= Days after imposing soil moisture stress

² W= Soil moisture level.

³ C= Cultivar

⁴ SEM= Standard error of the mean

Table 8.22 (b): Pooled analysis of variance results ($Pr>F$) for stomatal resistance measured by Delta T Porometer over the second and third harvests for adaxial, abaxial and total leaf surfaces.

	H ¹	Cu	Cu*H	W	W*H	W*C _u	W*H*C _u
							u
				Adaxial			
Pr>F	0.0001	0.0724	0.0207	0.0001	0.1076	0.0320	0.0331
SEM	0.0414	0.0506	0.0716	0.0414	0.0585	0.0716	0.1013
				Abaxial			
Pr>F	0.0885	0.3295	0.1664	0.9750	0.0537	0.5631	0.0281
SEM	0.2238	0.2742	0.3877	0.2238	0.3166	0.3877	0.5484
				Total			
Pr>F	0.0003	0.9389	0.9139	0.0001	0.6576	0.0481	0.0220
SEM	0.0315	0.0386	0.0546	0.0315	0.0445	0.0546	0.0772

¹ H= Harvest, Cu= Cultivar, W= Soil moisture level.

8.4.3.9 Stomatal frequency

Comparison of stomatal frequencies of adaxial and abaxial leaf surfaces using t-test showed that the frequencies for Eski (152 ± 8 vs 309 ± 30 per mm^2), and Remont (164 ± 32 vs 313 ± 22 per mm^2) were significantly different ($P < 0.01$) for the abaxial and adaxial leaf surfaces respectively. However lucerne had statistically similar stomatal frequencies on abaxial (272 ± 35 per mm^2) and adaxial (313 ± 22 per mm^2) surfaces of the leaves (Plate 8.4).

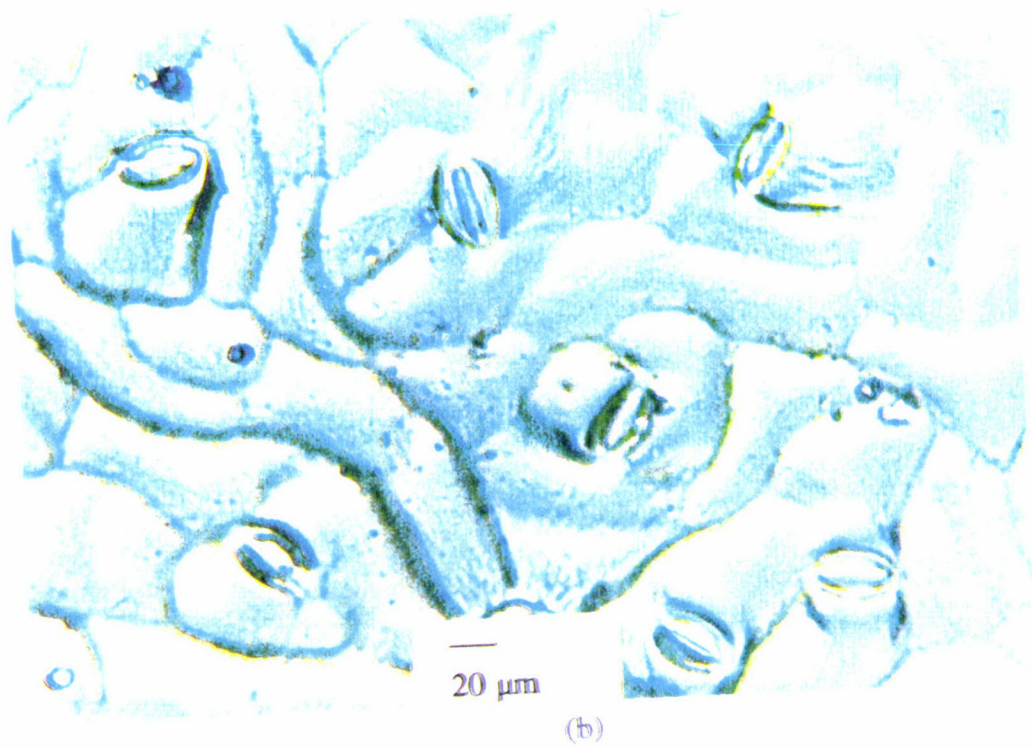
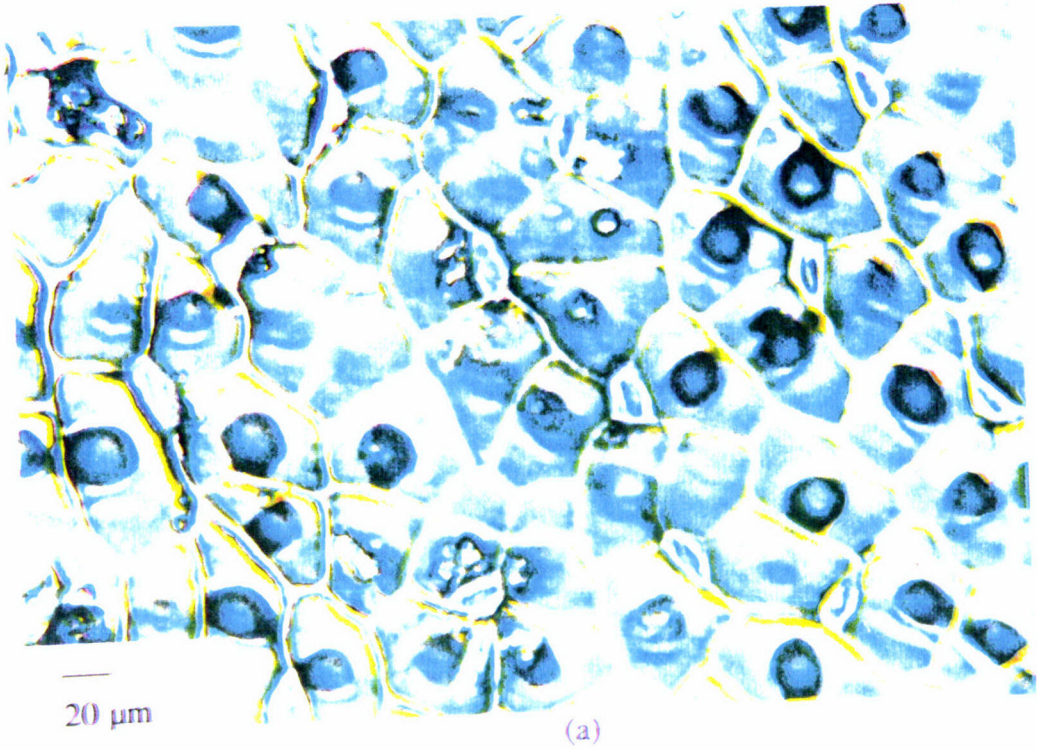


Plate 8.4: The stomatal frequencies of a) adaxial and b) abaxial surfaces of leaf of sainfoin (Eski).

8.5 Discussion

8.5.1 Soil moisture

Both experiments initially had similar soil moisture, however, evapotranspiration from the stressed experiment continually depleted soil moisture such that the (final) second regrowth harvest had the lowest soil moisture to 0.7 m depth.

Roots in the top soil (0-0.15 m depth) initially reduced soil moisture in this zone, however as roots grew deeper soil moisture was lost from lower in the profile (e.g. 0.3, 0.5 m depth). Relative to the non-stressed experiment, the average soil moisture reduction in the top 0.7 m of soil of the stressed experiment was 7.5, 14, and 24% during the 30, 60, and 90 days after imposing water stress, respectively (Table 8.1). The non-stressed experiment had soil moisture at field capacity during the experiment (Appendices 8.3, 8.1).

The estimation of evapotranspiration from the stressed experiment using soil moisture data showed that the evapotranspiration rate in the first to third months of growth was 0.8, 0.45, 1.9 (kg/m²/day) respectively. Scotter *et al.* (1979b) found evapotranspiration of perennial ryegrass was 6-7 (kg/m²/day). One explanation for the lower evapotranspiration rate in this study might be related to the type of plants. Perennial ryegrass was a continuous sward and used more soil moisture than the plants of this study which were spaced plants. The plants in the present study were deep rooted (Chapter 7) and might have depleted soil moisture from depths below 0.7 m, yet the soil moisture was only measured above 0.7 m depth (Scotter *et al.* 1979b). Barker *et al.* (1985) found that evapotranspiration rate was linearly related to soil moisture deficit. This is another reason for less evapotranspiration by plants in this study with a high soil moisture deficit compared with the results of Scotter *et al.* (1979b) measured at field capacity.

8.5.2 Morphology

Yield and yield components of the stressed plants were decreased as water stress progressed from the first to the third harvest. Water stress decreased the stem dry weight of both sainfoin and lucerne, as has been previously reported for lucerne (Cowett and Sprague 1962; Peterson *et al.* 1992; Halim *et al.* 1989; Mir-Hosseini-Dehabadi *et al.* 1993b) (Chapter 4), and sainfoin (Mir-Hosseini-Dehabadi *et al.* 1993c) (Chapter 5).

Over all harvests the leaf/stem ratio of Eski (0.62) was higher than for lucerne (0.5), in agreement with Mir-Hosseini-Dehabadi *et al.* (1993c) (Chapter 5) who found higher leaf/stem ratio for Eski than lucerne (1.14 vs 0.8). The higher leaf/stem ratio might have increased the forage quality. Leaf protein, in-vitro digestibility, Ca, and Mg were higher than for stems in sainfoin, and the percentage of cellulose, hemicellulose, lignin, cell wall constituents and K was lower than for stems (Koch *et al.* 1972). Kidambi *et al.* (1990) found an increase in the concentrations of Ca, Mg, and Zn, in sainfoin and lucerne with decreasing soil moisture supply. Peterson *et al.* (1992) indicated that the improved quality of water stressed legumes (lucerne, birdsfoot trefoil, red clover, cicer milkvetch) was related to greater leaf:stem weight ratio.

Lucerne yield and yield components were more affected by water stress than in the sainfoin cultivars. The yield of lucerne and sainfoin was decreased under water stress by 46% and 32% for lucerne and sainfoin cultivars respectively. A similar result was found in the glasshouse study (Chapter 7).

Lucerne SLA was higher than for the sainfoin cultivars. Similar results were found in the glasshouse study (Chapter 7), and by other researchers Sheehy and Popple (1981) and Mir-Hosseini-Dehabadi *et al.* (1993c). The greatest SLA was found for non-stressed plants from third harvest and regrowth plants. This result was similar to findings in Chapters 5-7.

Stem density was decreased by water stress in agreement with Bennett and Doss (1960) who also found a reduction in stem density of lucerne by water stress.

The stem density was not affected by water stress as much as stem and leaf dry weight, indicating thinner stems for stressed plants.

As expected, the different growth habit of the sainfoin cultivars resulted in a different distribution of yield through the harvests. Eski had the lowest yield at the first harvest. The yield distribution of Remont relative to Eski through all three harvests was more similar to lucerne, (Fig. 8.2), but the total yield for the season of both cultivars was similar. Similarly Cooper (1972b) found that Remont began growth earlier than Eski and yielded more than Eski on the first sampling (May 12) and the yield of Eski at the last sampling (21 June) was higher than that of Remont. Eski showed higher relative growth rate than Remont in summer (21 Jun). Cooper (1972b) concluded that Eski (a late-maturing "one-cut" type) would most efficiently utilize soil moisture and incident radiation in areas where soil moisture is available in spring but is limiting in summer.

Eski and Remont produced 70 and 53 % of their total yield, respectively by the first two harvests (Table 8.6). This was in agreement with Bolger *et al.* (1990) who found sainfoin had produced a high proportion of its seasonal yield (58-63 %) in spring.

Lucerne yield in both the stressed and non-stressed experiments was higher than that of the sainfoin cultivars (Fig 8.2), a result which has been reported in many studies (Hanna *et al.* 1977b; Percival and McQueen 1980; Bolger *et al.* 1990; Mir-Hosseini-Dehabadi *et al.* 1993c). Percival and McQueen (1980) found that the highest yielding sainfoin cultivar in their study (Melrose) had only half the yield of lucerne. Hanna *et al.* (1977a), in a dry region in Canada, found the average yield for sainfoin over five years was 6313 kg/ha whereas for lucerne, was 7105 kg/ha. In the other study (Peterson *et al.* 1992) lucerne out-yielded the other legume species under water stress conditions. For example, the average yield of drought stressed lucerne was 12% greater than for birdsfoot trefoil and cicer milkvetch, and 165% greater than red clover.

The lesser effect of water stress on sainfoin than lucerne could have resulted from both drought avoidance and drought tolerance strategies. Sainfoin produced the

higher proportion of its yield early in the season when in addition to enough soil moisture the lower VPD would increase plant WUE.

Relative to sainfoin, lucerne has a higher forage production and was able to adapt to water stress by osmotic adjustment and increased Rs. The yield of lucerne was affected by water stress more than for sainfoin (Tables 8.3, and 8.4) and its Ψ was lower than that of the sainfoin (Tables 8.10-8.13). The relatively greater effects of water stress on lucerne, than on sainfoin could have been partly due to its higher production in the absence of water stress.

In conclusion, sainfoin would be preferable, for areas with a soil moisture limitation in summer, since in addition to its equal yield in spring it is more palatable than lucerne (Smoliak and Hanna 1975), does not cause bloat in ruminant livestock (Gutek et al. 1974) and it is also not sensitive to lucerne pests and diseases eg. alfalfa weevil (*Hypera postica* L.) (Hanna et al. 1972).

8.5.3 Physiological factors:

The physiological factors like relative water content, osmotic potential, turgor potential, stomatal resistance, and solute accumulation were influenced by changing soil moisture. As water stress increased the stomatal resistance increased and leaf water potential and osmotic potential decreased. Similar results were found in the indoor experiments (Chapters 3-7). For example: over five harvests the RWC reduced by 9% in this study and 7% in the glasshouse study (Chapter 7). The difference between Ψ of the stressed and non-stressed plants of the present study was 0.4 MPa, and for the glasshouse experiment was also 0.4 MPa (Chapter 7). Relative to non-stressed plants the osmotic potential of the plants was decreased by 0.5 MPa, which was similar to the glasshouse study decrease of 0.4 MPa (Chapter 7).

The Rs of sainfoin and lucerne were similar, in agreement with the results of Sheehy and Popple (1981) and Bolger (1988). The Rs of the leaf surfaces (adaxial and abaxial) were different for the sainfoin cultivars, but not for lucerne. This difference was higher for sainfoin cultivars in the climate room (Chapter 6), than in the glass house (Chapter 7), or in the field (Chapter 8), which could have been

caused by environmental conditions. In climate room vpd was higher and light intensity was lower than glass house and field. Low light intensity increased the effects of vpd on stomatal closure and in high vpd the stomatal resistance of adaxial leaf surface decrease higher than that of the abaxial leaf surface (Pallardy and Kozlowski 1979). The R_s of the adaxial surface of sainfoin responded to water stress earlier than the abaxial surface, while the responses of the abaxial and abaxial surface of lucerne were similar. Pallardy and Kozlowski (1979) reported a greater sensitivity of the adaxial stomata of *Populus* to VPD and light density than for the abaxial stomata.

There are some possible explanations for the different stomatal resistance (R_s) of the adaxial and abaxial surfaces of the sainfoin leaves. A higher stomatal frequency was found for the adaxial leaf surfaces than the abaxial surfaces (Plate 8.4), whereas lucerne had a similar stomatal frequencies on both sides of the leaves. Lower stomatal frequency would increase the R_s , decrease water use, and increase water use efficiency (Jones 1983). However the effect of stomatal frequency on water use depends on pore size, which needs to be investigated in sainfoin for adaxial and abaxial leaf surfaces. Jones (1977) found selection for low stomatal frequency of barley was offset by increases in pore size (so that leaf R_s was unchanged).

A linear function of π on RWC (Fig8.3b) accounted for 97, 91, 95% of variation in π for Eski, Remont, and lucerne respectively, and showed the changes in the osmotic potential of the leaves were mostly due to dehydration rather than solute accumulation. The results showed that over five harvests the difference between π of stressed and non-stressed plants was 0.5 MPa whereas for π_{100} it was only 0.1 MPa, suggesting only 20% of change in π was due to solute accumulation in the leaves. In the glasshouse experiment (Chapter 7) the π of the stressed and control plants (1.6 vs 1.1) was similar to that of the present study whereas π_{100} was (1.16 vs 0.96 MPa) indicating that 40% of the changes in π were from solute accumulation.

During the experiment the water content of plants recovered at dawn. For example, relative water content over three harvests was 92 and 86 % at dawn and

midday respectively (Tables 8.8-8.19), and relative to midday it increased 12% at dawn (Fig. 8.3 a-d).

Lucerne was effected by water stress more than sainfoin, and had a lower leaf water potential and osmotic potential than for sainfoin (Tables 8.10-8.15). These results confirmed the results of the indoors studies (Mir-Hosseini-Dehabadi 1993a) (Chapter 3) and (Chapter 7). A higher Ψ for sainfoin than lucerne was also found by Sheehy and Popple (1981). Over the five harvests the difference between Ψ of these two species was about 0.6 MPa, a similar difference to that in the study of Sheehy and Popple (1981). They did not present any reason for this difference, due to insufficient information available to determine the basis of higher Ψ in sainfoin than lucerne.

The π of the plants changed dramatically at Ψ less than -1.5 MPa (Fig. 8.3 a-d), but at dawn when Ψ was about -1.5 MPa at a RWC of about 65% the reduction of π was only 0.5 MPa (Fig 8.3 a-d), indicating the earlier effect of water stress on Ψ than π . Lower Ψ and π at midday, than at dawn, at a given RWC (Fig. 8.3) suggested the effects of other factors (eg. temperature, humidity) on Ψ and π of the leaves.

More negative leaf water potential of the regrowth than growth plants could have been due to greater severity of water stress for regrowth plants in particular for the regrowth following the second harvest (Table 8.1).

In summary the following reasons might have resulted in the higher leaf water potential of sainfoin than of lucerne during the experiment:

- 1) Higher demand for water by lucerne than for sainfoin because of higher leaf area,
- and 2) The higher stomatal resistance of sainfoin than lucerne at a given leaf water potential, therefore giving sainfoin relatively greater drought tolerance.

8.3 Conclusions

Sainfoin cultivars had different growth patterns during the experiment. Eski initially grew more slowly but the total mass produced was similar for both cultivars. Lucerne out-yielded sainfoin cultivars in the stressed and non-stressed experiments, and had a higher LA, leaf dry weight, stem dry weight, stem density and SLA than sainfoin.

Lucerne yield was affected by soil moisture stress relatively more than sainfoin and also showed lower Ψ , and π than sainfoin, but similar R_s , RWC, and π_{100} for both species. Both species showed recovery from water stress during the night by having higher RWC, Ψ , and π , at dawn than midday.

Sainfoin produced a high proportion of its yield early in the season, and maintained its Ψ and π higher than lucerne and promised to be a desirable plant as an alternative to lucerne in dry conditions.

Chapter 9

Carbon Isotope Discrimination of leaves and roots of water stressed sainfoin

9. Carbon Isotope Discrimination of leaves and roots of water stressed sainfoin

9.1 Abstract

Carbon isotope discrimination (Δ) of leaves, and roots from different depths of sainfoin cultivars were studied in four indoor and outdoor experiments. The Δ of Eski, Remont (sainfoin cultivars), and lucerne was compared over three harvests in a field experiment under non-stressed and progressive water stress and conditions.

The results showed strong negative correlation between Δ and WUE as predicted by theory. A positive correlation was found between Δ and transpiration rate, turgor potential (P), relative water content (RWC), instantaneous transpiration rate, photosynthetic rate and yield. Discrimination (Δ) was negatively correlated with stomatal resistance (R_s), leaf water potential (Ψ), and osmotic potential (π) of the leaves. Roots had a lower Δ than leaves, and Δ was lower for deep roots than for shallow roots.

Discrimination (Δ), of Eski ("one-cut" type sainfoin) and lucerne was lower than for Remont ("two-cut" type sainfoin) across the three field harvests, which suggested that, similar to lucerne, Eski had a higher WUE than Remont over the three harvests. The results suggested that Δ was an effective method for comparing the WUE of sainfoin and lucerne cultivars. Plant Δ reflected the plant history of water use, and did not account for any effect of short term severe stress on plant production or survival.

9.2 Introduction

Among forage legumes lucerne is well known as a highly productive species in both dry and wet conditions. Although lucerne has received considerable research attention, information about sainfoin and its capacity in dry conditions is sparse (Bolger 1988).

Higher water use efficiency (WUE) is an attribute which is considered to result in superior yield during water stress conditions (Singh and Kumar 1981, Aggarwal and Sinha 1983, Misra and Chaudhary 1985). Variation in WUE among and within species has been shown by Briggs and Shantz (1914). Their results have been reinforced in the last decade, with numerous reports of substantial variation in WUE both between and within species e.g. in wheat (Farquhar and Richards 1984; Ehdaie *et al.* 1991; Ehdaie and Waines 1993), crested wheatgrass (Frank *et al.* 1985), barley (Hubick and Farquhar 1989), rice (Dingkuhn *et al.* 1991), sunflower (Virgona *et al.* 1990), peanut (Hubick *et al.* 1986a), and cowpea (Ismail and Hall 1992).

Variation in the WUE of sainfoin cultivars has not been reported so far, however, high WUE of sainfoin in spring relative to summer has been reported by Bolger and Matches (1990). Although this was similar to lucerne WUE in spring, lucerne had a higher WUE than sainfoin over the the whole season. Bolger and Matches (1990) used only above ground components of the plants for their calculations. Consideration of both roots and shoots might influence WUE of sainfoin relative to lucerne, particularly since sainfoin has a lower S/R ratio (Mir-Hosseini-Dehabadi *et al.* 1993c), (Chapter 5).

Root development in dry conditions is a favourable characteristic which helps plants obtain water from depth and survive during drought. Measurement of root mass is difficult, with high labour requirements and low accuracy. Furthermore, the accurate measurement of plant transpiration in the field is also difficult and contributes to error in the investigation of species and cultivar differences in WUE.

Carbon isotope discrimination (Δ) is a new approach for identifying variation in WUE of plants, and may overcome the considerable problems involved in field

measurement of total dry matter production and transpiration. The theory has been based on discrimination against ^{13}C by leaves during photosynthesis (Farquhar *et al.* 1982b; Farquhar and Richards 1984). In leaf tissue Δ has been shown to be negatively correlated with WUE in numerous species (Farquhar and Richards 1984; Hubick *et al.* 1986a; Hubick and Farquhar 1989; Hubick *et al.* 1988; Martin and Thorstensen 1988; Condon *et al.* 1990; Virgona *et al.* 1990; Johnson *et al.* 1990a; Read *et al.* 1991; Johnson and Bassett 1991; Ehdaie *et al.* 1991; Ehleringer 1991; Ismail and Hall 1992).

The objectives of this study were to understand:

- a) the effects of water stress on the Δ of the roots and shoots of sainfoin;
- b) variation in WUE between sainfoin cultivars indoors and outdoors under constant and progressive water stress;
- c) a comparison of the WUE of sainfoin and lucerne in the field, with progressive water stress; and
- d) relationships between leaf physiological characters and Δ during water stress.

9.3 Materials and Methods

9.3.1 Technique

Carbon isotope discrimination Δ was studied in the following :

- a) leaves of the four sainfoin cultivars (Cotswold-common, Eski, Fakir, and Remont) at three levels of soil moisture from the climate room experiment (Chapter 6)
- b) the leaves of the cultivars Grasslands G35 and Eski from second harvest of the glasshouse experiment (Chapter 7)
- c) roots of the cultivar Eski at 0-0.1 and 0.6-0.8 m depth from the second harvest of glasshouse experiment (Chapter 7).
- d) leaves of the cultivar Remont under two levels of soil moisture (Chapter 5)
- e) leaves of the cultivars Eski and Remont, and of lucerne (Grasslands Oranga) at the second, third and first regrowth harvests at two soil moisture levels, with three replicates in the field experiment (Chapter 8).

After harvest the samples of leaves/roots were dried immediately at 70 °C for 48 h and ground to a fine powder in a Wiley mill (40 µm) and then the molar ratio of C isotopes in the samples were determined using a ratio mass spectrometer as described by Hubick *et al.* (1986a). Leaves was bulked to give a minimum sample size of 1g dry weight.

The samples of stressed and control treatments from the climate room experiment (Chapter 6) were analyzed at the Australian National University (ANU)-Research School of Biological Science, and the rest of the samples were analyzed at the, Waikato Stable Isotope Unit Waikato University (New Zealand).

The Δ was calculated assuming a value of $-8 * 10^{-3}$ for the isotopic composition of the air relative to the standard Pee Dee formation of belemnite (Mook *et al.* 1983; Farquhar *et al.* 1989a).

9.3.2 Measurements

The following morphological and physiological treatments were measured as previously described (Chapters 5-8):

Yield, Ψ , π , RWC, R_s , T_r , and P_n , and the ratio of C_i/C_a was measured by Li-Cor 6200 from the same leaves used for R_s and P_n .

9.3.3: Statistical analysis

Analysis of variance was performed using the appropriate experimental design (see Chapters 5-8), using the General Linear Model (GLM) procedure of SAS (SAS Institute Inc. 1991). The relationships between plant characters and Δ were tested by linear regression (Steel and Torrie 1981).

9.4 Results

9.4.1 Climate room experiment

The Δ of plants (samples) from the climate room experiment ranged from 18.81 to 24.57 and showed significant differences between Δ for soil moisture levels ($P < 0.001$) (Table 9.1). The Δ of cultivars was not significantly different. The Δ of the moderately ($\Delta = 20.48$) and severely ($\Delta = 20.86$) stressed plants was lower than for the control plants ($\Delta = 23.18$) (Table 9.1).

Table 9.1: Carbon isotope discrimination ($\Delta \times 1000$) with Cotswold-Common, Eski, Fakir, and Remont, at three levels of soil moisture in a climate room.

	Control	Moderately stressed	Severely stressed
Cotswold-Common	23.49	21.05	21.30
Eski	22.81	20.61	19.63
Fakir	23.36	20.45	20.25
Remont	23.07	21.32	20.73

Soil moisture *** ($P < 0.001$) (SEM=0.27)

Cultivar ns

Soil moisture x cultivar ns. Numbers are mean of four replicates.

9.4.2 Glasshouse results

9.4.2.1 Carbon isotope discrimination of leaves in the glasshouse experiment (Chapter 7).

Carbon isotope discrimination of the leaves (samples) ranged between 21.77 and 23.79 and was significantly different between stressed and non-stressed treatments ($P < 0.05$) (Table 9.2). However, Δ was not significantly different between sainfoin cultivars (Grasslands G35, and Eski).

Table 9.2: Carbon isotope discrimination ($\Delta \times 1000$) with leaves of Eski and Grasslands G35 at two soil moisture levels in the glasshouse.

	Eski	Grasslands G35
Non-stressed	22.71	23.54
Stressed	22.46	22.41

Cultivar ns ($P < 0.05$)

Soil moisture * ($P < 0.05$) (SEM=0.19)

Cultivar x soil moisture ns ($P < 0.05$)

9.4.2.2 Carbon isotope discrimination of the roots

The Δ of the roots (samples) ranged between 20.19 and 22.15 and was significantly greater at 0-0.1 m than at 0.6-0.8 m depth ($P < 0.001$) (Table 9.3).

Stressed plants had significantly lower Δ (20.86) than the non-stressed plants (21.26) ($P < 0.05$). The interaction of soil moisture by depth was also significant ($P < 0.01$), while the differences in Δ for the stressed and non-stressed plants at 0.1 m depth (0.95) were higher than at 0.6-0.8 m depth (0.16) (Table 9.3).

Table 9.3: Carbon isotope discrimination ($\Delta \times 1000$) of roots of Eski, at two depths and two soil moisture levels in the Glasshouse.

	Depth (m)	
	0.0-0.1	0.6-0.8
Non-stressed	21.98	20.53
Stressed	21.03	20.69

Soil moisture * ($P < 0.05$) (SEM=0.129)

Depth **** ($P < 0.0001$) (SEM=0.129)

Soil moisture X depth ** ($P < 0.01$) (SEM=0.183)

Repeated measures analysis for comparison of the Δ of leaves and roots showed that Δ was significantly different for leaves and roots ($P < 0.05$). The Δ of leaves was 22.6, and of roots was 21.6.

9.4.3 Carbon isotope discrimination in the field

9.4.3.1 Carbon isotope discrimination of Remont

There was a significant difference between Δ of leaves of stressed (21.62 ± 0.16) and non-stressed Remont (22.6 ± 0.16) ($P < 0.05$). The range Δ for samples was 20.99-22.65.

9.4.3.2 Carbon isotope discrimination of Eski, Remont, and lucerne in the field.

The Δ ranged between 20.84, and 26.12 and pooled ANOVA over three harvests (second, third, and first regrowth harvest) showed a significant difference ($P < 0.05$) (Table 9.4) between Δ of stressed (23.01) and non-stressed (23.35) plants.

Over three harvests there was a significant difference ($P < 0.0001$) (Table 9.4) between cultivar/species. The highest Δ was for Remont (23.67) and the Δ of the Eski, and lucerne was statistically similar ($P < 0.05$) (Table 9.4).

Carbon isotope discrimination was affected by harvest ($P < 0.05$) and the regrowth harvest had the highest Δ (23.52), while the second (22.96) and third harvests (23.06) had statistically similar Δ (Table 9.4).

The interaction of soil moisture by cultivar was not significant, however a significant interaction was found between harvest and soil moisture ($P < 0.01$) (Table 9.4). The differences between Δ of stressed and non-stressed plants at the third harvest (1.16) were higher than at the second (0.14) and regrowth harvests (0.05).

Separate ANOVA for Δ of each harvest showed that Δ was significantly ($P < 0.05$) different only at the third harvest (Table 9.4). Cultivar/species had significantly different Δ only at the third and regrowth harvests, and the interaction of soil moisture and cultivar was not significant at any harvest.

Table 9.4: Carbon isotope discrimination ($\Delta \times 1000$) of Eski, Remont, and lucerne for non-stressed and stressed experiments at the second (60 days after imposing water stress, DS), third (90 DS), and regrowth (65 DS) harvests in the field.

	Second harvest (60 DS)	Third harvest (90 DS)	Regrowth (65 DS)
Non-stressed			
Eski	22.80 ¹	23.27	23.6
Remont	23.11	24.60	24.85
Lucerne	22.82	23.01	23.22
Stressed			
Eski	23.00	22.65	23.29
Remont	22.95	23.42	24.08
Lucerne	23.17	21.54	23.09
Pr>F W ²	0.605	0.021	0.786
Pr>F Cu	0.875	0.006	0.025
Pr>F W*Cu	0.515	0.630	0.527
SEM W	0.130	0.225	0.136
SEM Cu	0.160	0.280	0.167
SEM W*Cu	0.230	0.389	0.236

¹ Data are mean of three replicates.

² W= soil moisture levels, Cu= cultivar, W*Cu= interaction of soil moisture by cultivar, and SEM= standard error of the mean.

Pooled analysis of variance over all three harvests showed Pr>F Harvest (H)= 0.0107, SEM harvest=0.128 Pr>F Cu= 0.0001, SEM Cu= 0.128 Pr>F W=0.02, SEM of W=0.103 Pr>F W*H=0.0029 SEM W*H= 0.1785.

9.4.4 Relationships between Δ and Ψ , π , P , RWC, R_s , Tr , C_i/C_a and P_n

Leaf water potential (Ψ) ($r = -0.84$, $P < 0.001$), RWC ($r = -0.7$, $P < 0.05$), π ($r = -0.69$, $P < 0.05$) were negatively correlated with Δ , and P ($r = 0.65$, $P < 0.05$) had positive correlation with Δ in the climate room experiment (Chapter 6) (Fig. 9.1 a-d). However, none of these factors were significantly correlated with Δ in subsequent experiments.

Stomatal resistance of the whole leaf ($r = -0.87$, $P < 0.001$), adaxial ($r = -0.85$, $P < 0.001$), and abaxial ($r = -0.70$, $P < 0.05$) surfaces were negatively correlated with Δ in the climate room experiment (Fig. 9.2 a-c), but this relationship was not significant for other experiments.

Instantaneous transpiration ($\text{mol H}_2\text{O}/\text{m}^2/\text{s}$) ($r = 0.84$, $P > 0.001$) photosynthetic rate ($\mu\text{mol CO}_2/\text{m}^2/\text{s}$) ($r = 0.83$, $P < 0.001$), and average transpiration rate during experiment ($\text{ml H}_2\text{O}/\text{pot}/\text{day}$) ($r = 0.88$, $P < 0.001$) (Fig. 9.3 a-c) were all positively correlated with Δ . In the climate room experiment, WUE had a strong negative correlation with Δ ($r = -0.94$, $P < 0.0001$) (Fig 9.4).

In the third and regrowth harvests of the field experiment the external and internal partial pressures of CO_2 of the leaf, were positively correlated with Δ ($r = 0.93$) (Fig. 9.7).

9.4.5 Relationships of Δ with yield and SLA

Yield ($r = 0.72$, $P < 0.05$) (Fig 9.6) and SLA ($r = 0.62$, $P < 0.05$) of the plants in the climate room were positively correlated with Δ whereas in general, there was a poor correlation between these factors for other experiments. In the field this relationship was not consistent. For example the average Δ of sainfoin and lucerne was positively correlated with yield ($r = 0.96$, $P < 0.01$), and SLA ($r = 0.86$, $P < 0.05$), but separate analysis for sainfoin and lucerne showed non-significant correlation between Δ and yield and SLA. The yield of plants was also positively correlated with average transpiration rate (Fig. 9.7) ($r = 0.95$, $P < 0.001$).

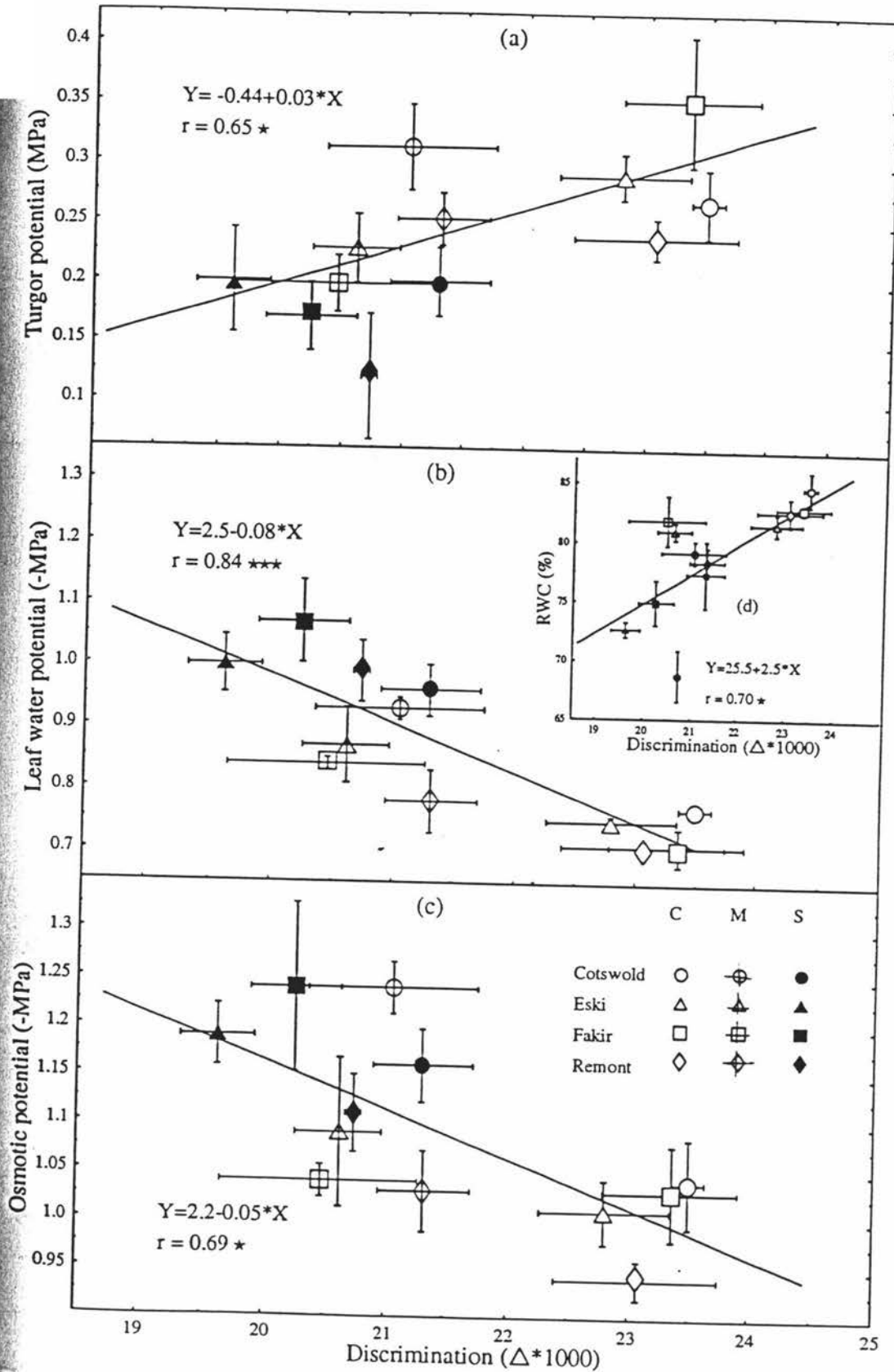


Fig. 9.1 : Correlation of Δ and a) turgor potential b) leaf water potential c) osmotic potential d) relative water content of four sainfoin cultivars (Cotswold-Common, Eski, Fakir, and Remont) under three constant soil moisture levels (C is control and M and S are moderately and severely stressed). Vertical and horizontal bars represent \pm SEM. Markers are means of four replicates; *, and *** show significance at 0.05, and 0.001 levels respectively.

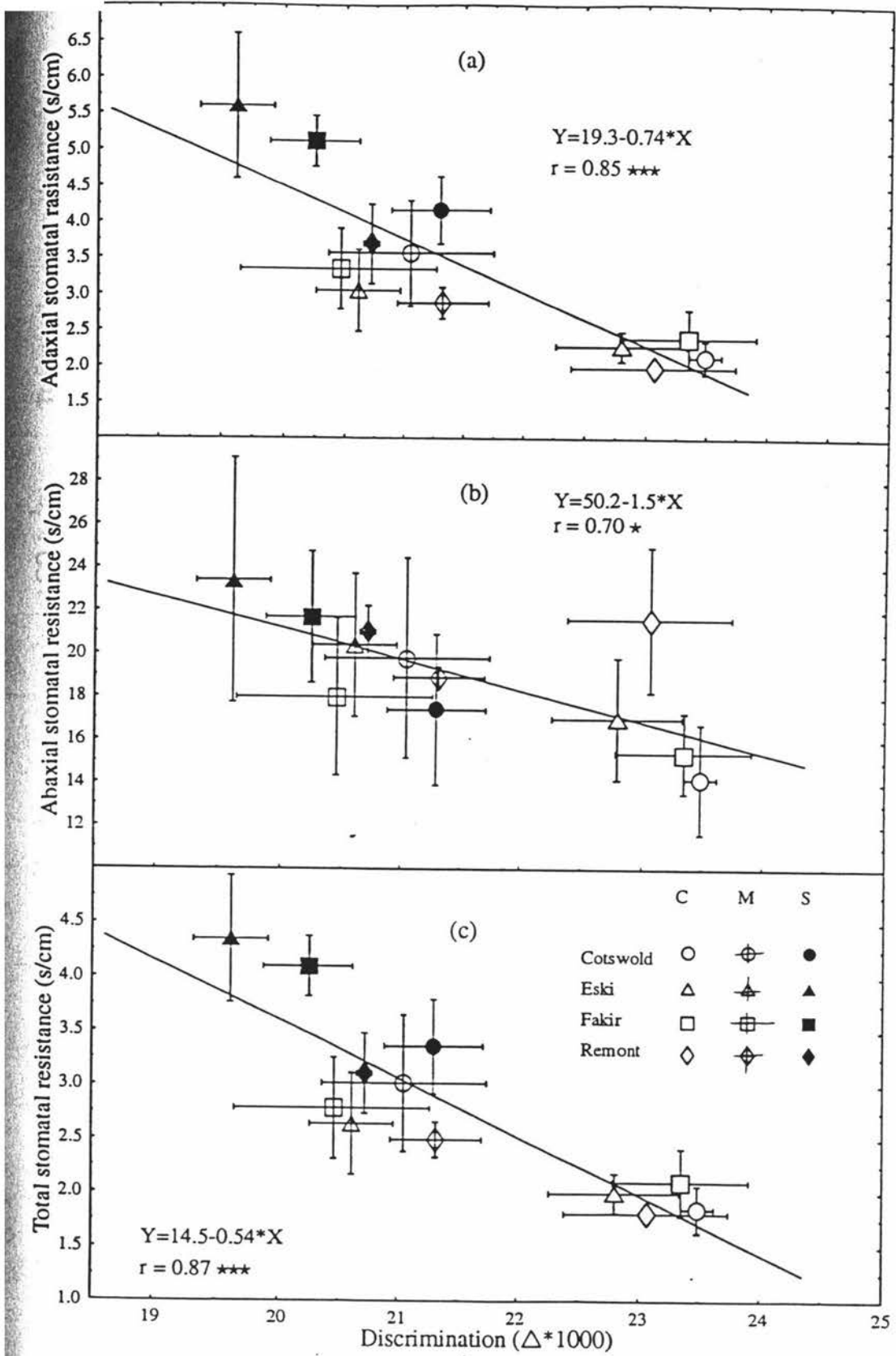


Fig 9.2: Correlation of Δ and stomatal resistance of a) abaxial leaf surface b) adaxial leaf surface c) whole leaf of the four sainfoin cultivars (Cotswold-Common, Eski, Fakir, and Remont) under three constant soil moisture levels (C is control, and M and S are moderately and severely stressed respectively). Vertical and horizontal bars represent \pm SEM. Markers are the means of four replicates; *, and *** show significance at 0.05, and 0.001 levels respectively.

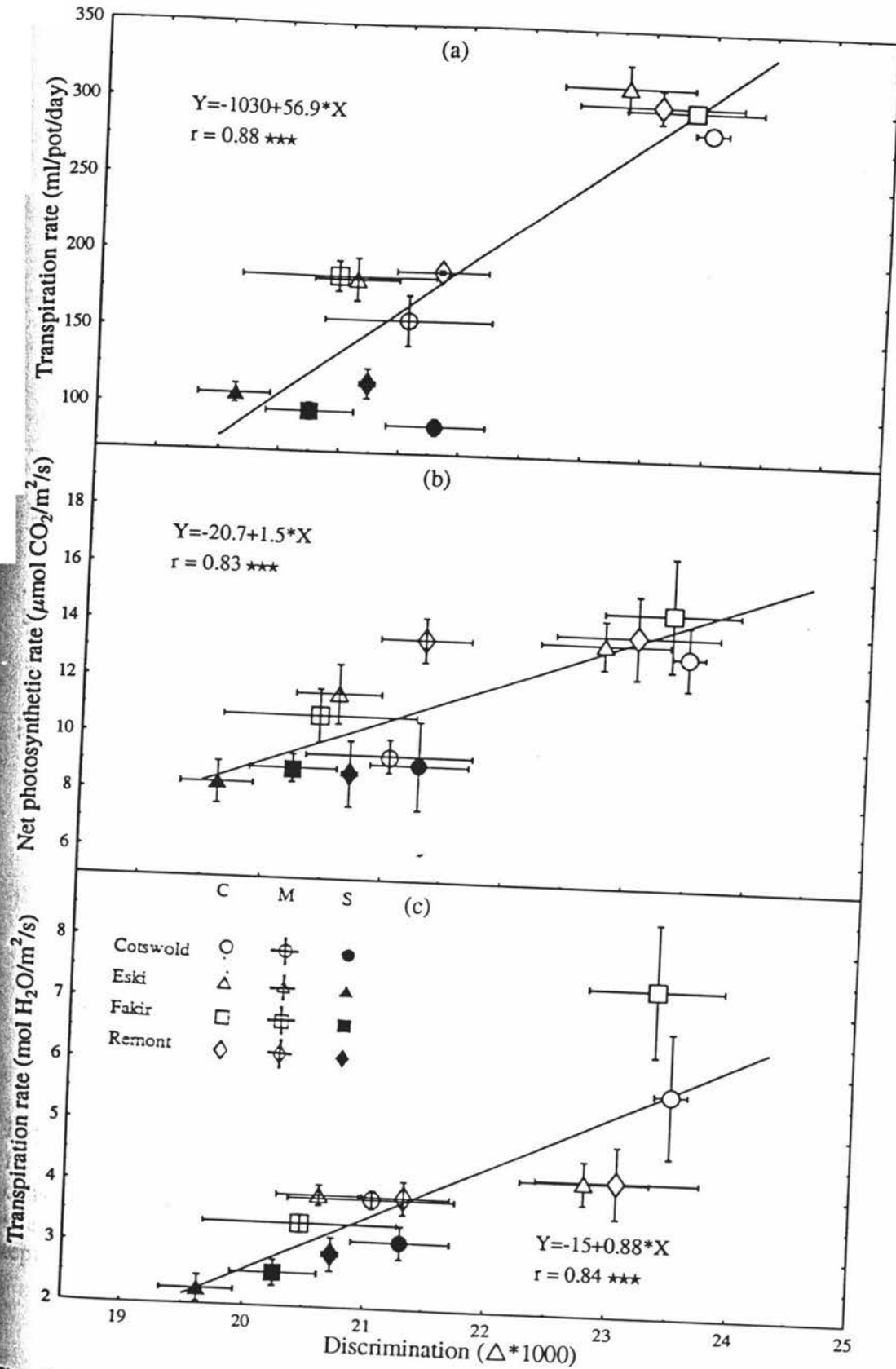


Fig. 9.3 : Correlation of Δ and a) transpiration b) net photosynthesis, and c) instantaneous transpiration of Cotswold-common, Eski, Fakir, and Remont under three constant soil moisture levels (C is control, and M and S are moderately and severely stressed, respectively). Vertical and horizontal bars represent \pm SEM. Markers are mean of four replicates; *** shows significance at 0.001 level

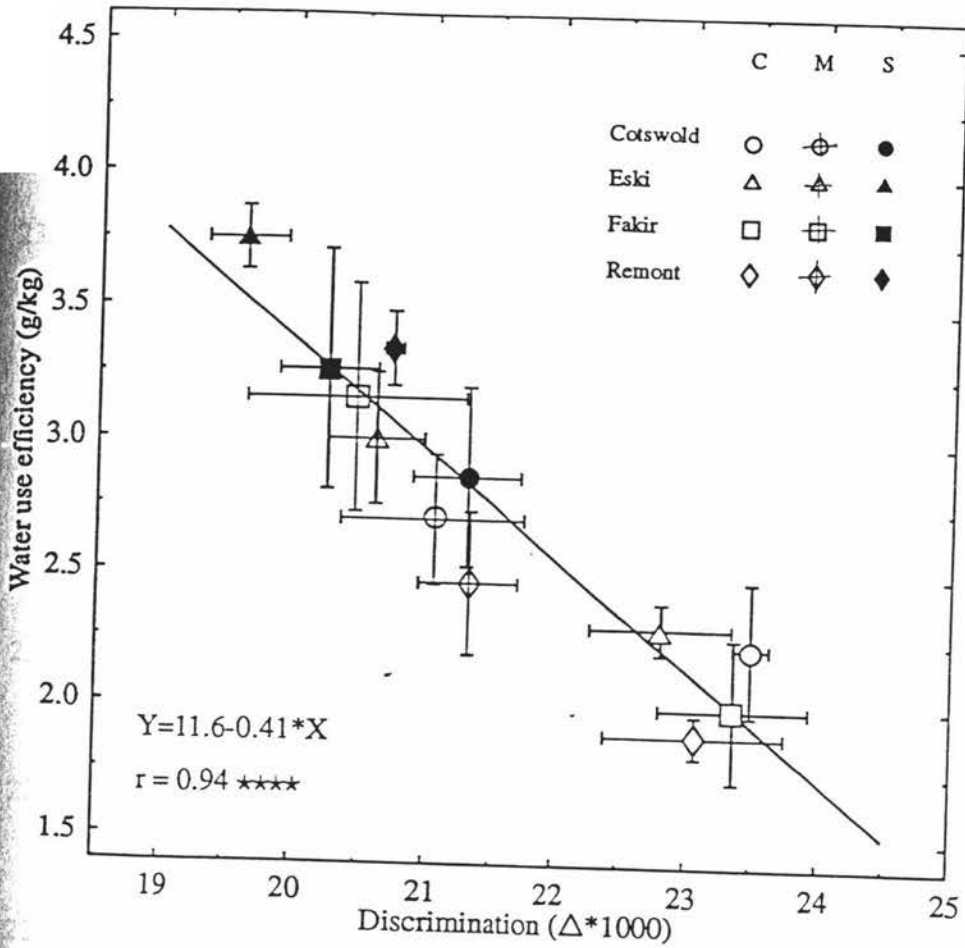


Fig. 9.4 Correlation of Δ and water use efficiency (g dry weight/kg water) of the four sainfoin cultivars (Cotswold-Common, Eski, Fakir, and Remont) under three constant soil moisture levels (C is control and M and S are moderately and severely stressed). Vertical and horizontal bars represent \pm SEM. Markers are means of four replicates; **** show significance at 0.0001 level.

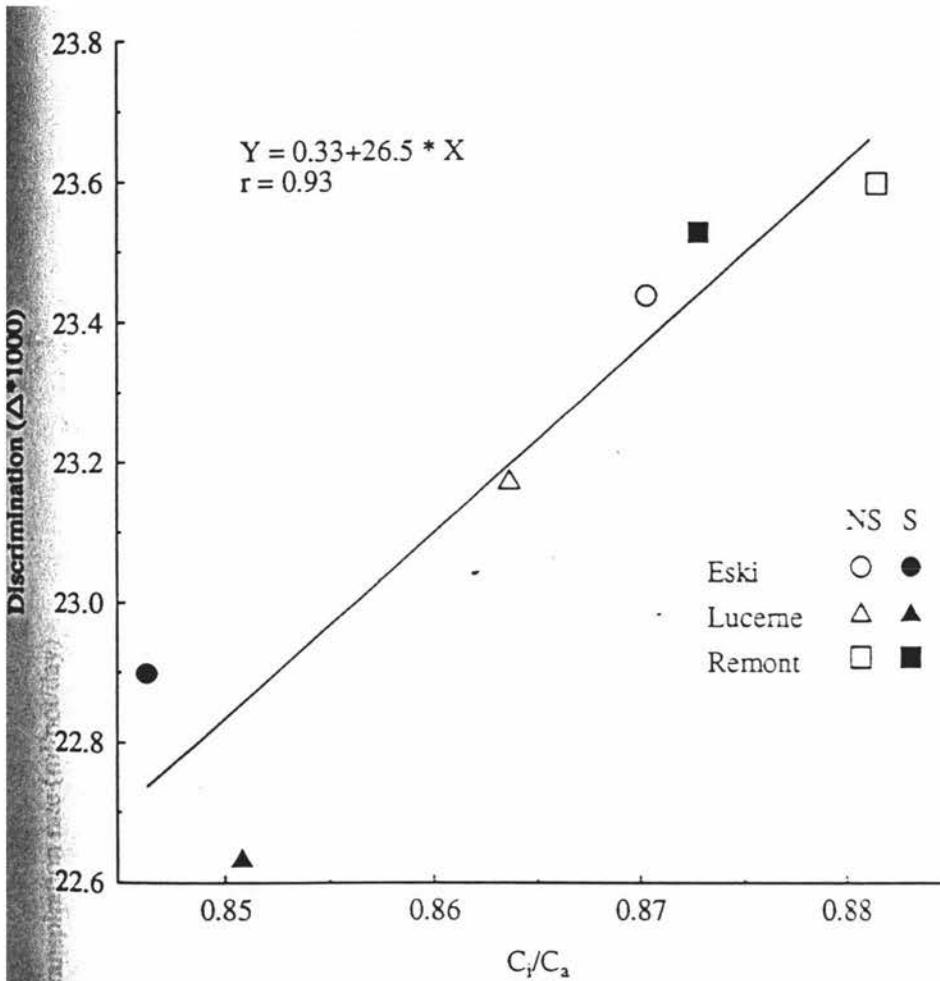


Fig. 9.5: Correlation of Δ and ratio of internal (C_i), and external (C_a) partial pressure of CO_2 concentration of leaves of Eski, Remont, and lucerne under stressed (S) and non-stressed (NS) conditions over the third and regrowth harvests in the field.

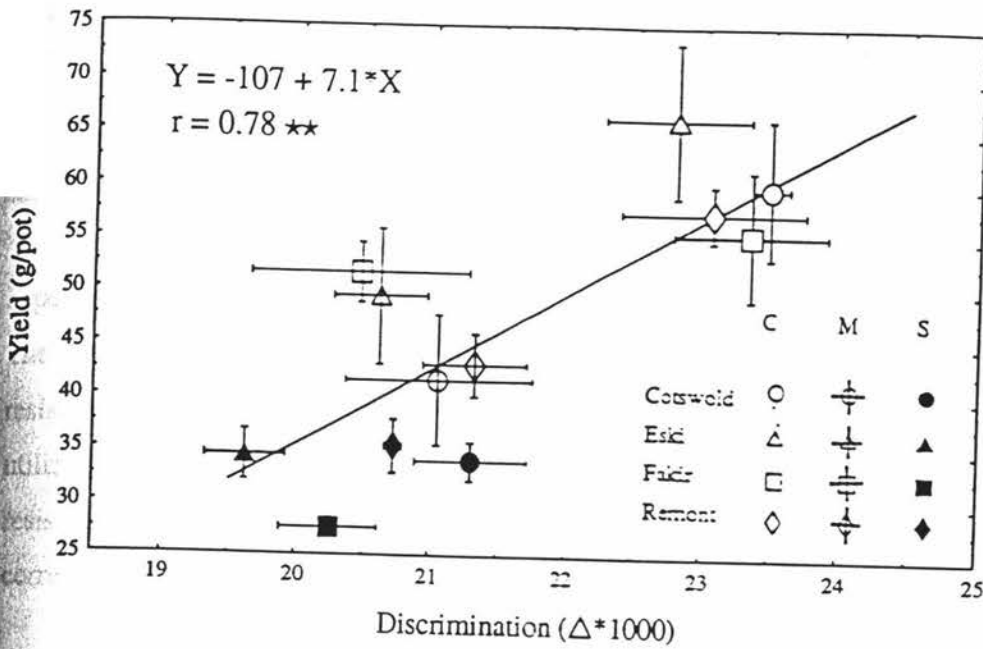


Fig. 9.6: Correlation of Δ and yield of four sainfoin cultivars (Cotswold–Common, Eski, Fakir, and Remont) under three constant soil moisture levels. Vertical and horizontal bars represent \pm SEM. Markers are the mean of four replicates ****** show significance at 0.01 level.

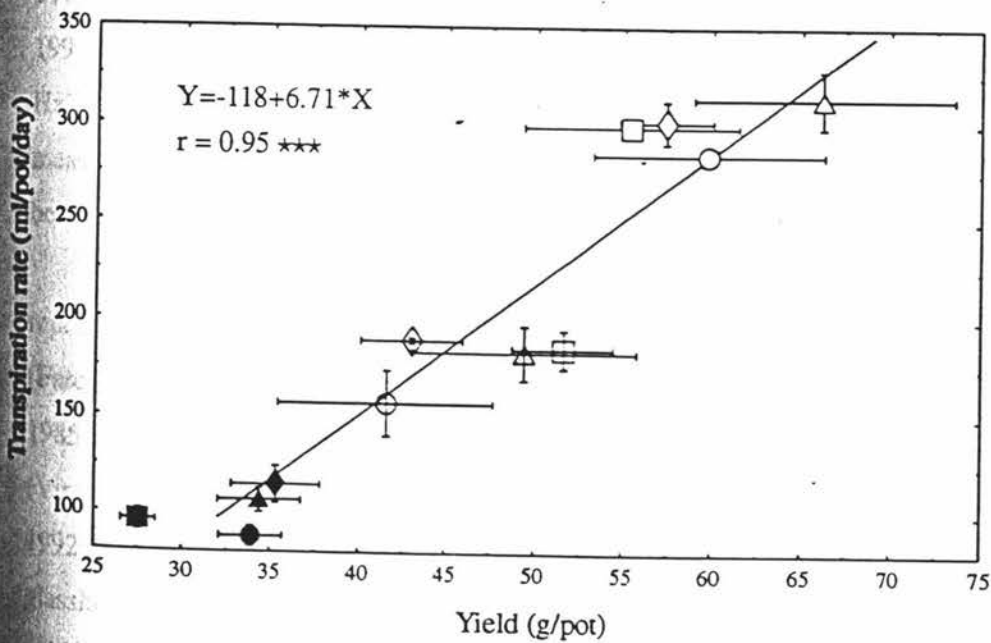


Fig. 9.7: Correlation of yield and transpiration rate of four sainfoin cultivars (Cotswold-Common, Eski, Fakir, and Remont) under three constant soil moisture levels. Vertical and horizontal bars represent \pm SEM. Markers are the mean of four replicates; ******* shows significance at 0.001 level.

9.5 Discussion

Carbon isotope discrimination was affected by soil moisture stress in all experiments, as predicted by the equation ($\Delta = a + (b-a)c_i/c_a$) of Farquhar *et al.* (1989a). The ratio of C_i/C_a was directly related to Δ and was influenced by stomatal resistance. At high stomatal resistance when the supply of CO_2 is less than utilization, C_i decreased and as a result decreased the ratio of C_i/C_a . Stomatal resistance was negatively correlated with Δ (Fig. 9.2). Similarly C_i/C_a was positively correlated with Δ as expected from theory (Fig. 9.5).

9.5.1 Discrimination and WUE

Farquhar and Richards (1984) proposed that Δ was negatively correlated with plant WUE. The same correlation has been reported by other investigators (Hubick *et al.* 1986a; Hubick *et al.* 1988; Martin and Thorstensen 1988; Hubick and Farquhar 1989; Condon *et al.* 1990; Virgona *et al.* 1990; Johnson *et al.* 1990; Read *et al.* 1991; Johnson and Bassett 1991; Ehdaie *et al.* 1991; Ehleringer 1991; Ismail and Hall 1992; 1993; and Ismail *et al.* 1994). The results of Δ were confirmed by actual measured WUE of the plants in this experiment (Fig 9.4). No significant differences between WUE of sainfoin cultivars was found by actual measured WUE or Δ data.

The differences between WUE within and between species were initially reported by Briggs and Shantz (1914), and have been reported subsequently for wheat (Farquhar and Richards 1984; Ehdaie *et al.* 1991), crested wheatgrass (Frank *et al.* 1985), barley (Hubick and Farquhar 1989), rice (Dingkuhn *et al.* 1991), sunflower (Virgona *et al.* 1990), peanut (Hubick *et al.* 1986a), and cowpea (Ismail and Hall 1992) and many other species. The similar Δ of Grasslands G35 and Eski, in the glasshouse experiment (Chapter 7), suggested the WUE of these two sainfoin cultivars was similar. In contrast to the pot experiments, the field experiment showed different Δ for cultivars/species (Table 9.4). Different results for Δ between pot and field experiments have been reported by (Ismail *et al.* 1993, 1994). The following reasons may have contributed to the different results from the field and pot

experiments:

- a) At the pot level, only one harvest was used whereas the field experiment comprised three harvests. In the field experiment the growth of the plants for each harvests occurred during different climate (e.g. air temperature, relative humidity, wind, light intensity), and the cultivars used in the field experiment had different distributions of yield of through the experiment (see Chapter 8).
- b) VPD affects RH and Δ (Condon *et al.* 1992) and WUE (Schulze *et al.* 1957). The pot experiment comprised constant environmental conditions for all cultivars whereas in the field the environmental conditions varied, (e.g. early in the season the vapour pressure deficit (VPD) was lower than that late in the season) and could have affected Δ .
- c) In the field, a lower boundary layer resistance to water vapour and heat transfer than in the pots might have allowed the canopy to to have increased transpiration for a given stomatal resistance, and water use efficiency could have been less for a given stomatal resistance, compared to isolated plants.

The results of Δ in the field showed a lower Δ for lucerne compared to Remont, indicating a higher WUE for lucerne. This agreed with Bolger and Matches (1990) who found a higher WUE of lucerne than sainfoin, through the whole season. The similar Δ of the cultivars/species at the second harvest of the field experiment (Spring) suggested a similar WUE for sainfoin cultivars and lucerne in spring. Bolger and Matches (1990) also found similar water use efficiency for sainfoin and lucerne in spring. In contrast Koch *et al.* (1972) found higher WUE for sainfoin in spring than in summer. A higher WUE for lucerne than for sainfoin has also been reported by Sheehy and Popple (1981).

The Δ for Eski and lucerne over all three harvests was similar, and higher than Remont. This might have been due to greater root length and Ψ of Eski and lucerne than Remont, which was found in the climate room experiment (Chapter 6). The greater root length of Eski might have increased water availability compared to Remont plants and moderated the effect of water stress at the regrowth harvest when the VPD was low, thereby increasing WUE and decreasing Δ (Collins *et al.* 1985).

Condon *et al.* 1992).

Another factor contributing to greater WUE of Eski might have been the slower early season growth compared with Remont, thus saving soil moisture for regrowth. Alternatively, the higher LAI of Eski at the regrowth harvest (0.86) compared with Remont (0.53) (Table 8.2) would have increased the light interception, and decreased the soil evaporation, and contributed to the higher WUE of Eski than Remont. Similar reasoning has been reported for the higher WUE of lucerne than sainfoin (Bolger and Matches 1990). The higher LAI of lucerne ($1.1 \text{ m}^2/\text{m}^2$) than sainfoin ($0.7 \text{ m}^2/\text{m}^2$) in the field study (Table 8.2) also was in agreement with Sheehy and Popple (1981), and Koch *et al.* (1972). Cooper (1972b) found slower early season growth for Eski than Remont (12 May), but higher late season growth of Eski (21 June).

9.5.2: Discrimination and Roots

Since roots are the result of CO_2 uptake by above ground parts of the plants it is expected that Δ of roots will also be affected by the environmental conditions (e.g. water stress, relative humidity, temperature) at the time of their growth. The analysis of Δ of roots found that roots of stressed plants had a lower Δ than the non-stressed plants. An interesting result was the difference between Δ of the roots at different depths, which might have been due to different soil water availability during development of the roots at different depths. The development of the roots at depth occurred at a greater soil moisture deficit than for the roots at shallow depth. Roots at the shallow depth essentially grew in the absence of water stress whereas root development at depth occurred during water stress conditions (Fig. 7.3, 7.4). The greater water deficit might have caused higher R_s and as a result, lower Δ for the carbon translocation to deep roots. The interaction of soil moisture by depth for roots Δ (Table 9.3) was caused by higher differences in Δ of stressed and non-stressed treatments at 0-0.1 m than 0.6-0.8 m. The reason for the low Δ of the roots at 0.6-0.8 for the non-stressed treatment needs further investigation. There was a gradient of Δ from leaves to roots at depth. At this stage the reason for this is unknown, however,

the higher Δ of leaves than roots could be due to differences in the time of development of these two components which happened when availability of soil moisture was different. During the early growth stages plants developed more leaves than roots when the soil moisture was not limiting, but root development occurred later as soil moisture deficits developed. Tables 7.2,7.3,7.12 show that between the first and second harvests (from low water stress to high water stress) about 80% of root development occurred. Thus the lower Δ was possibly a direct result of water stress on roots.

Discrimination (Δ) in roots was less than that of the leaves and it was in agreement with the results of Hubick *et al.* (1986a) who found a higher Δ for leaves than roots in peanut cultivars.

9.5.3: Yield, water status of the leaf and Δ

Yield was positively correlated with Δ in the climate room experiment, whereas in the field this correlation was not significant. The correlation of yield and biomass has not been consistent in other investigations. Condon *et al.* (1987), White *et al.* (1990), Craufurd *et al.* (1991), Ehdaie *et al.* (1991), Condon and Richards (1992), and Virgona (1993) found a positive correlation between biomass and Δ whereas Wright *et al.* (1988), Virgona (1990, 1993), Johnson and Bassett (1991), Ismail *et al.* (1993), and Virgona (1993), found a negative correlation between yield and biomass and Δ . Morgan *et al.* (1993) found no correlation between Δ and forage yield of yellow flowered falcata alfalfa clones.

One possibility for the inconsistent relationships between Δ and yield/biomass might be variation in the range of Δ . For example, in the climate room study there was a large range in Δ (18.81-24.57) which could have been due to large differences in severity of water stress in stressed and control plants. Due to low water stress in the early season for the glasshouse and field experiment, this difference in Δ was possibly not large enough to make this correlation significant. In the glasshouse experiment 30 days after imposing water stress there was still enough soil moisture at depth (0.5 m) (Fig.7.3), and in the field the average soil moisture at 0.0-0.7 m

depth 30 days after imposing water stress was still not significantly different between the stressed and non-stressed experiments. The smaller effect of water stress in the glasshouse and field experiments than in the climate room experiment might have caused less differences between the minimum and maximum Δ for these experiments than for the climate room experiment (see 9.4.1, 9.4.2, 9.4.3). In the study of Virgona (1993) there was also not a large range in Δ for legumes, which had a negative correlation between yield and Δ .

As a result of the gradual development of water stress in the glasshouse and field experiments, plants adapted to water stress conditions by osmotic adjustment (Tables 8.16-8.17), and moderated their growth response. Thus, Δ from the stressed plants in the field and glasshouse was higher than from the climate room experiment.

The correlations of yield and Δ across species and harvests were high and positive, but, were not significant within species. This result may have been due to the different growth habit of Eski and Remont (Chapter 8) and lucerne and sainfoin through the season. Sainfoin produced the greater proportion of its yield earlier in the season when the VPD was low, whereas lucerne had a more even distribution of the yield through the whole season.

Specific leaf area, which is negatively related to the thickness of the leaf, was closely and negatively correlated with WUE, and thus was positively correlated to Δ . This was in agreement with White *et al.* (1990) and Wright *et al.* (1988). The reduction of SLA due to water stress was observed in all experiments (Chapters 4-8) and could have been due to effects of water stress on leaf expansion and translocation of assimilate from the leaf (Hsiao 1973).

The Ψ and π were negatively correlated with Δ while RWC and P were positively correlated with Δ (Fig. 9.1). Turgor potential has been shown to effect the R_s of leaves, and, thus, Δ of plants can be affected by P indirectly through R_s .

Discrimination Δ was also positively correlated to photosynthesis and transpiration rate (Fig. 9.3), and a similar correlation existed between transpiration and yield suggesting the positive correlation of Δ and yield through both transpiration and photosynthetic rate. Stomatal apertures conducive to high P_n and Tr (low R_s) are conducive to high discrimination (Δ).

9.6 Conclusion

Discrimination of leaves and roots of sainfoin was decreased by water stress, and a gradient of Δ was found from leaves to roots at depth.

Discrimination was negatively correlated with WUE and the significant positive correlation of Δ and physiological responses of the leaves at pot level suggested Δ is a useful tool to understand the water relations and yield of the plants at this location.

Eski had a higher WUE than Remont and it would be preferred for forage production in dry conditions especially with a limitation on soil moisture during summer. The WUE of lucerne and Eski was similar, but since Eski produced a higher proportion of its yield early in the season, it might be preferred to lucerne in regions with precipitation only in winter and spring.

Inconsistent correlations between Δ and yield in the pot and field studies suggest this correlation should be considered carefully. It appears to depend on the growth habit of the plants relative to changes in environmental conditions (temperature, humidity, soil water available) during growth.

Chapter 10

General Discussion

10. General Discussion

In this chapter the physiological and morphological responses of sainfoin to water stress in all experiments are reviewed, and a comparison between sainfoin and lucerne drought resistance is made. The various methods used in the experiments are also compared and evaluated.

10.1 Responses of sainfoin to water stress

Sainfoin responded to water stress both physiologically and morphologically. The S/R ratio and the SLA of sainfoin decreased under water stress (Chapters 6,7). Roots and shoots of sainfoin showed osmotic adjustment to water stress and sainfoin also controlled transpiration rate in response to water stress by increasing stomatal resistance (Chapter 7). After 75 days of water stress the total root mass and length of stressed sainfoin was higher than for the controls, and water stress resulted in greater root growth at depth (Chapter 7). Although the total root mass and length of sainfoin and lucerne were similar, lucerne had greater root mass at 0-0.4 m depth, and the root growth of sainfoin below 0.6 m depth occurred earlier than in lucerne (Chapter 7).

Efficient use of water is an important attribute for forage species growing in dry conditions. Plants can improve their WUE by several approaches. One approach is for most of the growth to occur at a low vapour pressure deficit (VPD) (Turner 1986a). The VPD in spring is lower than in summer, therefore, plants that produce a greater proportion of their yield in spring would have a better WUE (Sinclair 1984). This was the case for sainfoin, where growth occurred earlier in the season when VPD was probably low. In contrast, forage production of lucerne was distributed through the whole season (Chapter 8). This attribute of sainfoin might be more important in areas where precipitation is limited to winter and early spring, for example 17% of the total land area of Iran (Choudhary 1992). In addition, the distribution of forage production by sainfoin also avoids the severe drought and heat stress of summer (Chapter 8, section 2.8).

Under water stress, carbon isotope discrimination of Eski and lucerne were

statistically similar, but it was higher for Remont. Discrimination was different for the leaves and roots of sainfoin, and, in addition, was lower for the roots at 0.6-0.8 m depth than for the roots at 0.0-0.1 m. Since plant material which grows in dry conditions has lower discrimination than that which grows in non-water-stressed conditions, the lower discrimination of roots at 0.6-0.8 m depth indicated these roots grew under more water stress than occurred above 0.1 m depth.

A significant correlation was generally found between carbon isotope discrimination and other physiological characters like Ψ , RWC, π , Tr, P_n and yield, demonstrating the usefulness of the method for predicting the response of sainfoin and lucerne to water stress (Chapter 9).

In dry conditions plants can use water more efficiently by reducing leaf area, rather than dry weight, and thereby decreasing SLA and increasing leaf thickness. Leaf thickness has been correlated with increases in the maximum rate of photosynthesis (Nobel *et al.* 1975, Dornhoff and Shibles 1976). Furthermore, a low SLA results in a lower transpiration surface per unit of dry weight and is a useful character for plants in dry conditions (Mooney 1980). Leaves will also have a lower boundary layer resistance and consequently a lower tendency for heat stress. Although water stress decreased the SLA of both sainfoin and lucerne (Chapters 4,5,6,7) sainfoin SLA was lower than lucerne SLA under both water stressed and non-stressed conditions. Sainfoin has shown lower SLA than lucerne in other studies (Sheehy and Popeele 1981; Bolger and Matches 1990).

A drought resistance attribute of plants is a high root to shoot ratio (Bielorai 1992b). A reduction in shoot mass and increase in root mass of sainfoin increased the water uptake capacity and decreased the water demand of sainfoin under water stress conditions (Chapters 6,7). Sainfoin decreased shoot but not root growth when exposed to water stress, which increased the root:shoot ratio (Chapter 7). Sainfoin root:shoot ratio was higher than for lucerne in most of the experiments (Chapters 3,5,7,8) suggesting a relatively greater allocation of carbohydrate to roots than shoots.

Although the root length and root mass density of sainfoin and lucerne were similar 105 days after the imposition of water stress, sainfoin roots had grown below

0.6 m depth earlier than lucerne roots (Chapter 7). This helped sainfoin to maintain Ψ and P. The root density was greater at depths below 0.6 m depth for sainfoin and above 0.4 m depth for lucerne (Chapter 7).

Lucerne and sainfoin R_s were increased by water stress. A similar R_s for sainfoin and lucerne had been observed by Sheehy and Popple (1981), and Bolger (1988). One notable difference between sainfoin and lucerne was that the R_s of the two species was not equally distributed between the adaxial and abaxial leaf surfaces. The R_s of the adaxial and abaxial leaf surfaces of lucerne were similar (Chapters 4,7,8). In sainfoin, the R_s of the abaxial leaf surface was higher than for the adaxial leaf surface (Chapters 5,6,7,8), partly as a consequence of differences in the stomatal frequencies of the respective leaf surfaces (Chapter 8).

An increase in midday stomatal resistance decreases transpiration rate and therefore decreases water loss. At midday in dry conditions VPD is high and, therefore, WUE would be low (Schulze *et al.* 1957, Hall *et al.* 1976). Sainfoin decreased transpiration rate at midday by increasing R_s (see Chapters 3,5,6,7,8) and this avoided production when a low WUE might be expected. The R_s of the abaxial leaf surface of sainfoin was less sensitive to water stress than that of the adaxial leaf surface (Chapters 5-8). Greater sensitivity of stomata on the adaxial leaf surface of *Populus* clones to environmental conditions has been reported by Pallardy and Kozlowski (1979).

Stomatal frequencies for the adaxial and abaxial surfaces of lucerne leaves were similar whereas the stomatal frequencies on the abaxial surface of the sainfoin leaves were lower than on the adaxial surface. Stomatal frequency was probably the most important factor affecting R_s of the respective leaf surfaces. Nevertheless, there are other factors which might influence R_s , e.g. pore size, and hairs. Further investigation to determine the cause and consequence of the differential R_s of the leaf surfaces of sainfoin is required.

Growth is influenced by turgor potential, and plants that are able to maintain turgor during water stress are more likely to continue growth under dry conditions. Sainfoin maintained its turgor in response to water stress, as well as lucerne by a

combination of high Ψ and osmotic adjustment of the leaves and the roots (Chapters 6,7,8).

Sainfoin and lucerne adapted to water stress in different approaches. Sainfoin Ψ was higher (less negative) than lucerne in most experiments (Chapters 3,6,7, and 8), but lucerne leaves showed higher osmotic adjustment than did sainfoin. In contrast, the osmotic adjustment of sainfoin roots was greater than for lucerne roots (see section 7.3.3.11.3). The high Ψ of sainfoin could be partly attributed to its roots growing below 0.6 m depth more quickly than for lucerne (Chapter 7). The RWC of sainfoin and lucerne were similar but the Ψ of sainfoin was higher than that of lucerne, suggesting the cell wall elasticity of these two species was possibly different. As the Ψ of sainfoin was always higher and the adaxial stomatal resistance of sainfoin was lower than that of lucerne, physiological adjustment of sainfoin to water stress differed from that of lucerne.

Sainfoin was more leafy than lucerne (Chapter 5) due to a proportionally lower stem dry weight but the total dry weight production and regrowth of lucerne was superior to that of sainfoin.

Among the sainfoin cultivars tested, Eski showed the best ability to grow under water stress. This cultivar showed both greater physiological and morphological adaptation than the other cultivars. Eski produced greater root mass than the other cultivars (Chapters 6,7) and maintained high leaf water potential. Although Eski had slower initial growth than Remont, it had a similar total yield (Chapter 8). In the field, Eski had a WUE higher than Remont, but similar to lucerne. Although Remont initially grew faster than all the other sainfoin cultivars it had a lower (more negative) RWC than the other cultivars (Chapter 6), and during the night its osmotic potential did not recover as well as for the others. These attributes of Remont suggested its productivity under non-stress conditions would be greater than under stressed conditions.

Remont production was higher than for Eski early in the season (Chapter 8), therefore for regions with soil moisture available and the need for forage early in the season Remont is preferable relative to Eski.

10.3 Comparison of the methods used in this study

Estimated Ψ , by both pressure chamber and the psychrometer (Wescor) were similar (see sections 6.11.5, and 7.5.3.3). Since the pressure bomb is easier to use and requires less time per measurement, it was more expedient than the Wescor. The Wescor psychrometer required an equilibration time per sample of 20 minutes, and the instrument also took time to set up. Sometimes between the first and the last sample took more than 2 hours using the Wescor, which could affect the results due to variations in the environment in that time. The other disadvantages of Wescor relative to the pressure bomb are its sensitivity to temperature, its poor portability, and its high cost (Brown and Oosterhuis 1992).

Three methods were used for soil moisture and transpiration rate measurements, at the pot level. The TDR produced similar results to total pot weight and gravimetric soil water content (See section 7.3.1.1, & 3.1.2). The TDR was easy to use and needed less time and labour than the other methods. The GSWC is a destructive method and not only took more time relative to TDR, but also disturbed the root. The TDR was suitable for adjustment of soil moisture through the depths since it was fast, and could measure at specific (seven) depths, whereas weighing the pot only measured total soil moisture of the pots.

Carbon isotope discrimination was a useful method for predicting WUE of sainfoin (see section 9.2). It also showed significant correlation with RWC, Ψ , π , P, transpiration rate, photosynthesis rate, and yield. Therefore the carbon isotope discrimination technique was useful in predicting most of the physiological characters of water stressed sainfoin over the whole life of the plant. An advantage of the carbon isotope discrimination technique is that the measurements are not time dependent so large numbers of samples can be measured after completion of the experiment.

Disadvantages of carbon isotope discrimination method include: a) the high cost of analysis, especially when large numbers of samples are involved b) the technique is not able to separate the effects of water stress at a specific time, since it integrates over the total life of the tested material (eg. leaf). The ability of plants to

survive extreme episodes of water stress is often important. Carbon isotope discrimination is not able to show the response of a plant at certain point when an extreme condition was imposed.

10.4 Conclusion

Sainfoin adapted to water stress by increasing stomatal resistance and water use efficiency, rooting deeper, decreasing osmotic potential and transpiration, osmotic adjustment of the leaves and roots, and decreased leaf area and stem dry weight. Under water stress sainfoin grew roots below 0.6 m faster than lucerne and during water stress maintained a higher leaf water potential than lucerne. Sainfoin roots showed higher osmotic adjustment than lucerne roots and also had higher (less negative) leaf water potential.

Sainfoin produced most of its yield earlier than lucerne, and therefore showed adaptation to regions with precipitation distributed in winter and spring. In both non-stressed and stressed conditions lucerne was superior to sainfoin, with higher productivity, regrowth, specific leaf area, and leaf area index, but lower root:shoot ratio than sainfoin. However, the LA and yield of lucerne decreased more than sainfoin in response to water stress.

Among sainfoin cultivars, Eski showed the best response to water stress with deep roots and high Ψ . Remont had greater growth under non-stressed conditions. Across the three field harvests carbon isotope discrimination of Eski and lucerne was lower than for Remont indicating the higher WUE of Eski and lucerne than Remont.

Carbon isotope discrimination was negatively correlated with water use efficiency, stomatal resistance, leaf water potential, and osmotic potential, whereas turgor potential, relative water content, transpiration rate, photosynthetic rate, and yield correlated positively with carbon isotope discrimination, and showed the capability of the method for predicting water status and WUE of the plants during the experiment.

Overall, the physiological and morphological responses of sainfoin to water stress were as good as or better than those of lucerne. Although lucerne has been improved in more extensive breeding programs than has sainfoin, sainfoin showed quicker deep rootedness, and higher leaf water potential, and root osmotic adjustment

than lucerne. Sainfoin showed a good ability for forage production in regions with little precipitation in summer, but precipitation in winter and early spring. Sainfoin has poorer seasonal yield and regrowth after cutting than lucerne, but has useful physiological and morphological adaptation to water stress. Work on the heritability of these responses to water stress of sainfoin is required but this study has demonstrated that sainfoin has potentially useful attributes.

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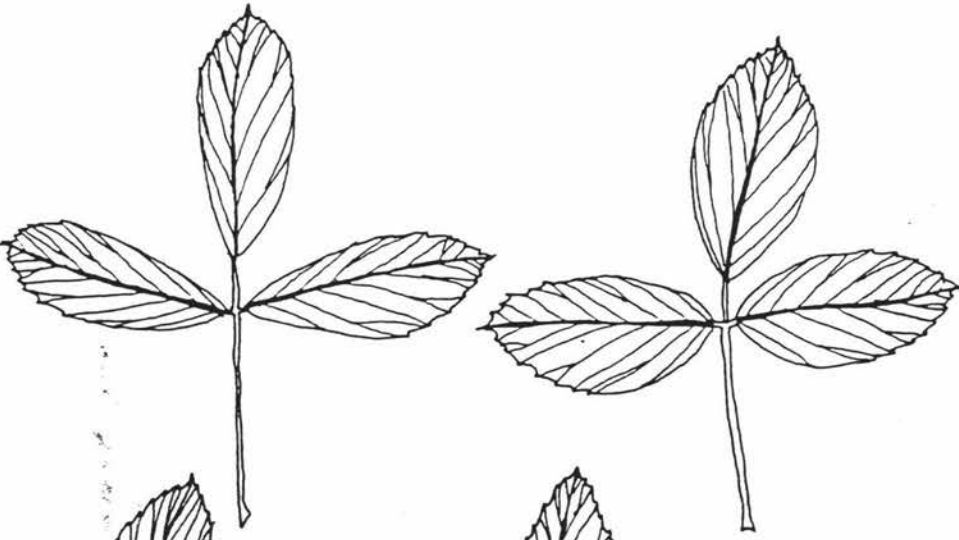
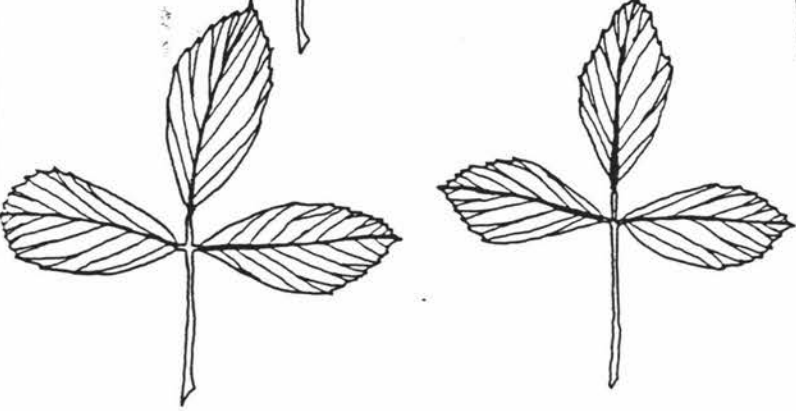
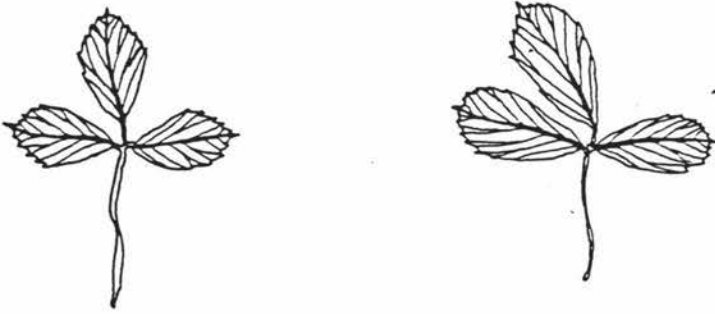
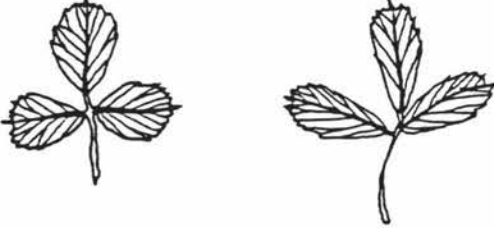

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Appendices

		AREA (cm ²)	
		9.23	1
		6.22	2
		2.63	3
		1.65	4
		1.02	5

Appendix 4.1: Template used to estimate leaf area of lucerne during growth.

Appendix 4.1: Template used to estimate leaf area of lucerne during growth.

Appendix 6.1 : Relative water content (RWC) of four sainfoin cultivar at three soil moisture levels.

Treatment	8/11	15/11	23/11	7/12	14/12	20/12	27/12
Cotswold-C	84.5 ¹	77.6	81.9	79.8	78.9ab	78.3	81.4a
Eski	82.9	78.3	77.3	80.5	77.7ab	74.5	78.0ab
Fakir	83.9	80.8	76.8	81.7	82.7a	77.8	76.0d
Remont	81.2	77.7	76.1	76.2	76.5b	75.9	72.9c
Control	84.1 ²	80.6	82.6a	85.7a	83.5a	82.0a	82.0a
Moderate	84.7	78.8	77.1b	80.5b	82.6b	78.5b	78.0b
Severe	80.6	76.0	74.4b	73.1b	70.5b	69.4b	71.2b
Pr > F Cu	0.2600	0.7100	0.1200	0.3400	0.0200	0.2300	0.0032
Pr > F W	0.0200	0.2700	0.0030	0.0004	0.0001	0.0001	0.0001
Pr>F W*Cu	0.2600	0.6200	0.7000	0.8000	0.0070	0.5300	0.0950
Cu SEM	1.56	2.20	1.83	2.2	1.36	1.43	1.52
W ² SEM	1.35	1.91	1.59	1.92	1.17	1.23	1.31
W*Cu SEM	2.71	3.82	3.17	3.84	2.35	2.46	2.62

¹ Numbers in cultivar rows are means of four replicates.

² W = soil moisture, Cu = cultivar, and SEM= standard error of the mean.

Number(s) within column with same letter(s) are not significantly different.

Appendix 6.2 : Stomatal resistance (s/cm) of the adaxial leaf surface of four sainfoin cultivars and three soil moisture levels.

Treatment	08/11	15/11	23/11	7/12	20/12	27/12
Cotswold-C.	3.28	4.71	2.58	3.17	3.06	2.98
Eski	4.31	3.10	2.96	3.98	4.13	3.36
Fakir	3.94	3.70	3.04	3.67	3.72	3.63
Remont	2.55	2.80	2.99	3.24	2.96	2.58
Control	2.20b ²	2.17b	1.95c	2.07c	2.63b	2.48b
Moderate	2.53b	3.66ab	2.96b	3.58b	3.23b	3.26ab
Severe	5.83a	4.902a	3.75a	4.89a	4.54a	3.99a
P>F CU	0.560	0.115	0.5600	0.4000	0.1100	0.390
P>F W ³	0.005	0.006	0.0001	0.0001	0.0007	0.009
P>F W*C	0.500	0.700	0.5200	0.6400	0.5700	0.430
CU SEM	0.930	0.640	0.250	0.370	0.370	0.460
W SEM	0.810	0.550	0.218	0.330	0.320	0.390
W*CU SEM	1.620	1.100	0.440	0.650	0.650	0.790

¹ Data are means of four replicates.

² Numbers with same letter(s) (within a column) are not significantly different.

³ W = soil moisture, CU = cultivar, and SEM= standard error of the mean.

Appendix 6.3 : Stomatal resistance (s/cm) of the abaxial leaf surface of four sainfoin cultivars and three soil moisture levels.

Cultivar	08/11	15/11	23/11	7/12	20/12	27/12
Cotswold-C.	15.38 ¹	25.3	15.70	18.39	14.36	13.72
Eski	17.75	21.20	23.32	26.08	18.56	14.57
Fakir	20.26	21.93	12.81	27.73	14.00	13.22
Remont	18.83	28.55	23.18	24.77	15.47	12.14
Control	15.12	16.80	18.23	21.07	17.15	13.80
Moderate	19.29	25.90	18.78	23.68	12.81	14.20
Severe	19.72	29.98	19.51	27.98	16.85	12.23
P>F CU	0.430	0.710	0.130	0.460	0.390	0.710
P>F W ²	0.160	0.102	0.950	0.430	0.165	0.520
P>F W*C	0.500	0.630	0.700	0.570	0.081	0.580
CU SE	2.10	4.97	3.45	4.37	2.03	1.48
W SE	1.83	4.30	2.99	3.79	1.76	1.28
W*CU SE	3.65	8.61	5.99	7.58	3.52	2.56

¹ Data are means of four replicates.

² W = soil moisture, CU = cultivar, and W*CU = interaction of soil moisture by cultivar.

Appendix 6.4: Leaf water potential (-MPa) of four sainfoin cultivars at three soil moisture levels at dawn in the glasshouse.

Treatment	5/9	12/9	19/9	26/9	5/10	12/10
Cotswold-C.	0.59 ¹	0.59	0.69a	0.81	0.80a	0.76
Eski	0.50	0.55	0.66a	0.59	0.62ab	0.74
Fakir	0.43	0.51	0.50b	0.54	0.57b	0.70
Remont	0.60	0.44	0.61ab	0.59	0.52b	0.70
Control	0.39b	0.43b	0.44b	0.48b	0.53b	0.63b
Moderate	0.46b	0.45b	0.53b	0.64ab	0.61ab	0.66b
Severe	0.73a	0.70a	0.87a	0.77a	0.74a	0.86a
Cultivar P>F	0.300	0.60	0.0400	0.09	0.02	0.70
Water P>F	0.001	0.03	0.0001	0.01	0.04	0.01
W ² *Cu P>F	0.080	0.60	0.0500	0.30	0.60	0.80
Cu SEM	0.071	0.081	0.047	0.077	0.063	0.066
W SEM	0.061	0.070	0.040	0.067	0.054	0.057
W*Cu SEM	0.122	0.139	0.086	0.134	0.110	0.114

¹ Data are means of four replicates.

² W = soil moisture levels, Cu = cultivar, and SEM= Standard error of the mean.

Appendix 6.5: Osmotic potential (-MPa) of four sainfoin cultivars at dawn under three soil moisture levels in the glasshouse

Treatment	5/9	12/9	19/9	26/9	5/10	12/10
Cotswold-C.	0.86	0.93a	0.89a	1.03	1.04a	1.01a
Eski	0.77	0.75b	0.78b	0.91	0.88b	0.93b
Fakir	0.78	0.81ab	0.76b	0.91	0.81b	0.96b
Remont	0.72	0.76b	0.80ab	0.95	0.80b	0.91b
Control	0.72	0.73b	0.70b	0.82b	0.76b	0.90b
Moderate	0.78	0.79b	0.74b	0.91b	0.86b	0.91b
Severe	0.85	0.91a	1.00a	1.10a	1.02a	1.10a
Cultivar P>F	0.30	0.05	0.0300	0.8000	0.0010	0.0300
Water P>F	0.13	0.01	0.0001	0.0001	0.0001	0.0010
W*P P>F	0.80	0.60	0.7000	0.7000	0.8000	0.0100
Cu SEM	0.05	0.05	0.03	0.04	0.04	0.04
W SEM	0.04	0.04	0.03	0.04	0.04	0.04
W*Cu SEM	0.09	0.08	0.05	0.07	0.08	0.07

¹ Data are means of four replicates.

² W = soil moisture, Cu = cultivar, and SEM= standard error of the means.

Appendix 6.6: Turgor potential (-MPa) of four sainfoin cultivars at dawn under three soil moisture levels in the glasshouse

Treatment	5/9	12/9	19/9	26/9	5/10	12/10
Cotswold-C.	0.27	0.33	0.17	0.23	0.24	0.25
Eski	0.27	0.20	0.12	0.32	0.26	0.19
Fakir	0.33	0.29	0.26	0.37	0.24	0.26
Remont	0.13	0.32	0.19	0.36	0.28	0.21
Control	0.32a	0.28a	0.26	0.34	0.23	0.26
Moderate	0.31a	0.34a	0.21	0.27	0.25	0.24
Severe	0.12b	0.21a	0.13	0.33	0.30	0.24
Cultivar P>F	0.060	0.130	0.060	0.400	0.90	0.300
Water P>F	0.002	0.330	0.200	0.600	0.400	0.200
W ² *P P>F	0.010	0.700	0.080	0.300	0.500	0.370
Cu SEM	0.05	0.05	0.04	0.06	0.04	0.05
W SEM	0.05	0.04	0.03	0.05	0.04	0.04
W*C _u SEM	0.09	0.09	0.06	0.10	0.07	0.08

¹ Numbers are means of four replicates.

² W = soil moisture levels, Cu = cultivar, and SEM= standard error of the mean.

Appendix 6.7: Leaf water potential (-MPa) of four sainfoin cultivars at dawn in the climate room under three soil moisture levels.

Treatment	08/11	15/11	23/11	07/12	14/12	20/12	27/12
Cotswold-C.	0.71 ¹	0.86a	0.89	0.93ab	0.94	0.92	0.91
Eski	0.79	0.73ab	0.74	1.03a	1.06	0.94	0.79
Fakir	0.82	0.78ab	0.76	1.03a	0.88	0.93	0.91
Remont	0.78	0.66b	0.75	0.81b	0.93	0.92	0.94
Control	0.68	0.63b	0.63b	0.85b	0.81b	0.76c	0.72c
Moderate	0.83	0.73b	0.80a	0.93ab	0.93b	0.94b	0.83b
Severe	0.83	0.93a	0.91a	1.06a	1.11a	1.09a	1.10a
Pr>F Cu ²	0.63	0.0400	0.0910	0.05	0.1100	0.9700	0.0700
Pr>F W	0.11	0.0001	0.0005	0.03	0.0005	0.0001	0.0001
Pr>F W*Cu	0.43	0.0190	0.5000	0.11	0.3900	0.3500	0.0400
W SEM	0.06	0.04	0.04	0.05	0.05	0.04	0.04
Cu SEM	0.06	0.05	0.05	0.06	0.05	0.05	0.04
W*Cu SEM	0.11	0.08	0.09	0.11	0.09	0.08	0.07

¹ Data are means of four replicates. Numbers with same letter within column are not significantly different.

² Cu= cultivar, W= soil moisture, and SEM= standard error of the mean.

Appendix 6.8: Osmotic potential (-MPa) of four sainfoin cultivars at dawn under three soil moisture levels in the climate room

Treatment	08/11	15/11	23/11	07/12	14/12	20/12	27/12
Cotswold-C.	1.06ab ¹	1.18a	1.15a	1.15ab	1.18a	1.15ab	1.15a
Eski	1.13a	0.97bc	1.01b	1.23a	1.12ab	1.2ab	1.10b
Fakir	0.99b	1.08ab	0.99ab	1.22ab	1.08b	1.24a	1.10b
Remont	1.05ab	0.93c	0.98b	1.05b	1.08b	1.09b	1.30b
Control	0.99b	0.97b	0.914b	1.09b	1.02b	1.06b	1.10b
Moderate	1.06ab	1.05ab	1.05a	1.13a	1.16a	1.2a	1.10b
Severe	1.12a	1.11a	1.14a	1.26a	1.16a	1.24a	1.18a
Pr>F Cu ²	0.19	0.001	0.054	0.130	0.120	0.100	0.0060
Pr>F W	0.05	0.64	0.002	0.052	0.001	0.005	0.0001
Pr>FW*Cu	0.10	0.35	0.190	0.300	0.33	0.017	0.8600
Cu SE	0.042	0.046	0.048	0.060	0.032	0.037	0.029
W SE	0.036	0.040	0.042	0.052	0.027	0.037	0.029
W*Cu SE	0.072	0.080	0.083	0.099	0.055	0.075	0.058

¹ Data in cultivar rows are means of four replicates. Numbers with same letter within column are not significantly different.

² Cu= Cultivar, W= soil moisture, and SEM= standard error of the mean.

Appendix 6.9: Turgor potential (-MPa) of four sainfoin cultivars at dawn under three soil moisture levels in the climate room

Cultivar	08/11	15/11	23/11	07/12	14/12	20/12	27/12
Cotswold-C.	0.35a	0.32a	0.26a	0.22a	0.24a	0.23ab	0.28a
Eski	0.33a	0.23a	0.28a	0.21a	0.06b	0.26ab	0.26a
Fakir	0.18a	0.30a	0.23a	0.19a	0.21ab	0.31a	0.21ab
Remont	0.27a	0.27a	0.24a	0.24a	0.15ab	0.17b	0.10b
Control	0.31a	0.34a	0.28	0.24	0.21	0.30a	0.29a
Moderate	0.24a	0.31a	0.25	0.20	0.23	0.27a	0.22b
Severe	0.28a	0.17b	0.23	0.21	0.05	0.15b	0.12c
Pr>F Cu ²	0.48	0.005	0.48	0.04	0.02	0.007	0.0005
Pr>F W	0.55	0.050	0.85	0.84	0.09	0.100	0.0047
Pr>F W*Cu	0.30	0.008	0.65	0.77	0.43	0.170	0.0210
Cu SE	0.05	0.04	0.03	0.04	0.05	0.04	0.04
W SE	0.05	0.03	0.03	0.04	0.05	0.03	0.03
W*Cu SE	0.09	0.06	0.06	0.07	0.09	0.07	0.07

¹ Data are means of four replicates. Numbers with same letter within column are not significantly different.

² Cu= Cultivar, W= soil moisture, and ESM= standard error of the means.

Appendix 6.10 : Leaf water potential (-MPa) of four sainfoin cultivars at three soil moisture levels at midday in climate room.

Treatment	7/12	12/12	17/12	22/12
Cotswold-C.	1.00	1.19	1.19	1.03
Eski	1.22	1.11	1.07	0.95
Fakir	1.09	1.07	1.03	1.02
Remont	1.15	1.10	1.27	0.96
Control	0.88b	0.84b	0.94b	0.82c
Moderate	1.12b	1.03b	1.15ab	0.98b
Severe	1.38a	1.47a	1.33a	1.17a
Pr> F Cu	0.6200	0.7200	0.45	0.6300
Pr> F W ²	0.0008	0.0001	0.03	0.0001
Pr> F W*Cu	0.0200	0.4000	0.12	0.1200
Cu SEM	0.10	0.08	0.12	0.06
W SEM	0.08	0.07	0.10	0.05
W*Cu SEM	0.17	0.14	0.20	0.10

¹ Number(s) with same letter(s) (within column) are not significantly different. Data in cultivar rows are means of four replicates.

² W = soil moisture, Cu = cultivar, and SEM= standard error of the mean.

Appendix 6.11 : Osmotic potential (-MPa) of four sainfoin cultivars at three soil moisture levels at midday in the climate room.

Treatment	7/12	12/12	17/12	22/12
Cotswold-C.	1.21	1.42	1.38	1.29
Eski	1.25	1.32	1.30	1.24
Fakir	1.21	1.30	1.30	1.27
Remont	1.20	1.34	1.20	1.18
Control	1.09b	1.20b	1.12b	1.15b
Moderate	1.16b	1.32b	1.37a	1.19b
Severe	1.41a	1.52a	1.40a	1.40a
Pr> F Cu	0.950	0.5100	0.1430	0.2800
Pr> F W ²	0.003	0.0004	0.0003	0.0001
Pr> F W*Cu	0.520	0.9800	0.4100	0.4300
Cu SEM	0.07	0.06	0.06	0.04
W SEM	0.06	0.05	0.05	0.04
W*Cu SEM	0.13	0.10	0.10	0.74

¹ Number(s) with same letter (within column) are not significantly different. Data are means of four replicates.

² W = soil moisture, Cu = cultivar, W*Cu = interaction of soil moisture and cultivar.

Appendix 6.12 : Turgor potential (-MPa) of four sainfoin cultivars at three soil moisture levels at midday in climate room.

Treatment	7/12	12/12	17/12	22/12
Cotswold-C.	0.21 ¹	0.23	0.20	0.27
Eski	0.03	0.21	0.23	0.29
Fakir	0.09	0.24	0.27	0.25
Remont	0.05	0.24	-0.07	0.22
Control	0.21	0.36a	0.18	0.34a
Moderate	0.04	0.29a	0.22	0.21b
Severe	0.03	0.05b	0.07	0.23b
Pr> F Cu	0.850	0.990-	0.210	0.580
Pr> F W ²	0.370	0.002	0.920	0.020
Pr> F W*Cu	0.130	0.290	0.210	0.580
Cu SEM	0.110	0.066	0.096	0.039
W SEM	0.098	0.058	0.083	0.034
W*Cu SEM	0.196	0.116	0.167	0.069

¹ Number(s) with same letter (within column) are not significantly different. Data are mean of four replicates.

² W = soil moisture, Cu = cultivar, and SEM= standard error of the mean.

Appendix 6.13 : Leaf water potential (-MPa) (measured by pressure chamber) of four sainfoin cultivars at three soil moisture levels at midday in the climate room .

Treatment	08/11	15/11	23/11	07/12	14/12	20/12	27/12
Cotswold-C.	0.25 ¹	0.15	0.29	0.42	0.41	0.72	0.88
Eski	0.25	0.35	0.15	0.41	0.25	0.78	0.73
Fakir	0.20	0.23	0.22	0.27	0.43	0.72	0.78
Remont	0.47	0.47	0.20	0.39	0.47	0.78	0.78
Control	0.12a	0.14b	0.10c	0.15b	0.17b	0.29c	0.29c
Moderate	0.32ab	0.26b	0.21b	0.40a	0.45a	0.67b	0.72b
Severe	0.45a	0.50a	0.34a	0.57a	0.56a	1.16a	1.36a
P>F Cu	0.12	1.0400	0.1800	0.4200	0.2299	0.4600	0.7500
P>F W ²	0.01	0.0031	0.0003	0.0001	0.0006	0.0001	0.0001
P>F W*Cu	0.83	0.0730	0.7800	0.5400	0.0530	0.1800	0.8800
Cu SEM	0.08	0.08	0.04	0.07	0.08	0.12	0.10
W SEM	0.07	0.07	0.04	0.06	0.07	0.10	0.08
W*Cu SEM	0.14	0.14	0.07	0.12	0.13	0.21	0.17

¹ Numbers (within columns) with same letter(s) are not significantly different. Data are means of four replicates.

² W = soil moisture, Cu = cultivar, and SEM= Standard error of the mean.

Appendix 6.14 : Photosynthesis rate $\mu\text{mol CO}_2/\text{m}^2/\text{s}$ of four sainfoin cultivars at three soil moisture levels during the last month in the climate room.

Treatment	27/11	4/12	10/12	13/12	18/12	20/12	25/12
Cotswold-C.	8.8 ¹	11.3	9.6	10.0	11.5	9.3	11.2
Eski	13.2	11.3	10.3	10.8	9.9	10.5	11.4
Fakir	12.0	13.1	11.0	9.2	13.9	8.7	11.9
Remont	11.6	14.8	10.0	11.8	11.5	10.5	12.6
Control	13.4	15.9	11.4a	12.3a	14.9a	11.6a	15.0
Moderate	11.8	12.5	12.8a	10.9ab	10.8ab	9.5ab	10.8
Severe	8.9	9.6	6.5b	8.26b	9.8b	8.3b	9.8
Pr> F Cu	0.17	0.1040	0.8217	0.170	0.390	0.56	0.93
Pr> F W ²	0.40	0.0003	0.0001	0.002	0.040	0.05	0.04
Pr> F W*Cu	0.70	0.9664	0.8173	0.720	0.052	0.28	0.91
Cu SEM	1.38	1.14	1.10	0.09	1.62	1.10	1.76
W SEM	1.19	0.98	0.94	0.75	1.40	0.95	1.53
Cu*W SEM	2.39	1.98	1.88	1.52	2.81	1.90	2.94

¹ Data in cultivar rows are means of four replicates. Numbers with same letter within column are not significantly different.

² W = soil moisture, Cu = cultivar, and SEM= standard error of the mean.

Appendix 6.15: Leaflet numbers/pot of four sainfoin cultivars at three soil moisture levels.

Treatment	03/09	04/10	14/10	30/10	15/11	30/11	15/12
Cotswold-C	138.2a ¹	314.0	411.0	546.3	627.0	666.8	766.1
Eski	96.1b	298.6	444.1	621.6	657.7	603.6	737.9
Fakir	125.3b	312.2	412.0	541.5	568.0	662.7	621.4
Remont	188.9a	409.6	522.1	673.3	678.6	677.3	697.7
Control	145.5	388.1a	531.0a	729.5a	827.8a	792.3a	924.2a
Moderate	131.4	349.1ab	480.8a	598.0ab	393.4b	610.1ab	616.6b
Severe	131.4	263.4b	330.0b	459.0b	484.2b	555.0b	576.6b
Pr> F Cu	0.0024	0.139	0.44	0.49	0.67	0.91	0.63
Pr> F W ²	0.75	0.026	0.014	0.013	0.001	0.059	0.0028
Pr> F W*Cu	0.42	0.155	0.44	0.59	0.46	0.19	0.46
Cu SEM	15.91	36.55	54.55	70.19	71.03	81.57	82.61
W SEM	13.78	31.65	47.24	60.78	61.52	70.64	71.54
W*Cu SEM	27.6	63.31	94.49	121.57	123.04	141.29	143.08

¹ Data are means of four replicates. Numbers with same letter (within column) are not significantly different.

² W= soil moisture, Cu= cultivar, and SEM= standard error of the mean.

Appendix 6.16: Estimated leaf area of four sainfoin cultivars at different soil moisture levels.

Treatment	03/09	04/10	14/10	30/10	15/11	30/11	15/12
Cotswold-C	88.1ab ¹	147.0	183.7	180.3b	217.8b	230.9	217.0
Eski	81.7b	166.0	221.7	256.4a	283.3a	271.0	289.0
Fakir	98.3ab	163.4	207.7	209.8ab	221.0b	269.0	227.5
Remont	104.1a	151.0	196.6	179.1b	210.7b	224.0	216.0
Control	96.2	204.0a	277.5a	287.0a	344.5a	344.1a	356.7a
Moderate	96.2	159.5b	197.6b	201.0b	211.7b	246.5b	207.0b
Severe	86.7	105.9c	132.3c	131.0c	143.3c	155.2c	147.8b
Pr>F Cu	0.04	0.5700	0.4500	0.0250	0.0800	0.6500	0.1300
Pr>F W2	0.31	0.0001	0.0001	0.0001	0.0001	0.0003	0.0001
Pr>F W*CU	0.59	0.2200	0.6400	0.1700	0.1900	0.5700	0.2900
Cu SEM	5.70	11.11	17.07	19.28	21.57	33.68	24.5
W SEM	4.90	9.60	14.80	16.60	18.70	29.20	21.20
W*Cu SEM	9.98	19.25	29.57	33.39	37.39	58.34	42.44

¹ Numbers with same letter(s) (within column) are not significantly different. Data are mean of four replicates.

² W= soil moisture, CU= cultivar, and W*Cu= Interaction of soil moisture and cultivar, and SEM= satandard error of the mean.

Appendix 6.17: The monthly transpiration rate (ml/pot/day) of four sainfoin cultivars at three soil moisture levels.

	Aug.	Sep.	Oct.	Nov.	Dec.
Cotswold-C	53b ¹	105b	206b	273c	245
Eski	51b	114ab	247a	322a	268
Fakir	54ab	136ab	239a	289bc	248
Remont	59a	150a	235ab	305ab	261
Control	64.7a	179.3a	343.4a	481a	430a
Moderate	51.2b	132.8b	227.0b	271b	211b
Severe	46.8c	70.9c	124.9c	141c	126c
Pr >F Cu	0.0400	0.0300	0.0400	0.0130	0.1500
Pr >F W	0.0001	0.0001	0.0001	0.0001	0.0001
Pr >F W*Cu	0.3900	18.700	0.6100	0.8200	0.1100
Cu SEM	1.84	10.89	10.18	10.15	7.28
W ² SEM	1.59	9.38	8.82	8.80	6.74
W*Cu SEM	3.20	0.99	17.60	17.60	13.48

¹ Data are means of four replicates. Numbers with same letter(s) are not significantly different.

² W= Soil moisture treatment, Cu= Cultivar, and SEM= Standard error of the mean.

Appendix 6.18: Repeated measure analysis of morphological and physiological characters of sainfoin cultivar under three soil moisture levels.

	Tests of hypothesis for within subject effects (Time effects and its interaction with other treatments)				Tests of hypothesis for between subjects effects (Pooled over time).		
	Pr> F (Time=T)	Pr> F (T*W ⁴)	Pr> F (T*C)	Pr> F (T*W*C)	Pr> F (W)	Pr> F (C)	Pr> F (W*C)
RWC ¹	0.0001	0.0056	0.2537	0.6118	0.0001	0.0143	0.0887
RS (Abaxial)	0.0001	0.1728	0.3601	0.5239	0.2518	0.5304	0.8118
Rs (Adaxial)	1.1300	1.4100	0.9100	0.8100	0.0001	0.2041	0.4452
Ψ (Gse)	0.0001	0.4110	0.5772	0.7603	0.0001	0.0488	0.1224
π (Gse)	0.0001	0.0780	0.6918	0.9152	0.0001	0.0004	0.4821
P (Gse)	0.0007	0.0117	0.0588	0.2204	0.1310	0.2822	0.1077
Ψ (CR)	0.0001	0.2486	0.0078	0.0286	0.0001	0.3834	0.2095
π (CR)	0.0001	0.5036	0.0023	0.0066	0.0001	0.0056	0.0623
P (CR)	0.0026	0.1013	0.0625	0.1608	0.0001	0.0864	0.0068
Ψ (Mid) ²	0.0488	0.4138	0.5303	0.0923	0.0001	0.8001	0.0195
π (Mid)	0.0029	0.1278	0.6971	0.8568	0.0001	0.4085	0.5305
P (Mid)	0.0153	0.1577	0.9326	0.2207	0.0147	0.5860	0.0335
Ψ (PB) ³	0.0001	0.0001	0.1862	0.0819	0.0001	0.2329	0.5213
Photosynthesis	0.0279	0.3921	0.6126	0.6348	0.0001	0.1748	0.2522
Leaflet number	0.0001	0.0001	0.3648	0.4767	0.0067	0.7015	0.3537
Leaf area	0.0001	0.0001	0.0979	0.7084	0.0001	0.2062	0.2691
Transpiration	0.0001	0.0001	0.0139	0.7719	0.0001	0.0057	0.8300

¹RWC, Rs, Ψ, π, P are: Relative water content, stomatal resistance, leaf water potential, osmotic potential, and turgor potential respectively and Gse, CR, show measurements at glasshouse and climate room respectively.

² Mid= measurements at midday.

³ Ψ (PB)= leaf water potential using Pressure bomb at midday.

⁴ W, and C are Soil moisture levels and cultivar respectively.

Appendix 6.19: The interaction between time * soil moisture * cultivar Ψ at dawn in the climate room.

Soil moisture	Cultivar	Ψ 1 (8/11)	Ψ 2 (15/11)	Ψ 3 (23/11)	Ψ 4 (07/12)	Ψ 5 (14/12)	Ψ 6 (20/12)	Ψ 7 (27/12)
Control	Cotswold-C.	6.50	6.75	6.65	7.98	8.66	8.15	8.56
	Eski	7.03	7.05	6.92	8.92	8.53	7.58	6.01
	Fakir	6.48	5.86	5.87	9.30	6.90	8.21	6.53
	Remont	7.21	5.32	6.03	8.06	8.64	6.56	7.58
Moderate	Cotswold-C.	8.30	9.58	10.32	11.44	8.00	9.80	7.70
	Eski	7.41	7.65	6.86	9.71	11.46	9.26	8.43
	Fakir	8.36	6.10	7.45	9.32	9.36	9.06	9.38
	Remont	8.98	5.90	7.3	6.89	8.40	9.31	7.93
Severe	Cotswold-C.	6.45	9.57	10.01	8.57	11.43	9.65	11.14
	Eski	9.48	7.43	8.28	12.14	11.88	11.48	9.36
	Fakir	9.73	11.43	9.18	12.36	10.00	10.76	11.26
	Remont	7.33	8.61	9.01	9.35	10.98	11.73	12.57

¹ Numbers are means of four replicates.

Appendix 6.20: The interaction between time * soil moisture * cultivar π at dawn in the climate room.

Soil moisture	Cultivar	$\pi 1$ (8/11)	$\pi 2$ (15/11)	$\pi 3$ (23/11)	$\pi 4$ (07/12)	$\pi 5$ (14/12)	$\pi 6$ (20/12)	$\pi 7$ (27/12)
Control	Cotswold-C.	9.49	10.45	9.69	10.78	11.28	10.49	11.27
	Eski	10.84	9.21	9.61	11.15	9.63	10.89	9.56
	Fakir	9.10	10.18	8.36	11.43	10.10	13.09	10.15
	Remont	10.19	8.93	8.92	10.29	10.03	8.25	9.50
Moderate	Cotswold-C.	11.76	12.30	13.36	13.58	12.34	12.42	11.32
	Eski	10.01	9.93	9.59	11.72	12.45	12.53	10.55
	Fakir	10.10	9.80	9.80	10.39	11.03	11.45	10.36
	Remont	11.19	9.79	9.26	9.58	10.68	11.76	9.75
Severe	Cotswold-C.	10.73	12.73	11.61	10.23	11.72	11.50	13.15
	Eski	12.98	9.88	11.12	14.19	11.62	12.68	11.45
	Fakir	10.76	12.59	11.56	14.80	11.42	12.80	12.92
	Remont	10.20	9.10	11.35	11.56	11.70	12.91	11.51

¹ Numbers are means of four replicates.



Area

2.35 cm²1.71 cm²1.23 cm²0.78 cm²0.27 cm²0.133 cm²

Appendix 6.21: Template used to estimate leaf area of sainfoin during growth.

Appendix 7.1: The following formulas were used to calculate the moisture demands (I_{1...I7}) for 0, 125, 275, 425, 587.5, 762.5, 925, mm depths respectively, at regrowth harvests according to VSWC of 20 cm (A), 50 cm (B), 85 cm (C) depths.

$$I_{(0)} = \frac{14-A}{100} * 6182 * \frac{12.5}{35}$$

$$I_{(125)} = \frac{14-A}{100} * 6182 * \frac{15}{35}$$

$$I_{(275)} = \frac{1}{2} * \left[\frac{14-A}{100} * 6182 * \frac{15}{35} \right] + \frac{1}{2} * \left[\frac{14-B}{100} * 5740 * \frac{15}{32.5} \right]$$

$$I_{(425)} = \frac{14-A}{100} * 5740 * \frac{16.25}{32.5}$$

$$I_{(587.5)} = \frac{1}{2} * \left[\frac{14-B}{100} * 5740 * \frac{15}{35} \right] + \frac{1}{2} * \left[\frac{14-C}{100} * 7740 * \frac{17.5}{32.5} \right]$$

$$I_{(762.5)} = \frac{14-C}{100} * 5740 * \frac{16.25}{32.5}$$

$$I_{(925)} = \frac{14-C}{100} * 5740 * \frac{15}{32.5}$$

Appendix 7.2 The average volumetric soil water content (cm^3/cm^3 , %) of pots to 70 cm depth measured by TDR at three harvests three plant types and two soil moisture treatments.

	Early harvest	Late harvest	Regrowth harvest
		Control	
Eski	28.75 ¹	27.83	30.00
G35	28.98	28.15	29.80
Lucerne	28.78	25.53	26.58
		Stress	
Eski	19.21	16.78	15.65
G35	20.63	14.33	13.30
Lucerne	18.85	13.60	10.40
Pr> F W ²	0.0001	0.0001	0.0001
Pr> F Cu	0.7168	0.3257	0.0150
Pr> F W*Cu	0.8131	0.7152	0.9049
SEM W	0.7550	0.9905	0.8346
SEM Cu	0.9167	1.2070	1.0221
SEM W*Cu	1.2989	1.7076	1.4455

¹ Data are means of four replicates.

² W= soil moisture level, Cu= cultivar, SEM= standard error of the mean.

Pooled ANOVA of VSWC over all three harvests showed Pr>F Harvest (H)= 0.0004, SEM H= 0.6248, Pr>F Cu= 0.0124, SEM Cu= 0.6248, Pr>F Cu*H= 0.3500, SEM Cu*H=1.1479, Pr>F W=0.0001, SEM W=0.5047, Pr>F W*H= 0.0015, SEM W*H= 0.8556, Pr>F W*Cu= 0.8330, SEM W*Cu= 0.8556, Pr W*Cu*H= 0.9019, SEM W*Cu*H= 1.4820

Appendix 7.3: Volumetric soil water content (cm^3/cm^3 %) of pots at early, late, and regrowth harvests under two soil moisture treatments at 0.2, 0.5, and 0.85 m depth at harvesting times.

Depth	Early harvest			Late harvest			Regrowth harvest		
	0.20 m	0.50 m	0.85 m	0.20 m	0.50 m	0.85 m	0.20 m	0.50 m	0.85 m
Eski	25.82 ¹	28.18	30.35	19.20	23.03	30.38	25.23	28.300	29.80
G35	25.73	26.63	29.68	20.18	23.93	27.13	25.43	27.15	29.65
Lucerne	24.53	26.65	29.58	20.23	24.58	28.15	25.33	23.43	24.03
					Control				
					Stress				
Eski	12.7	20.75	28.15	9.72	11.08	9.93	10.43	14.68	15.83
G35	10.73	18.33	28.63	9.27	12.93	12.70	9.88	9.93	13.23
Lucerne	9.80	19.15	29.30	9.45	12.20	11.03	8.30	10.93	11.20
Pr>F W ²	0.0001	0.0001	0.3289	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Pr>F Cu	0.3492	0.4564	0.9700	0.9616	0.6767	0.8326	0.4894	0.1682	0.1030
Pr>F W*Cu	0.7959	0.9553	0.7859	0.8481	0.8057	0.0531	0.4592	0.5457	0.7387
SEM W	0.8610	0.9620	0.8050	0.8300	1.0800	0.6500	0.5100	1.2700	1.3500
SEM Cu	1.0840	1.2120	1.0000	1.0000	1.2500	0.7500	0.6300	1.5500	1.6500
SEM W*Cu	1.6570	1.8520	1.309	1.3400	1.7600	1.0600	0.8800	2.1900	2.3400

¹ Numbers are mean of four replicates.

² W= soil moisture treatment, Cu= cultivar, and SEM= standard error of the mean.

Repeated measures analysis over harvests and three depths showed Pr>F Depth (D)=0.0001, Pr>F D * Harvest (H)=0.0001, Pr>F D * Cultivar (Cu)=0.7199, Pr>F D * Cu * H= 0.2251, Pr>F D * soil moisture (W)=0.0056, Pr>F D * W * H=0.0001, Pr>F D x W * Cu=0.3188, Pr>F D * W * Cu * H=0.7263

Appendix 7.4: Root length density (m/m^3)* 10^3 at seven depths at the early harvest (45 DAP) of Eski, Grasslands G35, and lucerne (Grasslands Oranga) at two soil moisture levels.

Treatment	Depth (cm)						
	0-10	10-20	20-30	30-40	40-60	60-80	80-100
	Control						
Eski	30.7 ²	30.1	21.6	14.4	5.2	0.5	0.0
G35	25.1	28.1	17.3	10.6	4.4	0.0	0.0
Lucerne	17.5	23.1	16.2	07.8	2.7	0.5	0.1
	Stress						
Eski	5.9	8.7	13.2	14.3	12.2	7.7	1.5
G35	6.2	8.7	10.4	14.9	13.5	7.6	1.7
Lucerne	8.5	12.6	12.2	13.6	11.2	1.7	0.1
Pr>F Cu ¹	0.5200	0.9400	0.4392	0.7463	0.7801	0.0792	0.4028
Pr>F W	0.0002	0.0004	0.0191	0.4075	0.0053	0.0002	0.0343
Pr>F Cu*W	0.2400	0.4722	0.7562	0.8130	0.9417	0.0495	0.3361
SEM Cu	3.2	3.2	2.1	3.4	2.2	0.9	0.4
SEM W	2.6	2.7	1.7	2.8	1.8	0.8	0.3
SEM Cu*W	4.5	4.6	3.0	4.8	3.1	1.3	0.6

¹ Cu= Cultivar, W= Soil moisture, and Cu*W= Interaction of cultivar and soil moisture.

² Numbers are the mean of four replicates.

Repeated measures analysis showed: Pr>F depth = 0.0001, Pr>F depth by W= 0.0001, Pr>F depth by cultivar = 0.9854, and Pr>F depth by W x Cu= 0.5408

Appendix 7.5: Root length density (m/m³)*10³ at seven depths at the late harvest (75 DAP) of Eski, and Grasslands G35, and lucerne (Grasslands Oranga) at two soil moisture levels.

Treatment	Depth (cm)						
	0-10	10-20	20-30	30-40	40-60	60-80	80-100
	Control						
Eski	47.0 ²	45.0	27.5	17.4	6.4	1.3	0.0
G35	44.7	37.1	30.2	9.2	7.8	1.7	0.0
Lucerne	39.1	51.9	44.7	37.7	15.8	1.5	0.0
	Stress						
Eski	31.4	18.0	17.2	16.9	20.5	21.0	42.6
G35	17.2	16.4	11.8	13.3	18.7	17.2	16.3
Lucerne	18.5	22.8	18.8	17.9	18.6	24.4	20.7
Pr>F Cu ¹	0.1332	0.4069	0.1777	0.0029	0.3445	0.4152	0.1999
Pr>F W	0.0001	0.0010	0.0020	0.1117	0.0014	0.0001	0.0001
Pr>F Cu*W	0.5215	0.8529	0.4466	0.0212	0.1693	0.3733	0.1999
SEM Cu	3.6	5.4	4.2	2.8	2.1	1.8	1.8
SEM W	3.0	4.4	3.4	2.3	1.7	1.5	1.5
SEM Cu*W	5.1	8.0	6.0	4.0	2.9	2.6	2.5

¹ Cu= Cultivar, W= Soil moisture, and Cu*W= Interaction of Cu and W.

² Numbers are means of four replicates.

Repeated measure analysis showed: Pr> F for depth = 0.0001, Pr > F depth by W= 0.0001 Pr>F depth by Cu= 0.1226 and Pr>F depth by W x Cu= 0.5442

Appendix 7.6 : Root length density (m/m^3)* 10^3 at seven depths at regrowth harvest (30 days cutting, 105 DAP) of Eski, and Grasslands G35, and lucerne (Grasslands Oranga) at two soil moisture levels.

treatment	Depth (cm)						
	0-10	10-20	20-30	30-40	40-60	60-80	80-100
	Control						
Eski	47.3 ²	47.7	31.9	12.1	6.6	2.9	1.1
G35	72.0	58.9	26.4	17.1	9.2	2.4	0.8
Lucerne	39.8	48.0	29.2	38.0	13.3	1.2	17.1
	Stress						
Eski	1.54	1.68	1.75	1.58	1.71	1.62	2.59
G35	2.07	1.74	1.67	1.77	2.08	1.96	1.86
Lucerne	1.97	1.99	2.07	2.88	2.16	2.31	2.65
Pr>F Cu ¹	0.0259	0.4161	0.7467	0.0017	0.3857	0.3245	0.0802
Pr>F W	0.0001	0.0001	0.0161	0.6725	0.0066	0.0022	0.0008
Pr>F Cu*W	0.0572	0.3103	0.8176	0.3687	0.9112	0.8906	0.3367
SEM Cu	4.2	3.1	3.5	3.3	2.8	3.4	3.6
SEM W	3.4	2.6	2.8	2.7	2.3	2.8	2.9
SEM Cu*W	6.0	4.4	4.9	4.6	3.9	4.8	5.0

¹ Cu= Cultivar, W= Soil moisture, and Cu*W= Interaction of cultivar and soil moisture.

² Numbers are means of four replicates.

Repeated measure analysis showed: Pr > F for depth= 0.0001, depth by moisture = 0.0001, Pr>F depth by cultivar = 0.0004, and Pr>F depth by moisture by cultivar = 0.1022.

Appendix 7.7 : Root weight density (g/m^3) at seven depths at the early harvest (45 DAP) of Eski, Grasslands G35, and lucerne (Grasslands Oranga) at two soil moisture levels.

Treatment	Depth (cm)						
	0-10	10-20	20-30	30-40	40-60	60-80	80-100
	Control						
Eski	538.0 ¹	258.0	123.0	85.0	34.0	2.0	0.0
G35	432.0	273.0	144.0	95.00	45.00	0.00	0.0
Lucerne	247.0	120.0	58.0	29.00	11.00	2.90	0.0
	Stress						
Eski	250.0	145.0	150.0	116.0	102.0	75.00	20.0
G35	302.0	152.0	151.0	166.0	159.0	76.00	10.0
Lucerne	337.0	158.0	106.0	101.0	79.0	15.00	2.0
Pr>F Cu ¹	0.3491	0.4035	0.0848	0.2950	0.1152	0.0569	0.2864
Pr>F W	0.0766	0.1757	0.2644	0.0952	0.0013	0.0001	0.0157
Pr>F Cu*W	0.0515	0.3150	0.7797	0.8411	0.5925	0.0400	0.2799
SEM Cu	50.0	40.0	20.0	28.0	18.0	10.00	3.0
SEM W	41.0	33.0	17.0	23.0	15.0	7.00	3.0
SEM Cu*W	70.0	57.0	29.0	40.0	26.0	13.00	5.0

¹ Numbers are means of four replicates.

² Cu= Cultivar, W= Soil moisture, and Cu*W= Interaction of cultivar and soil moisture. Repeated measures analysis showed: Pr>F for depth = 0.0001, Pr>F depth by moisture = 0.0006, Pr>F depth by cultivar = 0.6824, depth by cultivar by moisture = 0.0159

Appendix 7.8: Root weight density (g/m^3) at seven depths at the late harvest (75 DAP) of Eski, and Grasslands G35, and lucerne (Grasslands Oranga) at two soil moisture levels.

Treatment	Depth (cm)						
	0-10	10-20	20-30	30-40	40-60	60-80	80-100
	Control						
Eski	1,776.0	900.0	457.0	234.0	110.0	18.0	0.0
G35	1,510.0	610.0	469.0	167.0	123.0	40.0	24.0
Lucerne	1,960.0	968.0	410.0	249.0	141.0	60.0	0.0
	Stress						
Eski	1,495.0	701.0	560.0	433.0	445.0	377.0	614.0
G35	1,142.0	559.0	383.0	331.0	402.0	368.0	335.0
Lucerne	1,879.0	1,075.0	668.0	530.0	378.0	266.0	204.0
Pr>F Cu ¹	0.0396	0.0057	0.2740	0.0223	0.9134	0.6192	0.0006
Pr>F W	0.1761	0.6128	0.1239	0.0001	0.0001	0.0001	0.0001
Pr>F Cu*W	0.7865	0.4256	0.0726	0.4303	0.5388	0.2224	0.0005
SEM Cu	148.0	80.0	48.0	32.0	31.0	32.0	29.0
SEM W	121.0	65.0	39.0	26.0	25.0	25.0	23.0
SEM Cu*W	210.0	113.0	70.0	45.0	44.0	45.0	41.0

¹ Cu= Cultivar, W= Soil moisture, and Cu*W= Interaction of cultivar and soil moisture.

Numbers are means of four replicates.

Repeated measures analysis showed: Pr>F for depth = 0.0001, Pr>F depth by moisture = 0.0001, Pr>F depth by cultivar = 0.0004, depth by cultivar by moisture = 0.2691

Appendix 7.9: Root weight density (g/m^3) at seven depths, at regrowth harvest (105 DAP) of Eski, Grasslands G35, and lucerne (Grasslands Oranga) at two soil moisture levels.

Treatment	Depth (cm)						
	0-10	10-20	20-30	30-40	40-60	60-80	80-100
	Control						
Eski	1460 ¹	830	483	176	88	39	15.0
G35	2190	1167	428	241	127	50	13.0
Lucerne	1622	777	296	367	220	110	174.0
	Stress						
Eski	1185	719	622	464	414	519	546.0
G35	1151	538	499	424	363	453	410.0
Lucerne	1302	1078	662	694	326	311	257.0
Pr>F Cu ²	0.3986	0.6086	0.7529	0.1331	0.9329	0.7505	0.6237
Pr>F W	0.0179	0.2535	0.0778	0.0096	0.0035	0.0002	0.0001
Pr>F Cu*W	0.2638	0.0250	0.4822	0.7949	0.3935	0.3079	0.0362
SEM Cu	177	110	88	76	56	64	56.0
SEM W	145	87	71	63	45	52	46.0
SEM Cu*W	251	151	124	110	79	90	79.0

¹ Numbers are means of four replicates.

² Cu= Cultivar, W= Soil moisture, and Cu*W= Interaction of cultivar and soil moisture.

Repeated measures analysis showed: Pr>F for depth = 0.0001, Pr>F depth by moisture = 0.0001, Pr>F depth by cultivar = 0.1373, depth by cultivar by moisture = 0.0077

Appendix 7.10: P>F from pooled ANOVA over harvests for leaf water potential measured by Pressure bomb (Ψ_P), leaf water potential measured by Wescor (Ψ), osmotic potential of the leaf measured by Wescor (π), turgor potential of the leaf (P), and osmotic potential of the leaf at full turgor measured by Decagon ($\pi_{(100)}$), over all three harvests.

	H ¹	C	C*H	W	W*H	W*C	W*C*H
Ψ_P	0.0001	0.0001	0.0013	0.0001	0.0001	0.3842	0.9097
Ψ	0.0001	0.0295	0.1583	0.0001	0.0015	0.0051	0.8563
π	0.1521	0.0008	0.2946	0.0001	0.0078	0.0073	0.3393
P	0.0001	0.2431	0.2244	0.9350	0.1774	0.8774	0.8095
$\pi_{(100)}$	0.0519	0.1543	0.3039	0.0180	0.5473	0.2669	0.5156

¹ H= Harvest, C= Cultivar, W= Soil moisture level effects.

Appendix 7.11: Pr > F for the effect of time (dawn and midday) and time interactions for leaf water potential measured by Pressure bomb (Ψ_P), leaf water potential measured by Wescor (Ψ), osmotic potential of the leaf measured by Wescor (π), turgor potential of the leaf (P), and osmotic potential of the leaf at full turgor measured by Decagon (π_{100}), over three harvests.

	T ¹	T*H	T*C	T*Cu*H	T*W	T*W*H	T*W*C	T*W*C*H
Ψ_P	0.0001	0.0001	0.2191	0.4169	0.0002	0.0017	0.4168	0.3763
Ψ	0.0001	0.0001	0.3039	0.4531	0.0120	0.3451	0.8718	0.4913
π	0.0001	0.0001	0.0335	0.2692	0.2287	0.0419	0.8255	0.8242
P	0.3030	0.0477	0.4200	0.2981	0.0777	0.9771	0.6679	0.7304
$\pi_{(100)}$	0.0881	0.1220	0.9056	0.3000	0.0236	0.4810	0.0046	0.9028

¹ T= Time (dawn and midday), H= Harvest, C= Cultivar, W= Soil moisture level.

Appendix 7.12: Pr > F for pooled ANOVA over three harvests for root water potential (Ψ), osmotic potential (π), turgor potential (P), and root osmotic potential at full turgor (π_{100}), at two depths.

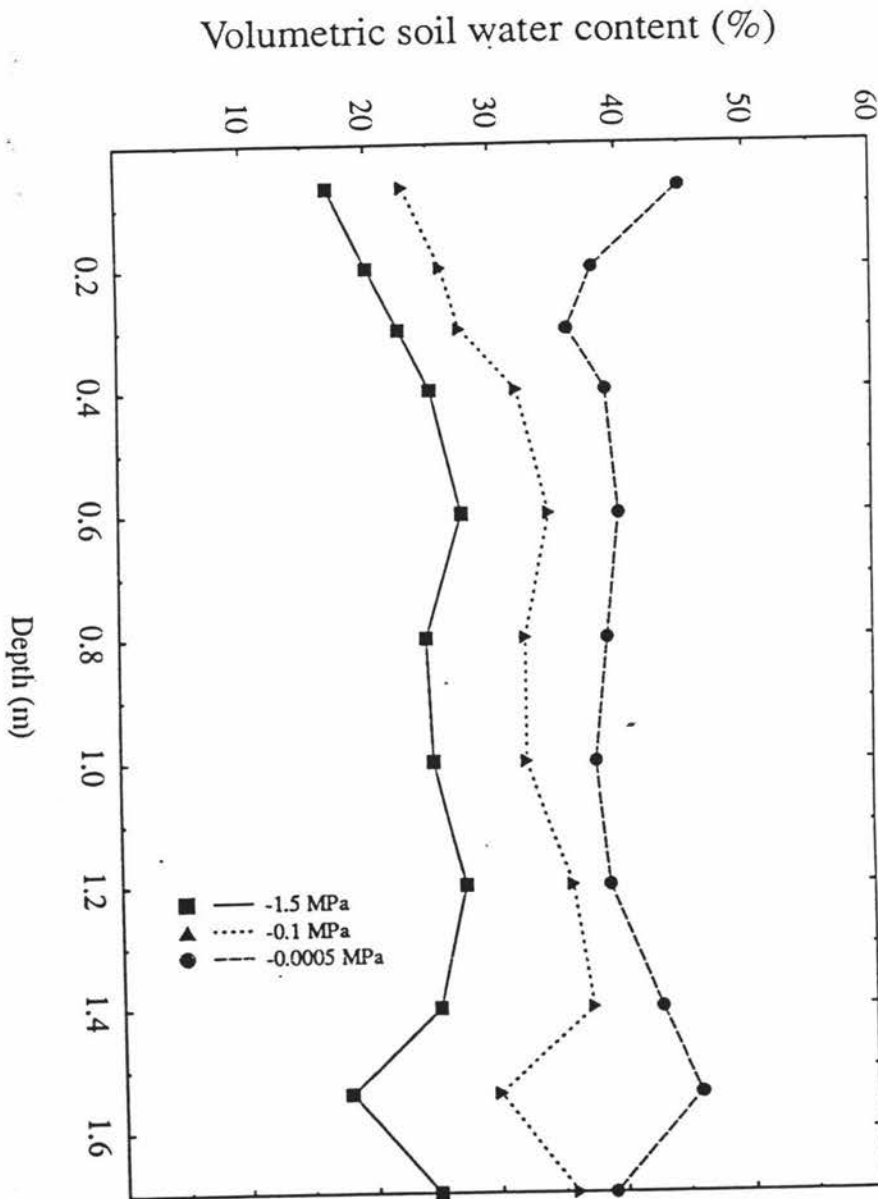
	Pr > F			
	Ψ	π	P	π_{100}
Moisture (W)	0.0001	0.0001	0.0388	0.0097
Cultivar (Cu)	0.9632	0.7721	0.5731	0.0236
Cu*W	0.2280	0.0452	0.3689	0.7235
Harvest (H)	0.1082	0.2150	0.7465	0.0002
W*H	0.1641	0.0737	0.2376	0.2455
Cu*H	0.0451	0.1166	0.3194	0.6888
W*Cu*H	0.6336	0.8861	0.5767	0.7814
Depth (D) ¹	0.1649	0.1511	0.4866	0.2236
W*D	0.4668	0.4045	0.6404	0.4410
Cu*D	0.7779	0.9606	0.9077	0.9756
W*Cu*D	0.3009	0.2157	0.5748	0.2267
H*D	0.7462	0.6198	0.5238	0.3659
Cu*H*D	0.3336	0.3972	0.5748	0.2046
W*H*D	0.1909	0.9761	0.4535	0.6702
W*Cu*H*D	0.9956	0.6513	0.3223	0.2621

¹ Depth= depth of sampling, (100-300, and 500-700 mm depth)

Appendix 7.13: Pr > F for pooled ANOVA over three harvests for roots and leaves for water potential (Ψ), osmotic potential (π), turgor potential (P), and root osmotic potential at full turgor (π_{100}).

	Pr > F			
	Ψ	π	P	π_{100}
Moisture (W)	0.0001	0.0001	0.0059	0.0001
Cultivar (Cu)	0.1512	0.0491	0.5671	0.8106
Cu*W	0.0685	0.0094	0.4893	0.7430
Harvest (H)	0.0001	0.0055	0.0349	0.0001
W*H	0.4737	0.3122	0.8407	0.9291
Cu*H	0.0915	0.2836	0.1375	0.7398
W*Cu*H	0.6811	0.8032	0.4551	0.6493
Location ¹ (L)	0.0001	0.0001	0.0001	0.0007
W*L	0.0264	0.1729	0.4284	0.5331
Cu*L	0.0224	0.0090	0.5262	0.0372
W*Cu*L	0.3395	0.3173	0.4909	0.0716
H*L	0.0007	0.6108	0.0098	0.3104
Cu*H*L	0.0135	0.0026	0.5725	0.2218
W*H*L	0.4789	0.5984	0.0381	0.8536
W*Cu*H*L	0.5334	0.9213	0.9054	0.9964

¹ Location= leaves and roots.



Appendix 8.1: Water content (cm^3/cm^3) of soil at 0.0 to 1.7m depth at soil water potential of -1.5, -0.1, and -0.0005 MPa. (Adapted from Scotter et al. 1979a).

Appendix 8.2: Mean of rainfall, temperature of the air and soil, and relative humidity (RH) (measured at Ag-Research, Grasslands¹ 500 m from the experimental area) during field experiment.

Month	Rainfall (mm)	Air temperature (°C)			soil Temperature (°C)		RH% ²
		Max	Min	Mean	0.1m depth	1.0m depth	
Dec (1992)	167.3	19.3	10.8	15.1	15.9	16.5	82
Jan (1993)	53.9	19.9	11.5	15.7	16.4	17.7	79
Feb (1993)	43.7	20.8	11.7	15.75	17.5	18.4	84
March (1993)	79.8	19.2	10.0	14.6	14.6	17.8	83
April (1993)	53.7	16.7	8.4	12.5	12.1	16.1	85

¹ Station AgResearch-Grassland Lat. °40.23S Lon. 175 °37E

² Relative humidity at 9.00 a.m.

- Water was applied eight times (about 20 mm each time) for non-stressed experiment when rainfall was not enough to keep soil moisture at field capacity.

Appendix 8.3: The volumetric soil moisture at depth 0-0.15, 0.15-0.30, 0.30-0.50, 0.50-0.70 m for the stressed and non-stressed experiments for all three harvests, and regrowth of second harvest.

	Non-stressed		Stressed		Pr>T
	Mean	SEM ¹	Mean	SEM	
		Depth 0-0.15 m			
Harvest 1	42.2	1.75	34.5	2.05	0.0463
Harvest 2	44.6	0.38	26.8	1.29	0.0002
Harvest 3	39.5	3.30	21.7	1.25	0.0010
Regrowth ²	35.5	3.84	11.4	2.7	0.0067
		Depth 0.15-0.30 m			
Harvest 1	26.2	0.75	27.1	1.18	0.5477
Harvest 2	22.23	1.54	25.68	1.32	0.1647
Harvest 3	33.7	1.29	23.9	1.43	0.0010
Regrowth 2	33.3	0.21	19.8	0.54	0.0001
		Depth 0.30-0.50 m			
Harvest 1	50.8	3.32	45.1	0.64	0.1638
Harvest 2	53.5	2.94	41.7	3.27	0.0555
Harvest 3	41.9	2.04	34.7	3.02	0.0841
Regrowth 2	37.95	0.31	24.57	0.54	0.0001
		Depth 0.50-0.70 m			
Harvest 1	35.80	1.68	36.60	0.63	0.6880
Harvest 2	32.90	0.98	38.00	0.82	0.0166
Harvest 3	36.00	0.75	34.70	1.14	0.3516
Regrowth 2	37.24	1.82	32.55	1.31	0.1049

¹ SEM= Standard error of the mean.

² The Regrowth 1 was similar period as Harvest 2

Appendix 8.4: Repeated measures analysis for comparison of the first (30 days after stress) and second (60 days after stress) harvests and their subsequent harvests for morphological characteristics.

	Time (T) ¹	T*Harvest	T*C ²	T*C*H	T*W ³	T*W*H	T*W*C	T*W*H*C
Leaf area	0.0002 ⁴	0.0001	0.0002	0.0063	0.1839	0.0061	0.5574	0.0152
Leaf DW	0.0002	0.0129	0.0060	0.0105	0.7748	0.0106	0.2225	0.0122
Stem No	0.5281	0.0124	0.0380	0.2486	0.0061	0.2923	0.0006	0.0330
Stem DW	0.0001	0.0001	0.4189	0.0169	0.1214	0.5743	0.5661	0.1120
Yield	0.0001	0.0001	0.8045	0.0221	0.3477	0.1382	0.6555	0.0080
SLA	0.4093	0.0001	0.4520	0.1031	0.0006	0.0732	0.3457	0.3732

¹ Time= First and second harvests compare to related regrowth

² C= Cultivar

³ W= soil moisture level

⁴ Numbers are probability of significance

⁵ SLA= Specific leaf area

Appendix 8.5: The results of repeated measures analysis for comparison of the first (30 days after stress) and second (60 days after stress) harvests and their subsequent harvests for physiological characteristics at dawn.

	Time (T) ¹	T*Harvest	T*C ²	T*C*H	T*W ³	T*W*H	T*W*C	T*W*H*C
ΨP ⁴	0.1787 ⁵	0.0344	0.0250	0.2090	0.0541	0.0495	0.0537	0.1499
ΨW	0.0001	0.0001	0.4375	0.3863	0.0001	0.1655	0.9263	0.1914
π	0.0001	0.0001	0.1401	0.3077	0.0313	0.0034	0.4618	0.8336
P	0.1085	0.0162	0.0369	0.3827	0.3377	0.1489	0.4826	0.0476
Solute	0.2402	0.0001	0.7931	0.9049	0.0619	0.0091	0.6508	0.7622
RWC	0.0001	0.0007	0.6545	0.1235	0.0001	0.0033	0.1847	0.5818

¹ Time= First and second harvests compare to related regrowth

² C= Cultivar

³ W= soil moisture level

⁴ ΨP, ΨW are leaf water potential by Pressure chamber and Wescore, π, P are osmotic potential and turgor potential, Solute is osmotic potential at fully turgor and RWC is relative water content.

⁵ Numbers are probability of significance

Appendix 8.6: The results of repeated measures analysis for comparison of the first (30 days after stress) and second (60 days after stress) harvests and their subsequent regrowth harvest for physiological characteristics at midday.

	Time (T) ¹	T*Harvest	T*C ²	T*C*H	T*W ³	T*W*H	T*W*C	T*W*H*C
ψP^4	0.3228 ⁵	0.0001	0.0001	0.1934	0.0003	0.0134	0.0001	0.5511
ψW	0.0001	0.0001	0.0119	0.7618	0.0001	0.1315	0.8103	0.2265
π	0.0001	0.0001	0.0638	0.2458	0.0001	0.3558	0.9414	0.2103
P	0.0001	0.2038	0.2789	0.2239	0.0633	0.4862	0.8900	0.0064
Solute	0.0697	0.0502	0.3113	0.7403	0.0351	0.4034	0.8082	0.2329
Photo	0.0961	0.2433	0.1190	0.7065	0.0008	0.0001	0.7096	0.3815
Rs	0.0002	0.0012	0.3290	0.0071	0.0001	0.0002	0.4550	0.5164
RWC	0.0001	0.0001	0.5870	0.0933	0.0001	0.0001	0.1905	0.0234

¹ Time= First and second harvests compare to related regrowth

² C= Cultivar

³ W= soil moisture level

⁴ ψP , ψW are leaf water potential by Pressure chamber and Wescor, π , P are osmotic potential and turgor potential, Solute is osmotic potential at fully turgor, and photo, Rs, and RWC are photosynthesis, stomatal resistance, and relative water content respectively.

⁵ Numbers are probability of significance

Appendix 8.7 : Probability of significance for comparison of stomatal resistance (Rs)(s/cm) of adaxial, abaxial surfaces and total leaf Rs from the second harvest and related regrowth harvests by repeated measures analysis.

	Time(T) ¹	T*C ²	T*W ³	T*W*C
Adaxial (Rs)	0.9731	0.0203	0.7745	0.0037
Abaxial (Rs)	0.1769	0.5301	0.0142	0.0050
Total (Rs)	0.7520	0.3340	0.1077	0.0009

¹ Time= Harvest and regrowth

² C= Cultivar

³ W= Soil moisture level