MEDITERRANEAN AND TEMPERATE TALL FESCUES: PHYSIOLOGICAL AND MORPHOLOGICAL RESPONSES TO WATER DEFICIT, AND THE EFFECT OF NITROGEN ON WINTER AND EARLY-SPRING FIELD PERFORMANCE UNDER GRAZING

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ABSTRACT

A major goal for grazing systems is to increase winter herbage growth. Tall fescue (*Festuca arundinacea* Schreb.) has been widely recognised as one of the most important cool season forage species. Among tall fescue populations, those of Mediterranean origin (e.g. *F. arundinacea* var. *letoumeuxiana* from North Africa) have shown higher growth rates in winter and early-spring than comparable germplasm from northen Europe. For this reason, the complementary use of Mediterranean and temperate tall fescue populations to improve continuity of seasonal forage supply has been suggested for the Pampa region, Argentina. It is known, however, that the low winter N availability of the region limits herbage growth and that water deficits are likely to occur. It has been found that endophyte-infected tall fescue plants tolerate drought better than endophyte-free ones and currently novel endophyte strains harmless to livestock are commercially available.

This study therefore sought to compare the responses of Mediterranean and temperate tall fescue cultivars to water deficit, to investigate the extent to which water deficit responses are modified by the presence or absence of endophyte, and to compare winter-early spring growth and animal production of two contrasting cultivars with and without N fertilisation in the Pampa region.

Three glasshouse experiments were carried out to evaluate the response of contrasting tall fescue cultivars to water deficit. In the first experiment two temperate cultivars, Grasslands Advance (GA) and El Palenque (EP), and a Mediterranean cultivar, Maris Kasba (MK), were studied. Water deficit was induced by complete cessation of watering and sequential harvests were made to follow the adjustment of the respective cultivars. Morphological measurements also included the distribution of mass and length of root with depth.

In the second experiment, only MK and EP were studied. Plants of both cultivars were grown in the same pots and received water daily with gradation in intensity of water deficit achieved by varying the daily water ration per pot. Reducing and fructosyl sugar concentrations were analysed to examine cultivar differences and to investigate whether these sugars were involved in osmotic adjustment.

The third experiment compared MK and EP cultivars under water deficit, but in addition the effects of two endophytes, Kentucky 31 wild type (KY31) and a commercial strain supplied by AgResearch (AR501), were studied.

Herbage growth and animal production in winter-early spring of MK and EP swards in response to N fertilisation were compared in a grazing experiment carried out in the SE of Buenos Aires Province, Argentina. The N treatments were zero and 100 kg N ha^{-1} applied in equal split dressings in mid autumn and early winter. The paddocks were grazed by a variable number of growing steers in order to maintain a similar leaf area index (LAI) in all treatments.

The results of the water deficit experiments indicated that in comparison with the temperate cultivars EP and GA, the Mediterranean cultivar MK was characterised by a smaller plant size, higher tiller number, high root : shoot ratio, a lower stomatal resistance, lower content of reducing and fructosyl sugars and a lower growth rate under high temperatures. All tall fescue cultivars exhibited decreased growth rates, diminished evaporative surface area, and increased root : shoot ratio and osmotic adjustment in response to water deficit. A similar water status was observed for the different cultivars under comparable soil water availability. There was evidence that MK was able to delay onset of water deficit through its morphological characteristics. By contrast, stomatal resistance of temperate cultivars was more responsive to soil moisture changes and these cultivars had a greater tendency for osmotic adjustment than MK under the most stressful water deficit conditions studied. For the particular cultivars and endophytes strains studied here, the experimental evidence suggests that MK-KY31 and EP-AR501 combinations would be expected to perform better under water deficit than other combinations.

Maris Kasba swards had a higher stocking rate during mid winter-early spring, and consequently, the beef production was 26% higher than in EP swards. With N fertilisation the response was markedly increased and beef production was increased by 66% during the same period. However, by late August no differences in tissue turnover were found between cultivars, while in September EP showed a higher net growth rate (NGR) than MK. For both periods N application increased the NGR of the swards.

Further studies would be necessary to evaluate the recovery capacity after drought of the cultivars evaluated and the ability for osmotic adjustment in the meristematic tissues as well as their behaviour under field conditions. Because the particular performance characteristics of each endophyte strain-grass genotype combination vary, it is recommended that any endophyte strain be evaluated in combination with the plant genotypes with which it is to be associated. The complementary use of temperate and Mediterranean cultivars in animal production systems of the SE of Buenos Aires Province improved feed supply during winter. In addition, winter herbage growth and animal production of both cultivar types can be considerably improved with N fertilisation.

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Plate 7.1. Views of the grazing experiment on 4 July (a) and 8 October (b), respectively. In (a) the greener stripe visible is a paddock that had received N on 6 May. Plate (c) shows technicians taking root samples with a corer of 5 cm internal diameter and (d) a close-up view of a sand-filled core marked with a red ring 146

CHAPTER 1. INTRODUCTION

1.1. Introduction

A major aim for temperate grazing systems is to achieve an increased winter herbage growth. In this regard, germplasm of tall fescue of Mediterranean origin (e.g. *Festuca arundinacea* var. *letourneuxiana from* North Africa) has shown higher growth rates in winter and early-spring than comparable germplasm from northern Europe (MacColl and Cooper, 1967; Frame *et al.*, 1970). However, due to being frost sensitive (Frame, 1972; Delgado and Tanco, 1980), Mediterranean germplasm persists only in regions characterised by mild winters.

The importance of tall fescue as a cool season forage in USA (De Battista, 1989; Richardson *et al.*, 1990; van Santen, 1992) and in Argentina (Gonzáles and Gardner, 1977; Mazzanti and Arosteguy, 1985) has been widely recognised. Even in New Zealand, tall fescue has been considered an option for regions where ryegrass persistence is limited by summer drought (Anderson, 1982, Anderson *et al.*, 1982, Brock, 1982).

A number of studies have compared the morphological and physiological response of Mediterranean and European tall fescue populations to environmental factors (Chatterjee, 1961; Eagles, 1967a; Robson and Jewiss, 1968a, b), but there is little published information on the effect of water deficit (Neal-Smith and Wright, 1969). Endophyte-infected tall fescue seems to tolerate drought better than when endophyte-free (Read and Camp, 1986; West and Gwinn, 1993). However, the responses were variable depending on plant genotype, endophyte strain and environmental factors (Bacon, 1993). Novel strains of endophyte that seem harmless to livestock have been isolated (West and Gwinn, 1993). No information appears to be available about endophyte infected-Mediterranean tall fescues, or about Mediterranean populations artificially infected with agronomically superior endophyte strains.

For the SE of Buenos Aires Province, Argentina, the complementary use of Mediterranean and temperate tall fescue cultivars has been suggested (Mazzanti and Arosteguy, 1985; Mazzanti *et al.*, 1985). Mediterranean cultivars would compensate for the low winter herbage growth of temperate ones, and vice versa in summer. It is known, however, that the low winter N availability of the region limits herbage growth (Fernández Grecco *et al.*, 1995; Marino *et al.*, 1995). Consequently, N fertilisation may be necessary to allow a species with winter growth capacity to perform to potential.

This study therefore sought to compare the responses of Mediterranean and temperate tall fescue cultivars to water deficit, to investigate the extent to which water deficit responses are modified by presence or absence of endophyte, and to compare winter-early spring growth and animal production of two cultivars with and without N fertilisation.

2.2. Objectives

The experimental objectives were:

1. To compare the morphological and physiological responses of contrasting endophyte-free tall fescue cultivars to water deficit.

2. To compare the morphological and physiological responses of a Mediterranean and a temperate tall fescue artificially infected with different endophyte strains to water deficit, and to obtain information on the comparative performance of the fungal strains studied.

3. To compare the herbage growth and animal production of swards of a Mediterranean and a temperate tall fescue, maintained at the same leaf area index, and to determine whether N fertilization is necessary for Mediterranean tall fescues to attain potential growth in winter and early-spring.

1.3. Thesis structure

This thesis is presented in eight chapters. The present introductory chapter is followed by a review of literature (Chapter 2). Chapters 3 and 4 report water deficit experiments using endophyte-free plants and Chapter 5 reports a third experiment in which plants were artificially infected with known strains of endophyte. There follows a general discussion on water deficit response in Mediterranean and temperate populations (Chapter 6). Chapter 7 describes a grazing experiment carried out in the SE of Buenos Aires Province, Argentina, to compare winter growth rates and animal production of two contrasting cultivars with and without N fertilisation . Final conclusions are presented in Chapter 8.

The glasshouse experiment reported in Chapter 3 was carried out at the Plant Growth Unit, Massey University, New Zealand. Two temperate cultivars, Grasslands Advance and El Palenque, and a Mediterranean cultivar, Maris Kasba, were studied. In this trial water deficit was induced by complete cessation of were studied. In this trial water deficit was induced by complete cessation of watering and sequential harvests were made to follow the adjustment of the respective cultivars. In this experiment, morphological measurements also included mass and length of root recovered from four soil depths.

In Chapter 4, a glasshouse experiment carried out at Unidad Integrada Balcarce INTA-FCA, Argentina, is reported. This experiment differed from the first in that the two cultivars studied, Maris Kasba and El Palenque, were grown in the same pots. Also, plants received water daily with gradation in intensity of water deficit achieved by varying the daily water ration per pot. In this experiment reducing and fructosyl sugar concentrations were analysed to check for cultivar differences and to investigate whether these sugars were involved in osmotic adjustment.

The glasshouse experiment reported in Chapter 5 was carried out at AgResearch, Palmerston North, New Zealand. It also compared endophyte-free Maris Kasba and El Palenque cultivars under water deficit, but in addition the effect of two endophytes, KY31 and AR501, was studied. The latter is a commercial strain with low ergovaline production, supplied by AgResearch.

The grazing experiment compared the herbage growth and animal production in winter-early spring of Maris Kasba and El Palenque swards in response to N fertilisation. Some estimates of root biomass and root production were also carried out.

Chapter 8 concludes this thesis by summarising the agronomic potential of Mediterranean tall fescues in the light of the information on water deficit responses and on the effect of N fertilisation on winter growth and animal production.

CHAPTER 2. LITERATURE REVIEW

2.1. Introduction

This review covers those aspects of the agronomy and physiology of tall fescue (*Festuca arundinacea* Schreb.) and the endophyte *Neotyphodium coenophialum* (Morgan-Jones and Gams) Glenn, Bacon and Hanlin, pertinent to the study. It commences by emphasising the importance of tall fescue as a grass species, with particular reference to research aimed at comparing Mediterranean and temperate populations, followed by some considerations on their relative feeding value and the importance of nitrogen to sward production. This is followed by a review of the components of tissue turnover in the sward, the importance and functions of the root system and methods of studying root systems. Considerations of plant water stress adaptation are presented, followed by a section concerning the morphological and physiological responses of tall fescue to the endophyte, especially under water deficit. Finally, a summary and some conclusions are presented.

2.2. Tall fescue

Tall fescue is a Eurasian grass species widely disseminated outside its natural range and successfully naturalised in Australia, New Zealand (Easton *et al.*, 1994) and Argentina (Mazzanti and Arosteguy, 1985). The genus *Festuca* is vast and diverse and it has been found recently that tall fescue and meadow fescue are more closely related to *Lolium perenne* L. than to other *Festuca* species (Easton *et al.*, 1994).

Tall fescue is the most important perennial cool season forage grass in the SE of USA (van Santen, 1992), where it occupies 14 million ha in humid, temperate regions (De Battista, 1989) and is used as a hay, pasture, turf and conservation species (Richardson *et al.*, 1990). In Argentina, tall fescue is the most widely grown perennial grass, with 3.2 million ha (Molina, 1988; INDEC, 1992), mainly in the Pampa region (Gonzáles and Gardner, 1977; Mazzanti and Arosteguy, 1985). Because of the variability in ecological areas of the humid and sub-humid Pampa region (Barsky, 1991), tall fescue occupies areas that can experience winter drought (e.g. Córdoba Province) or summer drought (e.g. Buenos Aires Province).

In New Zealand tall fescue has been recomended as an option where summer drought limits the persistence of ryegrass (Anderson, 1982, Anderson *et al.*, 1982, Brock, 1982). However, despite positive experimental results, many farmers remain uninformed of its potential or have had disappointing results, mainly due to establishment failure, its inability to compete in a grazed mixed sward with other grasses (Easton *et al.*, 1994), poor quality seed lines (Lancashire and Brock, 1983) and its reputation for toxicity (Brock, 1983). By contrast, results obtained in Hawkes Bay showed that under drought, tall fescue pastures were superior to Nui ryegrass in quantity and quality of feed for ewes (Wright *et al.*, 1985). Tall fescue was recommended as an alternative to ryegrass for the coastal south Taranaki region, characterised by mild, wet winters, summer dry periods and frequent grass grub damage. In this region, tall fescue gave superior annual production, a growth distribution that matched cow requirements and higher summer/autumn growth (Judd *et al.*, 1990). Thus, the sown area has increased as farmers have come to consider it a worthwhile species in difficult conditions and have gained confidence in their ability to establish and manage it (Easton *et al.*, 1994).

2.2.1. Mediterranean and temperate populations

For many forage species, germplasm of Mediterranean origin (e.g. *F. arundinacea* var. *letourneuxiana* from North Africa) differs from comparable British or North European germplasm in showing pronounced winter and early spring growth (MacColl and Cooper, 1967; Frame *et al.*, 1970). According to Delgado and Tanco (1980), Mediterranean tall fescues usually have slower establishment, greater frost sensitivity, greater tolerance to leaf pathogens and smoother leaves than the North European ones.

2.2.1.1. Studies comparing Mediterranean and temperate populations

2.2.1.1.1. Morphological and physiological responses to environmental factors

MacColl and Cooper (1967) in a glasshouse experiment observed a greater relative growth rate (RGR) in the Mediterranean than in northern European material (*L. perenne*, *Dactylis glomerata* L., and *F. arundinacea*) in winter. The higher RGR was due to a higher leaf area ratio (LAR) and also to a greater shoot/root ratio. This result agreed with Robson and Jewiss (1968a), but contrasted with Chatterjee (1961) and Eagles (1967a), who found that the greater

RGR in the Mediterranean populations was associated with a larger net assimilation rate (NAR). Eagles (1967a) suggested that differences in photoperiod, light energy and spectral composition between experiments could have affected NAR and LAR and, consequently, their relative contribution to RGR.

Many studies have compared two Mediterranean fescues, Syn I and Syn II, and one indigenous to Britain, S170, in controlled conditions (Morgan, 1964; Robson and Jewis, 1968a, b; Thomas and Lazenby, 1968 a, b, c). In general, these studies concluded that the seasonal growth pattern of the tall fescues was regulated largely by temperature and that the responses of the Mediterranean and North European populations to winter and summer conditions were different. Morgan (1964) observed that the Mediterranean grasses germinated more quickly than S170 when temperatures were similar to those usually present in the field in autumn and winter (5° and 10°C). Robson (1967) found that individual leaves of S170 grew at half the rate and to half the final length of Mediterranean varieties in winter. In the high temperature, high light intensity and long days of summer, S170 plants were more productive, erect and had fewer, larger tillers, while the Mediterranean varieties were prostrate, with many tillers (Morgan, 1964). Plants of S170 were less affected by short periods of low temperature than those from Mediterranean region (Robson and Jewiss, 1968a), and more of them survived under low temperature and severe defoliation (Thomas and Lazenby, 1968a). Therefore, it has been suggested that the ability to grow during winter was inversely related to the ability to survive low temperatures (Robson and Jewiss, 1968a). The cold-hardiness of the northem populations was attributed to the accumulation of soluble carbohydrates (Robson and Jewiss, 1968a, Thomas and Lazenby, 1968a) and to the more basal, thus more protected, location of apical and axillary meristems in S170 in comparison with the Mediterranean material (Thomas and Lazenby, 1968c) .

Chatterjee (1961) in Britain observed that three Mediterranean tall fescues, tillered from March to May (spring), while the European form, S170, considered among the more productive grasses in autumn and early spring, tillered from December to March (winter) and, after flowering, in July and August (summer). The leaf area index was higher in North African forms during winter but lower in summer. In summer, the elongation of vegetative tillers was greater in S170 but in winter the opposite occurred and, consequently, Mediterranean forms were more erect. During winter (November-March) organic matter production of S170 was only 25 % of that of the Mediterranean forms. The small increase in winter organic matter production of S170 was accompanied by a 75 % reduction in the total soluble carbohydrates. Both forms increased root weight during winter.

However, it has been observed that the root : shoot ratio of Mediterranean grasses increased with temperature, while the opposite occurred in north European grasses (Eagles, 1967a; MacColl and Cooper, 1967). It was suggested that this response was associated with an increased accumulation of assimilates in the root. The high concentrations of sugars in roots would help north European populations to tolerate low temperatures and produce photosynthetic tissue in the spring, and Mediterranean populations to allow plant survival during summer drought (Eagles, 1967a).

Supplementary light increased growth of the high altitude Mediterranean material grown outdoors and in the glasshouse, while S170 was unable to respond, except at higher temperature (Chatterjee, 1961). Nevertheless, Robson and Jewiss (1968b) observed that the differences in winter growth between Mediterranean varieties and S170 were not regulated by light intensity at the quantities of radiation received in England. However, it is not known whether S170 would be able to utilize the higher radiation of the North African summer.

Because of the findings above, Morgan (1964) proposed that a climatic adaptation had taken place. Grasses that had become successful in Britain were those adapted to grow vigorously in summer and survive the winter in a frost resistant but less active form. In Mediterranean climates, however, natural selection had promoted genotypes adapted to grow actively in the moist, comparatively warm winter season and to become prostrate, and relatively dormant as a drought-avoidance strategy in the dry summer. Moreover, in Mediterranean types, little natural selection for winter hardiness had taken place because temperatures in these areas rarely fall below freezing point.

Burner *et al.* (1988) evaluated variability among and within tall fescue accessions of different geographic origin (USA, Central Asian, Mediterranean, European). Characters evaluated were: water soluble carbohydrate levels of summer forage, first-cut and regrowth yields, disease resistance, relative maturity and winter injury. None of the Central Asian or Mediterranean introductions appeared promising as a source of germplasm for improving USA cultivars for the characters evaluated.

The disadvantage of Mediterranean populations being frost sensitive has been pointed out by Frame (1972) who concluded that under the West Scotland climate, any superiority in autumn/winter growth of the Mediterranean population (Syn I) was not significant from the agricultural point of view. However, in milder climates, like in the Argentinean Pampa, pastures of Mediterranean tall fescues more than

14 years old can be found in good condition. Similarly, in evaluative studies conducted in Australia, the Mediterranean fescues were not killed by winter cold (Neal-Smith and Wright, 1969; Schiller and Lazenby, 1975). Neal-Smith and Wright (1969), in Canberra, observed that Mediterranean tall fescues usually were more productive during autumn-winter-spring than the lines of temperate origin. Four Mediterranean lines of tall fescue exhibited high plant survival (92-97%) under drought, but herbage yields were drastically lowered. As a result, these authors suggested that Mediterranean tall fescues need a favourable soil moisture status from early autumn onwards to be able to express their full growth potential during winter.

While production of Mediterranean tall fescue was depressed by frequent severe winter defoliation and exhibited little response to summer defoliation regimes, the opposite was observed in S170 (Thomas and Lazenby, 1971). However, in Argentina, Mazzanti *et al.* (1985) working with a Mediterranean cultivar, Maris Kasba, and a temperate cultivar, El Palenque, selected from populations introduced to Argentina from USA, found that a higher grazing pressure during the previous summer decreased tiller number by around 22 % in Maris Kasba, but increased it by 30 % in El Palenque.

When Maris Kasba was compared with ten other cultivars under cutting at Barrow (Buenos Aires Province, Argentina), it had the highest yield from June 1985 to January 1986 (Duhalde and Forjan, 1986). At the same location, in another trial, Maris Kasba was the most productive during winter (65 kg DM ha^{-1} day^{-1} in mid- August) when 28.8 % of its total annual production was obtained. Similarly, Mazzanti and Arosteguy (1985) reported that no annual production differences were found between temperate and Mediterranean cultivars in a five-year cutting experiment in Argentina, but Mediterranean cultivars were more productive during winter.

2.2.1.1.2. Origin

Cultivars Kasba (Delgado and Tanco, 1980), Syn II (Morgan, 1964; Robson and Jewiss, 1968a, b; Thomas and Lazenby, 1968a, b, c) and Maris Kasba (Assuero, 1984; Escuder *et al.,* 1985; Mazzanti and Arosteguy, 1985; Mazzanti *et al.*,1985; Duhalde and Forjan, 1986; Escuder *et al.*,1992), are of similar origin. They are derived from material native to a wet zone, Arzou (Morocco), altitude 1,660m, and their chromosome number is 2n=70 (decaploid).

2.2.1.1.3. Feeding value

Escuder *et al.* (1985) compared the utilization of Maris Kasba and El Palenque in Balcarce (Buenos Aires Province, Argentina), and observed that winter and spring consumption of herbage was higher for the Mediterranean cultivar. This was attributed to its lower stem : leaf ratio. Animals grazing Maris Kasba exhibited a higher rate and extent of digestion than animals grazing El Palenque. Lower transit and total mean retention times in animals grazing the Mediterranean form were also found (Escuder *et al.,* 1992). These data are consistent with a greater feeding value for MK.

Van Santen (1992) found that there was considerable variation in animal preference among tall fescue populations. Low preference was a characteristic of populations containing Mediterranean germplasm and of populations selected in the southem USA. However, while digestibility of leafy tall fescue is similar to other pasture grasses, the fall in quality with maturity is early and severe in tall fescue (Easton *et al.*, 1994).

2.3. Nitrogen effect on the swards

Most N in grassland soils is present in the organic matter and is in an equilibrium where additions of organic residues from decay of roots and herbage are balanced by oxidation and losses (Ball and Ryden, 1976). The main inputs of N are from fertilizer and symbiotic N fixation. However, ruminants consume 60-75 % of herbage accumulation and excrete 75-95 % of the N ingested, mainly in the urine. N in the urine, mostly as urea, is biologically labile. By contrast, dung-N, largely organic compounds of bacterial origin, is more slowly hydrolysable than urea and presumably incorporated into soil by the action of soil fauna. Thus urine-patch areas are associated with the greatest potential loss of N (Ball and Ryden, 1976).

N is required for leaf enzymes, especially those associated with the photosynthetic apparatus, and N supply is often the factor limiting rate of development of new leaves (Sinclair, 1990). In many swards N is the main factor restricting tiller production (Davies, 1977). Nitrogen not only stimulates tillering, but also accelerates leaf appearance rate (Ryle, 1964), increases leaf extension rate (Gastal and Lemaire,1988) and also accelerates the rate of leaf decay in grass swards (Simpson and Stobbs, 1981; Wilman and Wright, 1983). However, Laidlaw and Steen (1989) found that when swards were grazed to a given herbage mass, a higher senescence rate per tiller in the low-N swards than in high-N swards was measured. The effect of N application on growth and chemical composition of temperate grasses has been reviewed by Wilman and Wright (1983).

Under controlled conditions or in field-grown spaced plants, most studies have shown that under N deficiency the relative carbon partitioning to the roots is increased (Bélanger *et al.*, 1992b). In other words, the root : shoot ratio is usually related inversely to plant N concentration (Hilbert, 1990). It was also reported that N increased root diameter in grasses (Oswalt *et al.*, 1959).

Under field conditions Bélanger *et al.* (1992a) observed that N fertilization increased photosynthetically active radiation (PAR) intercepted and radiation use efficiency (RUE) in Clarine tall fescue. The authors attributed the former response to a faster leaf area development and the latter to improved photosynthetic efficiency and/or increased C partitioning to shoots. It was also observed that the advantage for shoot growth at higher rates of N fertilization decreased as the relative N concentration approached its maximal value. Their results suggested that the effect of N deficiency on radiation interception occurred only when radiation interception was incomplete, but when most of the incoming radiation was intercepted, only RUE was affected by N fertilization.

In Buenos Aires Province, low soil nitrate levels during winter and spring have been found. For example, concentrations of 6.2 ppm for the 0-180 mm stratum and 3.2 ppm for the 180-300 mm stratum during mid-spring were reported for soils under pasture (Vázquez y Barberis, 1982). In Balcarce (S.E. of Buenos Aires Province), Navarro (1966) observed that the soil nitrate content parallelled the temperature curve, with minimum values in winter. Restricted forage growth rates in this region during winter due to the unavailability of N have also been reported (Fernández Grecco *et al.*, 1995; Marino *et al.*, 1995). Mazzanti *et al.* (1985) studied the effect of N applied in spring and autumn (50 kg N ha^{-1} in each application) on tiller density of Maris Kasba and El Palenque. They observed that the Mediterranean cultivar had a higher tiller number throughout the experimental period and that N increased tiller density by the end of the next winter by approximately 30% in Maris Kasba swards but did not affect El Palenque swards. It has been suggested by Schiller and Lazenby (1975) that, in temperate conditions, a high level of N appeared necessary for the full expression of the winter growth potential of the Mediterranean populations. Chatterjee (1961) found that Mediterranean tall fescues were more responsive to N than S170, and that an increased N level increased NAR, leaf area, and plants became greener, the response being higher under low light and low temperature conditions.

Mazzanti *et al.* (1994) carried out a grazing experiment in France to compare the effects of N on swards of two morphologically contrasting European cultivars of tall fescue. The cultivars were: Clarine, characterized by a low tiller density and large tillers, and Barcel, with a higher tiller density and smaller tillers. Swards were grazed by sheep and maintained at a similar LAI. The absence of any difference in herbage accumulation rate between the cultivars they studied was attributed to a homeostatic mechanism that ensured the differences in growth rate per tiller were counterbalanced by differences in tiller density. They concluded that in continuously grazed swards, the response to N application can be expected to be not only from increased leaf elongation rate, but also from promotion of tillering rate. Linked with the increased herbage accumulation, N application also resulted in increased herbage consumption and efficiency of herbage utilization (Mazzanti and Lemaire, 1994; Mazzanti *et al.*, 1994; Laidlaw and Steen, 1989). The main effect of N was observed on the increase in the frequency of defoliation, the two youngest leaves being more frequently defoliated than the older ones. However, the intensity of defoliation of individual leaves was almost constant (approximately equal to 0.5) for the five periods considered and similar for all leaf age categories (Mazzanti *et al.*, 1994).

2.4. Sward tissue turnover

All plant parts grow and die, or are harvested, resulting in fluxes of leaf and other material. The net change in weight of shoot material over a given period can be expressed as follows (Davies, 1993):

$$\Delta W = \Delta G - \Delta D \quad (2.4.1)$$

where:
ΔW = change in living herbage mass or shoot material.
ΔG = formation of new tissue.
ΔD = death and decomposition of older tissue

Changes in tiller number, leaf number and leaf area can be calculated in the same way. Dry matter flux in swards can be determined as the measure of new tissue formation rates (or senescence rates, or consumption rates) per population unit (e.g. per tiller) associated with the estimates of changes in the population units (Davies, 1993; Mazzanti and Lemaire, 1994). This approach has been described by ecologists as ‘phenometric analysis’ (Davies, 1993). The advantage of using this method to measure growth, senescence and net production rates is that this approach increases the understanding of component sward processes (Grant *et al.*, 1983). Where leaf formation and leaf death are not measured separately, net accumulation cannot be resolved into its constituent components. In earlier studies (e.g. Brougham, 1956) net accumulation was not resolved into components, leaving some ambiguity of interpretation. The interrelationships between the components of the dry matter flux have been proposed as shown in Fig. 2.1.

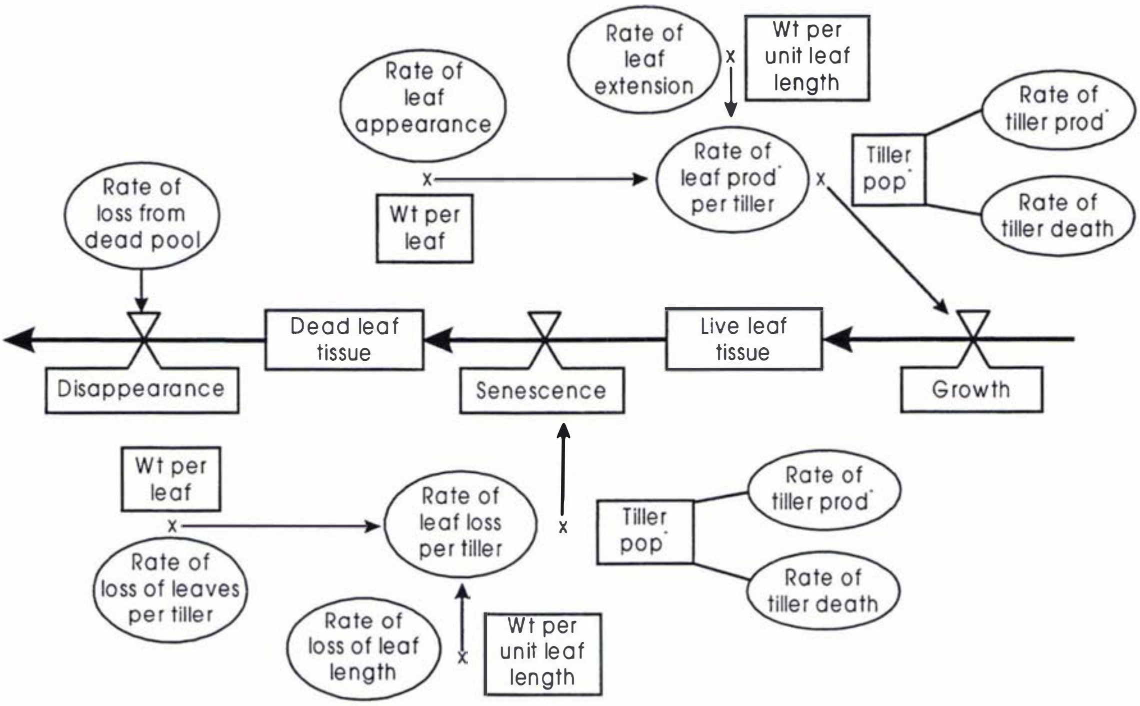

Figure 2.1. The interrelationships between various components of dry-matter flux. The arrows indicate the fluxes that contribute to a given pool of information. (From Davies, 1993)

2.4.1. Morphological basis and components of tissue growth and loss

The morphology of grasses has been described by Silsbury (1970). Essentially, the grass structure is based on the 'phytomer'. The phytomer differentiates at the stem apex and includes four organs: a leaf, an internode, an axillary bud (tiller) and one or more adventitious roots. While leaves and internodes are incapable of infinite growth, axillary buds, at least theoretically, are indeterminate. This is because each one is a replicate of the parent apical meristem and, similarly, can produce new phytomers up to a point when the apex becomes reproductive. Internode elongation occurs as part of the vegetative growth of some grasses, but in others, takes place only with reproductive development. The axillary bud is not foliar in origin and develops in the subapical regions. Tillering is important because it enhances the rate of LAI recovery after defoliation and provides a means of vegetative replacement of dead tillers.

2.4.1.1. Leaf growth

There are five stages in the growth of a grass leaf. They are: initiation, pre-appearance, post-appearance, maturity, and senescence. The leaf primordia initiate as lateral appendages on the apex in acropetal succession. The interval between two successive units is called a plastochron. At first, the leaf primordium grows as a diffuse meristem and increases in size mainly by cell division. When it is around 10-20 mm long two meristematic regions can be distinguished. Lamina growth is due to the meristematic region above the ligule, while sheath growth is due to the other at the leaf base (Silsbury, 1970). In tall fescue, leaf blades did not increase in length after their ligule had emerged, and usually a particular leaf blade did not emerge until its predecessor had almost finished expanding (Robson, 1967). Leaf initiation is terminated when the apex became reproductive after floral induction. Sometimes, grasses may become dormant in which case the apex ceases its growth or temporarily reduces its activity (Silsbury, 1970).

2.4.1.1.1. Leaf appearance rate

In grasses, where the number of leaves per tiller remains relatively stable for prolonged periods (Langer, 1954), the leaf appearance rate (LAR) gives a general idea of the magnitude of tissue tumover. LAR defines the potential tiller production because tillers appear in the axis of leaves. It is apparent that LAR is constant under controlled conditions and also in the field over a considerable part of the growth period for most species (Silsbury, 1970). The LAR has a close relationship with temperature in grasses (Langer, 1954; Davies and Calder, 1969; Peacock, 1975b).

2.4.1.1.2. Leaf extension rate

Peacock (1975b) has shown that perennial ryegrass leaf extension rate (LER) was determined by the temperature of a distinct zone at the level of the stem apex, and not by a general soil or air temperature. However, at a similar temperature, LER was higher in spring than in autumn suggesting that some other factor was limiting

LER in the latter (Peacock, 1975c). On the other hand, Lemaire and Chapman (1996) have reported that for most temperate grasses the response of LER to temperature is exponential in the range 0-12 °C average daily temperature, and linear above this until the optimum at 20-25 °C. Moreover, leaf size increased with temperature, and at a similar temperature was larger for reproductive than for vegetative swards. Peacock (1975c) reported that light irradiance level did not affect LER.

Davidson and Milthorpe (1966) observed in cocksfoot (*D. glomerata*) that removal of the blades of fully expanded leaves (two thirds of the total leaf area) did not affect leaf extension under high external nutrient supply, but affected it when nutrient supply was low. The removal of younger elongating leaves had no significant effect.

The effect of defoliation on LER in *L. perenne* depends on the severity of the defoliation. If only laminae were removed, no significant effect on LER per tiller was observed (Arosteguy, 1982). The length of the sheath tube through which the leaves emerge affects the length of the new leaves. In this way, if the upper part of the sheath is cut, the new leaves will be shorter (Davies, 1988).

LER is strongly affected by N nutrition. Under field conditions three- to four-fold differences in LER for contrasting N nutrition levels have been observed in tall fescue (Gastal *et al.*, 1992).

Reductions in LER have been proposed as indicative that the plant is under stress, and therefore have been utilised specially in water deficit studies (Davies and Zhang, 1991). The procedure of measuring leaf extension using a ruler has been detailed by Peacock (1975a), Arosteguy (1982) and Mazzanti and Lemaire (1994), and using auxanometers by Williams and Biddiscombe (1965). While it has been reported that marker rings and handling of the leaves do not affect dry matter production, green leaf per tiller or number of tillers (Hodgson and Ollerenshaw, 1969), later experiments have shown that handling seems to promote tiller initiation (Matthew, 1992; Hernández Garay, 1995).

2.4.1.2. Leaf senescence

In vegetative grass plants a maximum number of living leaves per tiller appears to be genetically determined (e.g. 3 for *L. perenne*, 2.5 for *F. arundinacea*). Thus, in a steady state, an equilibrium between LAR and leaf death is reached. In the same way as LAR, leaf senescence and lifespan are affected by temperature (Lemaire and Chapman, 1996). The senescence rate increases with the level of N fertilization in the later states of a sward regrowth cycle because of the effect of N on LER and leaf size. Consequently, if N fertilization is not followed by a proper management, dead material can accumulate in the sward (Lemaire and Chapman, 1996).

Any sward management that extends the defoliation interval in order to obtain a high leaf area index and high gross photosynthesis will increase senescence losses, the losses due to senescence and decomposition being maximised if the sward is allowed to grow uninterrupted (Hodgson *et al.*, 1981).

2.4.1.3. Tiller number

The equilibrium between tiller appearance rate (TAR) and tiller death rate determines the density of tillers in grazed swards (Lemaire and Chapman, 1996). Tiller density influences sward regrowth (Davies, 1988) and is strongly affected by light intensity, but is insensitive to temperature and day length (Williams and Biddiscombe, 1965).

Tiller loss is increased by intense shading, and tiller population increases as the frequency of defoliation increases. Intermediate levels of defoliation tend to promote a greater tiller population (Hodgson *et al.*, 1981). Consequently, under continuous grazing, shorter temperate swards have a high number of smaller tillers and a lower rate of growth per tiller than longer swards. However, the rate of growth per unit land area (i.e. the product of tiller number and tiller growth) remains almost the same (Davies, 1988) which implies that the scope for improving net production on continuously stocked swards is relatively limited (Hodgson *et al.*, 1981; Bircham and Hodgson, 1983; Grant *et al.*, 1983).

Experimental evidence suggests that tiller development is controlled by the phytochrome system in response to the red/far-red ratio of the incident light (Deregibus *et al.*, 1983). Experiments on many grass species have shown that the tillering rate slows as the leaf area index (LAI) reaches a value of three, and stops at higher LAI, when the light is almost extinguished at the bud level (Simon and Lemaire, 1987).

Tiller size and tiller density are inversely related. Yoda *et al.* (1963) found that for several plant species logarithmic plots of shoot weight against shoot density showed a -3/2 slope (or -1/2 if the relationship is between herbage mass and shoot density, according to Weller, 1987). This relationship has been confirmed for grass swards (Bircham and Hodgson, 1983) The relationship obtained is known as 'size/density compensation line', and can be considered as defining a line of constant LAI (Sackville Hamilton *et al.*, 1995). In this way, swards plotted to the right (e.g. above) an arbitrarily positioned size/density compensation line will have a higher LAI and swards plotted to the left (e.g. below) a lower LAI. Matthew *et al.* (1996) have observed in pastures of perennial ryegrass in Palmerston North (New Zealand) that below a certain threshold herbage mass (e.g. 2000 kg DM ha^{-1} in winter and near 5000kg DM ha^{-1} during reproductive growth), increases in tiller density related to heavy defoliation pressure are unable to compensate for the associated loss of leaf area. Consequently, below that threshold in herbage mass, the tiller density measured is lower than the expected, and the slope of the empirical line is greater than the theoretical one.

In isolated plants, tiller appearance can be related to leaf appearance. Specifically, Davies (1974) has initiated the concept of 'site filling', as the relationship between relative tillering rate and leaf appearance that allows the determination of the potential tillering rate (Simon and Lemaire, 1987). Nitrogen deficiency lowers site filling and restricts TAR below its potential value, even in swards with low LAI. But at the same time, N fertiliser can reduce tiller densities because of the rapid development of LAI and acceleration of tiller death (Lemaire and Chapman, 1996).

2.5. Root systems

2.5.1. Function and characteristics of roots systems

The primary functions of root systems of terrestrial plants are the uptake of soil-based resources, mainly inorganic nutrients and water, and anchorage (Evans, 1976; Fitter, 1996). Secondary functions are storage, synthesis of growth regulators, and plant propagation and dispersal (Fitter, 1996). In addition, input of senesced tissues to the soil constitutes an important contribution to the total ecosystem cycling of nutrients and to the maintenance of the soil microfauna and microflora populations at the rhizosphere level (Lynch, 1987; Nedwell and Gray, 1987; Bloomfield *et al.*, 1996).

Among the main features that show systematic variation are diameter, surface texture, colour and growth potential. The number, size and duration of root hairs, the persistence of the epidermis and cortex, and the nature of the bark of woody roots, all affect root surface area. Thicker roots might have advantages in penetrating compacted soils. Because fine roots have a more rapid turnover than thick roots, they can impose a significant metabolic cost to the plant (Fitter, 1996).

In pasture most fine roots occur in the upper few centimetres of the soil, where the nutrient concentration is higher and where the chemical and physical features of the soil are more suitable for root growth (Evans, 1976). However, Garwood and Sinclair (1979) found that roots of tall fescue were evenly distributed in the soil profile and at 60 cm depth this species had considerably more roots than perennial ryegrass, cocksfoot or timothy. They also noticed that tall fescue roots tended to be thicker than roots of other species. Nevertheless, detailed comparisons of root systems of Mediterranean and temperate tall fescues seem to be absent in the literature.

2.5.2. Root growth

Brouwer (1983) has stated that shoots and roots grow in rigorous harmony with one another and that the ratio between them is highly predictable under a variety of external conditions. In the case of grasses, it is expected that root mass and tiller density are correlated because almost every tiller can be considered as an individual plant with its own root system, even when the connection between mother and daughter tiller is still functional (Deinum, 1985). It is also known that, for a given plant, the relation between root and shoot growth varies during the different phases of its life cycle, in a consistent manner (Brouwer, 1983).

The following observations have been found in relation to the regulation of the distribution patterns between roots and shoots Brouwer (1983):

- Root growth is favoured when growth is limited by a substance to be absorbed by the roots; while above-ground parts are favoured when the limiting factor is absorbed by the shoot.
- When the shoot:root ratio under steady state conditions is disturbed by root cutting or defoliation, the original ratio is rapidly restored by changes in the growth pattern.
- When plants are transferred from one condition to another, the distribution pattern changes so that the equilibrium pertaining to the new situation is reached.

Root systems of grasses have been subjected to a number of studies in order to investigate seasonal pattems of growth (Jacques, 1956; Garwood, 1967; Evans, 1976; Matthew *et al.*, 1991), and the effects of defoliation (Oswalt *et al.* 1959; Evans, 1976; Troughton, 1977; Garwood and Sinclair, 1979; Brouwer, 1983; Deinum, 1985; Matthew, 1992), root pruning (Evans, 1973; Brouwer, 1983), light (Troughton, 1977), water availability (Davidson, 1969; Troughton, 1977; Troughton, 1981), oxygen (Evans, 1973) and mineral nutrients (Oswalt *et al.*, 1959; Davidson, 1969; Evans, 1970 , 1973 and 1976; Troughton, 1977; Brouwer, 1983; Steen, 1984; Steen, 1985; Boot and Mensink, 1991). However, detailed studies of root systems of Mediterranean and temperate tall fescues under water deficit, or their winter root production under grazing in response of N fertilisation do not seem to have been carried out.

2.5.3. Methods of studying root systems

Many methods have been described to study root systems (Böhm, 1979). Here, only the auger methods (i.e. soil cores), the ingrowth methods (i.e. mesh bags or refilled cores) and containers (i.e. tubes) will be discussed.

According to Böhm (1979), auger methods are the most appropriate for taking volumetric soil-root samples. They involve time and labour, partly for the extraction of the samples, but mainly for the additional work associated with washing the roots and measuring the necessary parameters, such as root length. However, Neil (1992) considered that soil coring techniques were the simplest and gave the most reasonable and consistent results, although a disadvantage of these techniques is the difficulty of distinguishing live and dead roots.

Another method of estimating root production involves measuring the ingrowth into root free sieved soil enclosed in mesh bags incubated in the field (Steen, 1991; Neill, 1992). The method has been suggested as useful for comparisons in field trials when relative instead of absolute estimates are sought. Biased effects can result, at least initially, from the pruning of roots, drainage of water along the walls of the drilled hole, different soil compaction within and outside the bag and different nutrient concentrations, among other problems (Steen, 1991; Neill, 1992). Biomass measured using long-term bags was consistently higher than the sum of root biomass for short-term bags, which confirmed a disturbance effect associated with the bag installation (Neill, 1992).

Matthew *et al.* (1986) conducted a study to compare different techniques for measuring root growth of a perennial ryegrass dominant pasture. The authors observed a significant difference in root length between sand-filled and silt-filled cores, showing that a difference in growth between refilled cores and undisturbed soil could also be expected. However, the patterns of change of root length of both kinds of refilled cores were consistent throughout the 80 days of measurements. The researchers concluded that the refilled core technique was useful to obtain information of comparative root growth rates for plots subjected to different treatments, and that when used in combination with root length measured in intact cores, it could provide an estimate of root turnover rate. In a later calibration Matthew (1992) found that the number of new root tips observed in 'minirhizotron' tubes placed in refilled cores or in undisturbed soil usually did not differ greatly, although there was some seasonal variation.

Container-grown root systems have been used to study the morphology, physiology, biochemistry and ecology of root systems. Böhm (1979) has summarised the advantages and disadvantages of this method. The fact that containers are easily handled and that growing conditions can be replicated many times are among the major advantages. But a disadvantage is that these methods are based on unnatural growing conditions due to the use of disturbed soil or artificial rooting media, possible lack of room for root spread and distribution, lack of competition from other plants, and presence or absence of certain microorganisms, among others. The size and shape of the container limits the root spread and penetration. Long vertical boxes or tubes of relatively small to moderate diameter are recommended in ecological root studies because roots are characterised by their tendency to turn and grow downwards (Böhm, 1979).

2.6. Water stress

All levels of organization of the plant (e.g. metabolism, physiology and gross morphology) are affected by water deficit. Higher plants unavoidably lose water during carbon dioxide assimilation. However, the roots and vascular system enable plants to extract water from the soil, and the presence of a cuticle with stomata permits them to conserve water when the supply from the roots is less than the evaporative demand (Passioura, 1982).

According to Passioura (1982), the term 'water status' is vague, even though it is useful. It has no units and it may allude to the water potential of the plant, its relative water content or its turgor. He considered that the term water status is perhaps best used to describe turgor or relative water content (RWC), rather than water potential. Total water potential (Slatyer, 1967) is the usual measurement of water status because it is relatively easy to measure, but it has not been proved that it is directly linked with physiological processes (Turner, 1981; Passioura, 1982). On the other hand, control systems should have a signal that changes over a time scale similar to the process being controlled. Consequently, as water potential can abruptly change, it does not appear to be an appropriate control signal for developmental processes that take place in response to water deficits (Davies and Zhang, 1991). Some basic theory related to plant water relations, as well as the methods used to measure water content and water potential components, are presented in Appendix 2.1.

It is important for the short-term productivity of a plant to maintain its photosynthetic tissue at a high water status while its stomata remain open. In order to do this it is vital for the plant to maintain positive turgor and, as discussed below, plant tissue seems to adapt to water stress by decreasing symplastic osmotic potential as water potential decreases, allowing turgor to remain positive (Passioura, 1982). Experimental evidence supports the hypothesis that turgor and osmotic components of total water potential are the physiologically active ones (Turner, 1981).

The relationship between RWC and water potential has been used as a measure of the dehydration tolerance of tissues. It has been suggested that those tissues capable of maintaining a high RWC as water potential declines are more tolerant to dehydration (Irigoyen *et al.*, 1992).

Plants growing in dry soil showed reduced growth rates, even though they maintained a high turgor (Davies and Zhang, 1991). Hsiao *et al.* (1976) observed that cell extension was more sensitive than photosynthetic activity to water deficit. As a result, it has been proposed that measurements such as leaf stomatal conductance and leaf extension rate would be good indicators of plant water deficit stress (Davies and Zhang, 1991). In addition, White *et al.* (1992b) observed that leaf rolling score was a good index to estimate tall fescue water status, and proposed its use as a selection criterion instead of more laborious water potential measurements.

2.6.1. Water stress adaptation

Three strategies for adaptation to water stress have been described by Turner (1986). They are: drought escape, drought tolerance with low water potential and drought tolerance with high water potential. Drought escape is represented by annual plants which complete their life cycle before critical soil and plant water deficit occurs, and in perennials which avoid drought by becoming dormant. Plants with the second mechanism can persist under water deficit because they can tolerate dehydration, either by turgor maintenance or by desiccation tolerance. In turn, the primary mechanism of turgor maintenance is osmotic adjustment. Desiccation tolerance appears to be related to the ability of the cells to support mechanical injuries, membrane degradation and membrane and cytoplasm protein denaturation, and again, shoot osmotic adjustment seems to be involved. Finally, plants that tolerate drought with high water potential are those that can maintain a high water status by, for example, delaying dehydration. This can result from an increased water intake or a reduced water loss. The water intake can be increased by means of osmotic adjustment, which permits root growth, allowing the plant to explore a greater volume of soil, or by changed hydraulic resistance to water flow in the plant. Among the factors that have been found to reduce water loss are: stomatal closure (apparently under the control of both abscisic acid and cytokinins), leaf rolling (under the control of leaf turgor), glaucescence, pubescence and reduction in leaf area (Turner, 1986).

It has been proposed that water availability in the soil is sensed by the roots and that this detection involves the transference of chemical information from roots to shoots. Low soil water availability and high soil bulk density have shown similar effects on plant growth indicating the possibility of soil water content being sensed by a change in soil strength (Davies and Zhang, 1991).

Abscisic acid (ABA) synthesis in the roots and concentration in plants increases in response to even mild soil drying (Turner, 1986; Davies and Zhang, 1991). Some experimental evidence suggests ABA not only modulates stomatal behaviour (Quarrie, 1989), and developmental processes (e.g. leaf initiation and expansion), but also increases proline accumulation (Aspinall and Paleg, 1981). From the latter responses, those that reduce the rate of plant size increase would help the plant to maintain its water status by decreasing the water demand (Quarrie, 1989).

Reductions in stomatal aperture initially affect water loss more than carbon dioxide (CO_2) uptake because of the differences in their diffusion pathways. For that reason, an increase in endogenous ABA levels would tend to increase water use efficiency (WUE), but the response would ultimately depend on the balance between the effect of ABA on stomatal aperture and photosynthetic processes (Quarrie, 1989).

2.6.2. Osmotic adjustment

Osmotic adjustment is an important mechanism of plant adaptation to water deficit. It consists of the accumulation of solutes with a concomitant decrease in the osmotic potential (Ψ_o) and an increase in the turgor pressure (Ψ_p) of cells. It allows the maintenance of metabolic processes (Thomas, 1987; Kramer and Boyer, 1995) through the preservation of stomatal opening, photosynthesis, and the postponement of leaf rolling and leaf death (Turner, 1986). However, osmotic adjustment is not necessarily associated with maintenance of growth (Thomas, 1987). When dehydration progresses, photosynthesis becomes inhibited, solute supply is diminished and osmotic adjustment is curtailed. As a consequence, under continued water limitation, osmotic adjustment can delay dehydration but cannot prevent it (Kramer and Boyer, 1995). Similarly, Elmi and West (1995) suggested that turgor maintenance might delay irreversible cell membrane injury and maintain the apparatus necessary for rapid recovery of growth after alleviation of the stress. They also hypothesised that, under water stress, osmotic adjustment may be more important in tissue survival than in tissue growth, the first being critical for the persistence of perennial grasses. Barker *et al.* (1993) observed a lower ability for osmotic adjustment in C_3 than in C_4 grasses, but the former had less rigid cell walls that helped to maintain turgor in spite of lower water potential.

It has been proposed that osmotic adjustment in the meristematic and elongating leaf tissues may enable surviving tillers to have sufficient turgor in the growing zone to delay dehydration and permit rapid resumption of leaf growth upon relief of stress (West *et al.*, 1990; West and Gwinn, 1993). Also root tip osmotic adjustment, which has been observed in some species (Sharp and Davies, 1979; Wullscheger and Oosterhuis, 1991), was thought to be of prime importance because of the role of root tips as desiccation sensors, as reported above (Turner, 1986).

Munns and Weir (1981) considered that decreases in growth (growth being defined as increase in protein or ethanol-insoluble dry weight) are expected to take place during drought because osmotic adjustment occurs at expense of organic solutes. These workers found that all carbohydrate pools were expanding in response to water deficit and observed a greater osmotic adjustment in elongating tissue than in fully expanded tissue. However, although increases in soluble sugars in wheat plants are considered an important component of response to water stress, it is not known whether these changes in soluble sugar concentration are a response to normal metabolic controls related to the supply and demand, or if they are a specific condition associated with water deficit (Munns and Weir, 1981).

White *et al.* (1992b) observed osmotic adjustment of around 0.25 MPa in leaf blades of three tall fescue populations. Similarly, West *et al.* (1990) found that osmotic adjustment occurred in all leaf tissue of tall fescue sampled and the magnitude of the adjustment decreased from youngest to oldest tissues. The osmotic adjustment remained in immature tissues for three to six days after rewatering. However, the mechanism of solute accumulation in tall fescue basal leaf tissue acclimated to water stress is not known. In this regard, the magnitude of the decline in water potential (Ψ_w) of basal leaf tissue was considered unlikely to be the signal that promotes solute accumulation.

Osmotic adjustment was found to be highly heritable in ryegrass (Thomas,1990). The accumulation of carbohydrate, amino acids, proline and inorganic ions was involved. However, selection for improved osmotic adjustment and turgor maintenance did not imply improved leaf growth during water deficit (Thomas and Evans, 1989; Thomas, 1990). Thomas and Evans (1991) considered that it was over-simplistic to believe that selection on the basis of bulk tissue osmotic potential (which is a measure of cytosol, vacuole and apoplast solute contents) would improve growth and survival during water deficits.

2.6.2.1. Fructans

The greater part of the world flora accumulates starch as the major reserve carbohydrate, but a significant group of plants accumulate fructans instead (Hendry, 1987). However, fructan presence does not appear to have any correlation with the presence or absence of starch (Pontis and del Campillo, 1985). Grasses of tropical and subtropical origin accumulate starch, but grasses of temperate origin accumulate fructans and concentrations higher than 50 % of the dry weight of a particular tissue have been reported (Pontis and del Campillo, 1985). A difference between fructans and starch lies in their subcellular location, while starch is accumulated in the chloroplasts in the short term and in the amyloplasts in the long-term (Hendry, 1987), fructans are said to be stored exclusively in the vacuole (Hendry, 1987; Pollock, 1986). Large quantities of fructans were found in elongation zones of wheat (*Triticum aestivum* L.), tall fescue and timothy (*Phleum pratense* L.) by Spollen and Nelson (1988).

Fructans are polymers of D-fructose carrying a D-glucosyl residue at the end joined by a (2→1) linkage as in sucrose and they are commonly represented by the formula G-F-$(F)_n$, where G-F refers to the sucrosyl group and n denotes the number of fructose units in the molecule (Axelrod, 1965; Pontis and del Campillo, 1985). The number of fructose units has been found to vary with plant species and their life cycle, but up to 260 have been reported (Pontis and del Campillo, 1985). Physical and chemical properties of fructans were summarised by Pontis and del Campillo (1985).

According to Hendry (1987) and Pollock (1986) conditions that favour biosynthesis and accumulation of fructans are those that promote photosynthesis (e.g. long day length) but do not favour growth (e.g. low temperature, low N fertiliser treatment, drought, fungal infection and removal of tillers or fruits, infrequent cutting or grazing). Thus, accumulation of storage carbohydrates would be expected when the supply through photosynthesis exceeds the plant demand for growth (Brown and Blaser, 1965).

The distribution of fructans within plants varies significantly. In grasses, a differential distribution appeared to occur with respect to the degree of polymerization within the plant. Tall fescue has short-chain fructans (Smith, 1973). However, Volenec (1986) found a positive association between the degree of polymerization of fructans and their concentration during the fructan accumulation phase in newly formed tall fescue leaf sheath tissue. He also observed relatively short-chain fructans in the base of elongating leaves and, similar to the report of Smith (1973), relatively long-chain fructans in leaf sheaths, suggesting that a separate function may exist for these compounds. He hypothesised that while the long-chain fructans may be merely part of the carbohydrate storage, the short-chain fructans could be used in osmoregulation or transient carbohydrate storage.

It has been suggested that fructans may increase resistance to freezing or desiccation since they are present in plants that tolerate a cold or a dry period during their life cycle (Pontis and del Campillo, 1985). Polymerization or breakdown of fructans would modify vacuolar Ψ_o, and then alter turgor pressure (Pollock, 1986). Another function that has been attributed to fructans is unloading of sucrose from phloem (Spollen and Nelson, 1988).

In an experiment carried out by Spollen and Nelson (1994) changes in the water-soluble carbohydrates, especially fructan, in the basal 25 mm of expanding tall fescue leaf blades were examined. The contents of both low- and high-degree of polymerization fructans were reduced during water deficit. The timing in the changes of both pools and the differences in their magnitude suggested that the regulation of the size of these two pools were specific. The authors also observed that the decrease in fructan was very likely correlated with increases in sucrose and hexose, which have a greater contribution to Ψ_o than fructans. It remained unclear whether the decrease in fructan content was due to decreased synthesis, increased depolymerization, or both. The results obtained lead to the conclusion that although fructan metabolism may be closely associated with the leaf base osmotic adjustment, hexose and sucrose contributed in a more direct way.

Virgona and Barlow (1991) studied changes in non-structural carbohydrate composition of wheat stems after anthesis. They found that under drought conditions fructans were depolymerised and promoted osmoregulation and maintenance of Ψ_p. However, the researchers were not able to elucidate whether this depolymerization was a response to water stress or just an incidental consequence of fructan depolymerisation related to maturation.

2.6.3. Morphological changes under water stress

Usually, a major mechanism for control of water economy in a plant is the regulation of leaf area and morphology. The control of leaf area has been suggested as a more efficient means for conserving water than varying stomatal conductance. It has frequently been observed that drought increases the root : shoot ratio (Passioura, 1982). A higher root : shoot ratio helps the plant to obtain more water to meet the evaporative demand on its leaves, but also implies a higher respiratory cost which may reduce the WUE. An explanation for this change in root : shoot ratio is that as water stress affects shoot growth more than photosynthesis, resulting in increased assimilate being available for the roots (Passioura, 1982).

Cruz *et al.* (1992) observed that severe water stress in the upper portion of sorghum rooting medium encourages lignification and suberization closer to the root apex. The workers suggested that these changes helped to maintain the bulk turgor in the basal root regions by limiting the loss of water to the surrounding medium.

2.7. Tall Fescue and endophyte

Some species of grasses like *L. perenne*, *F. arundinacea*, *F. versuta* Beal. and *Stipa robusta* (Vasey) Scribn. are known to establish mutualistic associations with *Neotyphodium* Glenn, Bacon, & Hanlin endophytes (formerly *Acremonium* Link sect. *albo-lanosa* Morgan-Jones & Gams). In this association stromata have not been found on infected plants and presumably are not produced (White et al., 1993). The endophyte is transmitted solely by penetration of mycelium into the embryo within the seed. These endophytes cannot be considered pathogens since no evidence of any detrimental impact on host plants has been shown. The prevalence of the seed-borne process within species of genera like *Festuca*, *Lolium*, *Poa* and *Stipa* suggest that their mutualistic relationship originated before speciation within the genera (Clay, 1990) and then the relationship became a coevolutionary one (White *et al.,* 1993). The mechanism of endophyte seed transmission is explained in detail by White *et al.* (1993).

The associations between *Neotyphodium* endophytes and tall fescue (*F. arundinacea*) and perennial ryegrass (*L. perenne*) have been widely studied. The mutualism confers on both the host and microbe a higher ecological fitness, which sustains the association against many abiotic and biotic stresses (West and Gwinn, 1993). In the specific case of tall fescue, infected plants have shown enhanced pest and drought resistance (Bacon, 1993). It is expected, however, that the behaviour described for single clones studied in isolation will be different from that of a pasture where the diversity of expression shown by each seed and its endophyte will reflect the entire genotypic range of each constituent within the cultivar complex (Arachevaleta *et al.*, 1989). This implies that different responses will also be expected among contrasting tall fescue populations, for example between temperate and Mediterranean cultivars.

According to Bacon (1993), the association of tall fescue and *Neotyphodium coenophialum* (Morgan-Jones & Gams) Glenn, Bacon & Hanlin can be summarised as a defensive mutualism characterised by specificity, biotrophic nutrition, and possible genetic and metabolic interactions with the grass. The endophyte receives nutrients, protection, reproduction and dissemination, while the plant is assisted by toxin production which deters herbivores (Arachevaleta *et al.*, 1989), by an enhanced mineral acquisition under mineral nutrient stress (Malinowski *et al.*, 1998) and by an increased drought tolerance (De Battista, 1989).

Mycelium of *Neotyphodium* spp. is typically in close association with vascular elements of the host (Belesky *et al.*, 1989) and it is highly abundant intercellularly in leaf sheaths and stems, and usually sparsely present in leaf blades. It seems to be absent in roots of grasses (White *et al.*, 1993) and pollen (De Battista, 1989).

It is expected that all plants grown from seeds of an endophyte-infected grass will contain the same endophyte present in the maternal plant. However, in some tillers, shoot meristems may elude the infection, thus producing endophyte-free seed (White *et al.*, 1993). Since seed-borne *Neotyphodium* endophytes do not sexually reproduce and they are reproductively isolated from other endophytes, they become genetically differentiated by the accumulation of random mutations. In this respect, it has been proposed that artificial selection of cultivated grasses has eradicated much genetic variation of endophytes (Clay, 1990).

The ecological significance of endophytes was first documented in 1977 when Bacon and associates in Georgia reported the relationship between presence of an endophyte in tall fescue and poor cattle performance (White *et al.*, 1993). This poor animal performance was a consequence of the presence of fungal alkaloids in infected grasses. Four alkaloid classes have been associated with fungal endophytes: the ergot alkaloids (e.g. lysergic acid and ergovaline), the indolediterpenes (e.g. paxilline and lolitrem B), a pyrrolopyrazine (peramine) and the saturated aminopyrrolizidines (loline, norloline, *N*-acetilloline, and *N*-formilloline, etc. known as 'lolines'). All these alkaloids show anti-insect activities, while activities against mammals have been found for all but peramine. Peramine and lolines are the most potent against insects, and indolediterpenes and ergot alkaloids are the most potent against grazing mammals (Schardl and Phillips, 1997). Lolitrem B is the alkaloid associated with the malady 'ryegrass staggers', commonly observed in sheep grazing perennial ryegrass in New Zealand, and ergovaline is the alkaloid related to a group of maladies collectively called '(tall) fescue toxicosis' often observed in cattle grazing endophyte-infected tall fescue (Schardl and Phillips, 1997).

2.7.1. Morphological and physiological responses of tall fescue to *Neotyphodium coenophialum*

Some studies have demonstrated variability in response to the endophyte among grass genotypes of the same species, suggesting that it is not possible to generalize about the endophyte influence upon tall fescue productivity and persistence at a population level (Belesky *et al.*, 1989; De Battista *et al.*, 1990;

Belesky and Fedders, 1996; Elbersen and West, 1996; Marks and Clay, 1996, Malinowski *et al.*, 1998). Interactions between the grass, fungus and the environment, enlarge phenotypic variation among symbiotic plants. For instance, Hill *et al.* (1990) found that responses of five tall fescue genotypes to endophyte were inconsistent for the measurements they performed (tiller production, crown weight, dry matter production, specific leaf weight, ergovaline production and total nonstructural carbohydrates concentration or quantity). Similarly, the endophyte effect on biomass production varied depending on plant genotype (Richardson *et al.*,1990) and fungus infection (De Battista *et al.*, 1990), indicating genotypic differences among individuals of both plant and endophyte. In addition, the fungus was found to promote tall fescue survival but dry matter production was variable between years (Read and Walker, 1990). The endophyte seems to generate an increased phenotypic expression that would allow mixed populations of endophyte-infected (E+) and endophyte-free (E-) tall fescue to increase the phenological diversity of the population without increasing genetic variability (Hill *et al.*, 1990), and while not all the infected plants will have all the mechanisms of fitness imparted by the endophyte, the sum of all the plants in the population should express fitness characteristics which will give symbiotic grasses more plasticity and adaptability (Bacon, 1994).

As the endophyte is located intercellularly and it does not have any structure to absorb nutrients, it has been suggested that the endophyte may utilize apoplasmic nutrients, which are mainly sugars and N compounds that diffuse out of the cytoplasm (Bacon, 1993). For instance, Belesky *et al.* (1991) observed that the endophyte did not affect tall fescue acid-extractable carbohydrate concentration or yield (concentration x dry matter) which led to the conclusion that endophyte was not harmful to the tall fescue host. However, Cheplick *et al.* (1989) found that endophyte-infected tall fescue seedlings exhibited a significant decrease in plant growth at the lowest nutrient availability level, while the opposite was found at high nutrient levels. In the case of adult plants, E+ plants were more productive at all nutrient levels, but the differences were smaller at low nutrient levels. This was the first experimental evidence of any detrimental effect of endophyte in tall fescue. The workers speculated that the diminished growth at low nutrient concentrations could involve a competition between the fungus and the plant for the scarce supply of photosynthate or nutrients. However, Cheplick *et al.* (1989) considered that under most circumstances, the maintenance cost of the endophyte should be counterbalanced by increased plant vigour and herbivore resistance. Nevertheless, in the case of seedlings growing in non-fertile environments, endophyte may slow seedling growth, without confering an increased vigour to the plant. Malinowski *et al.* (1998) observed an increased cumulative herbage yield in tall fescue E+ plants at low soil phosphorus (P) levels, while the opposite was found at high P levels.

De Battista *et al.* (1990) found that tall fescue responded similarly to Nui ryegrass plants (Latch *et al.,* 1985). Endophyte increased tiller number, herbage growth, and root yield, but shoot : root ratio was not changed. Arachevaleta *et al.* (1989) also observed a higher production of tillers in E+ tall fescue plants, but only at a high N level.

The endophyte seems to modulate the leaf growth-zone dynamics resulting in the alteration of cell size. However, Elbersen and West (1993; quoted by West and Gwinn, 1993) found that this effect was different in two clones studied. In one clone, young cell size was reduced while, in the other clone, final cell size was reduced by endophyte. Arachevaleta *et al.* (1989) found that leaf blades of E+ KY31 tall fescue plants were thicker and narrower than E- plants. It was suggested that this attribute may facilitate leaf rolling in E+ plants, which may reduce the loss of water under drought conditions. They also observed that the ultrastructural morphology of mesophyll tissue of E+ leaf sheaths of young plants differed from that of E- plants in that the former had intercellular air spaces, which was a characteristic of mature plants. It was concluded that endophyte may accelerate plant maturation rate by the production of an unknown plant growth regulator. That E+ plants used in the experiment flowered two weeks earlier than E- plants, supports this hypothesis.

Hill *et al.* (1990) reported that in a glasshouse experiment, crowns of tall fescue E+ plants were deeper in the soil. It was conjectured that a deeper crown location would make the plant more cold hardy in winter and heat tolerant during summer. These workers also observed that yield per tiller was greater in E+ plants than in their E- clones while, generally, total nonstructural carbohydrate concentrations were either the same or lower in the E+ clones than in E- clones, suggesting that the fungus provided a mechanism by which regrowth was increased such that carbohydrate reserves were utilized more efficiently.

Carbon exchange rate (CER) of tall fescue plants at high temperature (above 35 °C and in short-term acclimation to temperature), was higher in E+ than in E- plants (Marks and Clay, 1996). However, the higher CER was not the result of differences in leaf conductance or availability of carbon dioxide. Consequently, it was suggested the fungus presence could diminish photorespiration, or that the fungus could act as a sink for sucrose (e.g. to synthesise sugar alcohols), and thereby would reduce or prevent the inhibitory feed back of photosynthates on photosynthesis rates. On the other hand, De Battista *et al.* (1990) reported that

Lyons found an increased glutamine synthetase activity in E+ tall fescue plants, suggesting an improved reassimilation of ammonium released by deamination or photorespiration. Moreover, an increased carbon assimilation into amino acids was observed in E+ plants by Lyons *et al.* (1990) while protein concentrations remained unchanged.

It is not known how the fungus alters plant physiology, but a hormonal mechanism could be involved since *N. coenophialum* isolates with variable ability to produce indole acetic acid (IAA) in vitro have been found (Hill *et al.,* 1990). It was proposed that tillering rate would be stimulated or inhibited depending on the amount of IAA produced by the endophyte, and that this effect could vary, also, with plant genotype (Hill *et al.,* 1990).

2.7.2. Endophyte and water stress

Read and Camp (1986) reported the first evidence that tall fescue with a high rate of endophyte infection maintained a higher plant density than low-endophyte tall fescue during a drought. Since then much research has investigated the mechanisms involved, to develop agronomically superior associations that are harmless to livestock (West and Gwinn, 1993). However, Bacon (1993) proposed that when dealing with a mutualistic symbiosis, like the one between tall fescue and *N. coenophialum*, specific stress tolerances are not expected to be present in all endophyte-infected individuals because the different fungal and plant associations exhibit different physiological and biochemical responses as reported above (Section 2.7).

Belesky *et al.* (1989) hypothesised that as water stress modifies transpiration and photosynthate flow, endophyte activity could be affected in a way that can influence host growth and development. While the mechanisms by which the endophyte increases host survival during drought are not yet fully understood, West and Gwinn (1993) have proposed that the endophyte induces a kind of incipient stress that preconditions the plant to drought so it would be able to display earlier adaptive responses.

2.7.2.1. Morphological and growth effects

West *et al.* (1993) reported that endophyte-infected fully established tall fescue stands (80 % infection) had a greater tiller density recovery when a short term drought stress was relieved, and also exhibited a lower rate of tiller density decline during a subsequent drought in mid- to late-summer. Later, after an autumn recovery period, tiller density of endophyte-free stands was only 62 % that of endophyte-infected stands and remaining tillers were usually less vigorous. West and Gwinn (1993) suggested that these results indicate that endophyte infection raises the water-deficit threshold, measured by tiller and plant survival, for inducing stress in tall fescue. Similarly, Bouton *et al.* (1993) observed a higher mortality of E- tall fescue plants because of their lower summer drought tolerance, which resulted in greater infection levels of the sward, when percentages were based on the remaining plants. In addition, Arachevaleta *et al.* (1989), in a glasshouse experiment, found that while under severe water stress the endophyte did not provide any benefit in yield, three out of four E- replicates died while all E+ plants survived, demonstrating that the endophyte presence in tall fescue was still an advantage.

The influence of endophyte on perennial ryegrass leaf extension rate was variable and generally not significant (Eerens *et al.*,1993). However, in agreement with results of Belesky *et al.* (1989) in tall fescue, E- plants had, in most cases, similar or slightly greater leaf extension rate than E+ plants. Belesky *et al.* (1989) hypothesised that the maintenance of leaf elongation under stress could have an adverse consequence on plant persistence. They considered that production of a greater evaporative surface, produced by a higher leaf extension, may make a plant more liable to perish in a long term or successive stress events. However, as endophyte status did not affect all accessions consistently it was concluded that developing agronomically productive and persistent endophyte-free tall fescue genotypes would be possible (Belesky *et al.*,1989).

Maclean *et al.* (1993), in a growth cabinet experiment, compared combinations of three tall fescue 'Grassland Roa' plants, each infected with one of three different endophyte strains, and found no morphological effect of endophyte under water stress. Their results corroborate previous findings in that endophyte seemed to favour plant growth under more favourable conditions (Arachevaleta *et al.*,1989), but this was in contrast with Hume *et al.* (1993), who found no effect of endophyte on tillering rate. Hume *et al.* (1993) considered that the lack of response was because water stress was only moderate, and that the plants had been artificially infected with endophyte strains from different host plants, which could have resulted in poor mycelium growth and partial infection of the plant tillers.

Arachevaleta *et al.* (1989) observed that cloned E+ tall fescue plants showed leaf rolling sooner than E- plants under water deficit in the glasshouse. According to West and Gwinn (1993), the significance of endophyte in the stimulation of leaf rolling is unclear, however, they speculate that it is probably of transitory benefit and not essentially associated with crown survival. Nevertheless no difference in leaf rolling between E+ and E- plants was observed by Elmi and West (1995) or by White *et al.* (1992a).

Evidence of greater root biomass production in well-watered E+ tall fescue KY31 plants was provided in a series of experiments performed by Kelrick *et al.* (1990). Their results also suggested that hormone-like compounds may leak from E+ plants stimulating root growth of plants in the vicinity. The workers conjectured that, when E+ plants predominate in the mixture, their relative and absolute investment in root production would give them an advantage that would allow them to suppress the growth of local E- plants, despite the potential supply of a root-growth-promoting compound. However, as the ratio of E+ plants to E- plants declines, the capacity of E+ plants to suppress growth of neighbour E- plants should be expected to decrease accordingly and the effect of the hypothetical root growth promoter would be observable in E- plants.

Inconsistent results have also been found between experiments that studied root growth. For example, Richardson (1992) observed a greater root growth under water stress in E+ tall fescue plants than in E- plants. Belesky *et al.* (1989) found that root dry matter varied among accessions of KY31 tall fescue, but endophyte status and water regime did not affect shoot : root ratio. On the other hand, De Battista *et al.* (1990) found that endophyte infection tended to increase root dry matter in tall fescue.

2.7.2.2. Physiological effects

Belesky *et al.* (1987) reported lower stomatal conductance in E+ than in E- tall fescue genotypes subjected to water stress and a concomitant reduction in transpiration rate. Similar results were found by Elmi *et al.* (1990) and Elmi (1992; quoted by West and Gwinn,1993). It was speculated that early stomatal closure might originate either by some phytohormone signal from root-to-shoot of soil drying, or via fungal secretion of antitranspirant compounds into the apoplasm (West and Gwinn, 1993). However, results of a later experiment did not indicate convincingly that endophyte enhanced drought tolerance by inducing stomata closure (Elmi and West, 1995). In the same way, Richardson *et al.* (1993b) found no endophyte effect on stomatal conductance in two genotypes subjected to water stress. Moreover, in a field experiment conducted by West *et al.* (1988), E+ tall fescue plants seemed to have a higher stomatal conductance than E- plants when water supply decreased, as indicated by the difference between canopy and air temperature.

In a glasshouse study, Bates and Joost (1990) found that E- plants showed higher instantaneous water use efficiency (WUE), measured as the ratio between photosynthesis and transpiration rates, but this difference disappeared at low water availabilities when E+ and E- showed similar low WUE values. It was concluded, that when water was available, the infection of tall fescue by *N. coenophialum* takes place at the expense of the plant. Conversely, West and Gwinn (1993) found no endophyte effect on ^{13}C discrimination, implying no real endophyte effect on WUE. The latest results are in agreement with Richardson *et al.* (1990) who suggested that, in symbiotic tall fescue, water use efficiency and drought resistance may be unrelated. They argued that WUE under water stress is maintained despite a high stomatal conductance, because of an increased photosynthesis, which in turn, may confer other drought resistance mechanisms (e.g. promote root growth or osmotic adjustment) that would give E+ plants a greater fitness during drought periods.

Elmi *et al.* (1989; 1990) and Elmi and West (1995) reported that tall fescue E+ plants exhibited osmotic adjustment in leaves and basal vegetative growing zones relative to E- plants in KY31 populations and in clonal material. This phenomenon, that seemed to be common in plants derived from KY31 populations (Elmi *et al.*, 1990; Elmi and West, 1995), was also observed by Maclean *et al.* (1993) in plants of cultivar 'Grassland Roa'. Elmi (1992; quoted by West and Gwinn, 1993), found positive correlations between growing-zone osmotic adjustment during two drying cycles and percentage of tillers surviving severe drought in a single genotype. However, Hill *et al.* (1996) and White *et al.*(1992a) found no endophyte mediated change in osmotic potential in tall fescue. Nevertheless, a difference was found between E+ and E- plants in the bulk modulus of tissue elasticity and in the ratio of turgid weight to dry weight, suggesting that *Neotyphodium* endophytes modify tall fescue cell wall characteristics (White *et al.,* 1992a) . Because of these findings the authors hypothesised that the endophyte-mediated drought resistance may arise from modification of the drought avoidance strategy.

Richardson *et al.* (1992) observed that E+ tall fescue plants accumulated higher concentrations of glucose and fructose in leaf sheaths and glucose in blades compared to E- clones, and that they were accumulated at a level that could contribute to osmotic adjustment, but not account completely for it (Richardson, 1992). Simple sugars, derived from depolymerised fructans, provide a more effective osmoticum than fructans (Richardson,1991). Furthermore, Richardson *et al.* (1992) observed that arabitol was found only in water-stressed E+ plants. Although hexoses were more important than sugar alcohols in accounting for the osmotic adjustment, sugar alcohols may have contributed to protection of host enzymes and membranes from desiccation damage and also served as antioxidants (Bacon, 1993).

Interestingly, Richardson *et al.* (1993a) studied growth characteristics and water relations of an isolate of *N. coenophialum* obtained from a population of KY31 tall fescue and found that the fungal hyphae osmotically adjusted. It was suggested that under water stress the endophyte might induce changes in host plant metabolism despite its extracellular nature. The authors pointed out, however, that the chemical compounds responsible for the fungal osmotic adjustment remain unknown.

Turgor pressure in E+ plants was also found to be enhanced in stressed plants and the increase was larger in the basal 2-cm growing zone than in the leaf blade (Elmi, 1992; quoted by West and Gwinn, 1993). However the basis of the turgor maintenance in E+ tall fescue is still unknown (Richarson *et al.*, 1993b). Elbersen and West (1996) reported an increase in sheath water content of E+ plants. This effect might contribute to a postponement of the desiccation of the enclosed growing zone and apical meristem, extending tiller survival during water deficit.

2.8. Summary and conclusions

Tall fescue is the most important cool season forage grass in USA (van Santen, 1992), and in Argentina (Mazzanti and Arosteguy, 1985). In New Zealand it has been recommended as an alternative grass species in areas where summer drought (Wright *et al.*, 1985) and grass grub damage (Judd *et al.*, 1990) limit ryegrass persistence.

Mediterranean and temperate populations of tall fescue differ in their seasonal production (MacColl and Cooper, 1967; Frame *et al.*, 1970). Mediterranean populations show a greater growth rate during winter and early spring, while temperate ones grow more actively during summer, when Mediterranean populations remain relatively dormant (Morgan, 1964). Mediterranean germplasms, however, have the disadvantage of being frost sensitive, which makes them unsuitable for environments characterised by very hard winters, like Britain (Frame, 1972).

Many experiments have been conducted to compare the morphological and physiological responses of Mediterranean and temperate tall fescues to environmental factors (Chatterjee, 1961; Robson and Jewis, 1968 a and b; etc.). However, although tall fescue is known as a drought tolerant species, comparisons of the effect of water deficit on these contrasting populations are scarce.

Winter growth is of great interest since alternative strategies for producing additional feed, such as winter forage crops, are expensive and involve risk. In this regard, tall fescue of Mediterranean origin persists very well in regions characterised by mild winters like the S.E. of Buenos Aires Province. In this region, Mediterranean cultivars have shown a greater winter growth than temperate ones (Mazzanti and Arosteguy, 1985). Furthermore, the feeding value in winter and spring seems higher in Mediterranean cultivars (Escuder *et al.*, 1992). Consequently, if the Mediterranean and temperate cultivars were used in a complementary way, a more stable forage supply than that produced by either of the cultivars alone could be obtained (Mazzanti and Arosteguy, 1985). In addition, because of a low N availability in the soil of the above region during winter (Navarro, 1966), forage growth could be improved if N was applied (Fernández Grecco *et al.*, 1995, Marino *et al.*, 1995). However, detailed field studies of shoot tissue turnover, root production and animal production comparing tall fescues of Mediterranean and temperate origins, and their response to N fertilisation, have not yet been carried out.

The presence of endophyte *N. coenophialum* has been reported as beneficial for the plant to tolerate drought (Read and Camp, 1986; West and Gwinn, 1993). However, the studies reported suggest that the grass response to the endophyte is not consistent, and depends on plant genotype, endophyte strain and environmental factors (e.g. nutrient availability). Novel strains of endophyte that seem harmless to livestock have been identified (West and Gwinn, 1993). Again, it seems that no information is available about endophyte infected Mediterranean tall fescues, or about Mediterranean populations artificially infected with agronomically superior endophyte strains.

CHAPTER 3. MORPHOLOGICAL AND PHYSIOLOGICAL RESPONSES TO WATER DEFICIT OF ENDOPHYTE-FREE MEDITERRANEAN AND TEMPERATE TALL FESCUES

3.1. Introduction

Tall fescue (*Festuca arundinacea* Schreb.) is the most important perennial cool season forage in the south-eastern USA (van Santen, 1992; De Battista, 1989). In Argentina, tall fescue is the most widely grown perennial grass, (Molina, 1988; INDEC, 1993) and is cultivated mainly in the Pampa region (Gonzáles and Gardner, 1977; Mazzanti and Arosteguy, 1985). Because of the variability in ecological areas of the humid and sub-humid Pampa region (Barsky, 1991, Soriano *et al.,* 1992), tall fescue occupies areas that can experience winter drought (e.g. Córdoba Province) or summer drought (e.g. Buenos Aires). In New Zealand tall fescue has been recommended as an option where summer drought limits the persistence of ryegrass (Anderson, 1982, Anderson *et al.*, 1982, Brock, 1982).

Tall fescues of Mediterranean origin have shown a higher winter and early spring growth than those from European origin (MacColl and Cooper, 1967; Frame *et al.*, 1970). Although Mediterranean tall fescues were frost sensitive (Delgado and Tanco,1980), in milder climates, like in the Argentinean Pampa, pastures of Mediterranean tall fescues have persisted for more than 14 years in good condition. Similarly, in studies conducted in Australia, the Mediterranean fescues have not been killed by winter cold (Neal-Smith and Wright, 1969; Schiller and Lazenby, 1975).

As reported in Chapter 2, previous research has compared the growth responses to temperature, especially to low temperature (Chatterjee, 1961; Morgan, 1964; MacColl and Cooper, 1967; Robson, 1967; Robson and Jewiss 1968a, b), and daylength (Robson, 1967, Robson and Jewiss 1968b) of Mediterranean and temperate tall fescue populations. However, there is rather less information available (Neal-Smith and Wright, 1969) about the comparative responses of Mediterranean and temperate tall fescues to water deficit.

3.2. Objective

The objective of this experiment was to compare morphological and physiological responses of one Mediterranean and two temperate tall fescues cultivars during optimal and sub-optimal soil water availability.

3.3. Materials and Methods

3.3.1. Experimental design and statistical analysis

The experiment comprised three cultivars (see below) subjected to two watering treatments (fully watered and water deficit) with sequential destructive harvests over time. A randomised complete block design with three replicates was used to control environmental differences created by the position of the ventilators in the glasshouse. Re-randomisation of pots within blocks did not take place because of their size and weight. The treatments were combined in a factorial arrangement: cultivars (3), water treatment (2) and harvest time (4). The first harvest preceded imposition of the water treatments, so only the three replicates for each cultivar were harvested. Hence, Harvest 1 was analysed separately as a RCB (Steel and Torrie, 1980) to compare cultivars at time zero. Data from Harvests 2 to 4 were combined and analysed as a RCB. The SAS General Linear Models (GLM) procedure was used for both analyses. Weighted ANOVAs were run for variables in which variance heterogeneity between water treatments was found when the mean square errors, from preliminary analyses carried out on each individual water treatment and harvest combination, were analysed by Bartlett's test (Steel and Torrie, 1980). The reciprocals of the above error variances were used as weight. Percentages were arcsin square root transformed for statistical analysis. Means were separated using the LSD (Steel and Torrie, 1980) at the 5 % significance level. Where weighted analyses were used, to simplify tables layout, the standard error shown is the average standard error, calculated as $\sqrt{}(\Sigma^{n}_{i=1}(\mathrm{s.e.})_i^2/n)$, where n is the number of standard errors (s.e.) involved in the calculation. Quadratic regressions were fitted using SigmaPlot for Windows Version 4.

The experimental management schedule, conditions of plant culture and harvest dates are shown in Fig. 3.1. Photographs illustrating part of the experimental layout in the glasshouse and close-up views of plants from the different treatments are shown in Plate 3.1.

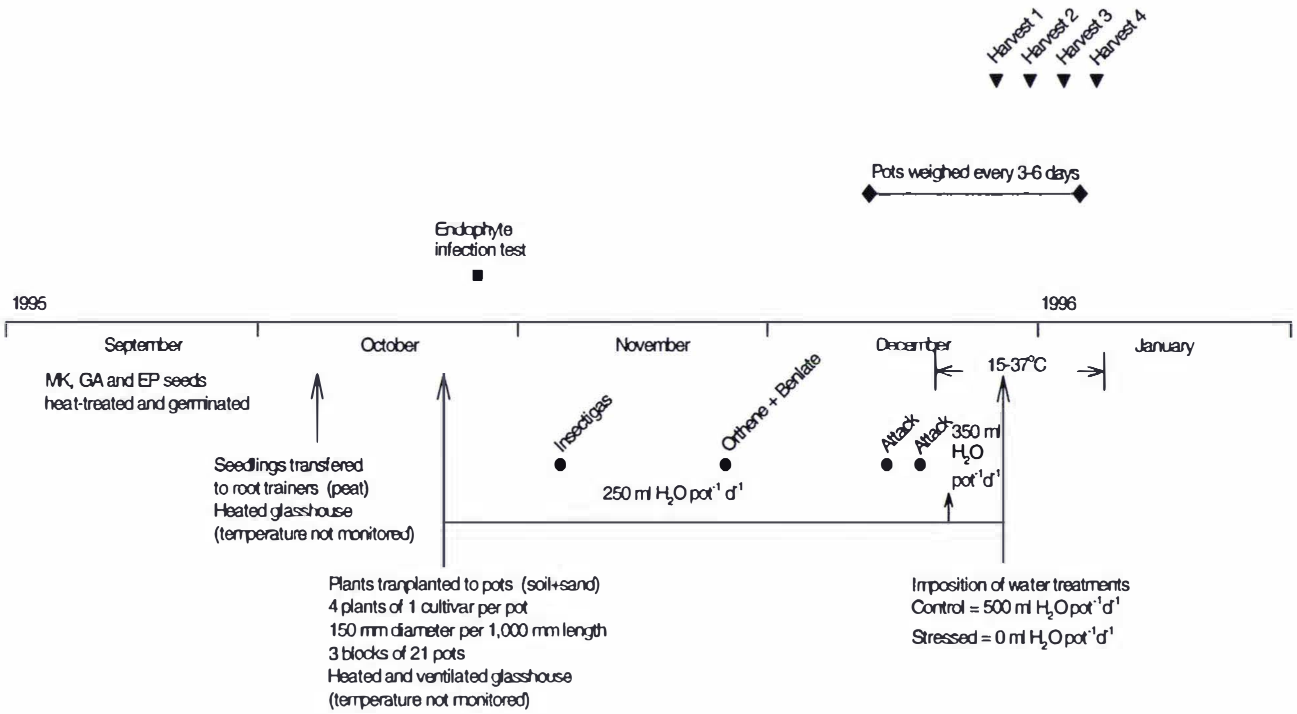

Figure 3.1. Experimental management schedule, conditions of plant culture and harvest dates for the first water deficit experiment. Massey University, New Zealand.

Plate 3.1. Experimental conditions in the glasshouse (a,b) and water treatment effects on the three cultivars (c-h).

3.3.2. Plant culture

The tall fescue cultivars studied in this experiment were: Grasslands Advance (AgResearch, New Zealand) and El Palenque (INTA[1] Pergamino, Argentina) of temperate origin, and cultivar Maris Kasba (from INRA[2], France) of Mediterranean origin. Seeds were incubated at 47 °C and 45 % relative humidity for 25 days to kill the endophyte *Neotyphodium coenophialum* (Bouton *et al.*, 1993; Dr. G. Latch, personal communication). They were then germinated and seedlings were transferred to root-trainers. After approximately 15 days of growth, on 23 October, the seedlings were transplanted to pots of 150 mm diameter and 1,000 mm height, filled with a mixture of Karapoti silt loam soil (B horizon) and sand (1 : 1 by volume), fertilised with 'Osmocote' (NPK 15-4.8-10.8) at 3 g l^{-1} soil. Four seedlings were planted in each pot. Plants were located in a glasshouse at Massey University, Palmerston North (40.3 ° Lat. S), under natural photoperiod that during the experimental period ranged from approximately 13h 30min to 15h. The glasshouse used in this experiment had limited temperature control. Cooling was set to operate above 20 °C and heaters to operate when temperature fell below 10 °C.

Plants were examined to ensure that endophyte elimination was successful (Latch and Christensen, 1982). Plants were sprayed to control aphids with Insectigas® (dichlorvos in liquid carbon dioxide aerosol) for six seconds on 5 November, with 1 g Orthene®75 (750 g kg^{-1} acephate in the form of a soluble powder) per litre H_2O on 25 November and with 1.0 ml Attack® (25 g l^{-1} permethrin plus 475 g l^{-1} pirimiphos-methyl in the form of an emulsifiable concentrate) per litre H_2O on 14 and 18 December. Plants were also treated to control mildew with 3 g Benlate (500 g kg^{-1} benomyl in the form of a water dispersible powder) per litre H_2O on 25 November.

Pots were watered in the morning with 250 ml water pot^{-1} d^{-1} until 22 December, then with 350 ml water pot^{-1} d^{-1} until 28 December and from then on 500 ml water pot^{-1} d^{-1} for Control plants only (see below). Pots were weighed every 3-6 days between 12 December and 6 January, and water was added as required to bring the pot weight to estimated soil field capacity.

An initial harvest of one pot per cultivar per block was carried out (Harvest 1).

[1] Instituto Nacional de Tecnología Agropecuaria.

[2] Institut National de la Recherche Agronomique.

Blocks were harvested on consecutive days from 26 December because of the practical difficulty of harvesting the three blocks on the same day. Following this harvest water was completely withheld from water deficit pots. Three more harvests were carried out (i.e. Harvest 2 from 30 December-1January, Harvest 3 from 3 to 5 January and Harvest 4 from 7 to 9 January).

Maximum and minimum daily temperatures were recorded from 20 December to 9 January (Fig. 3.2).

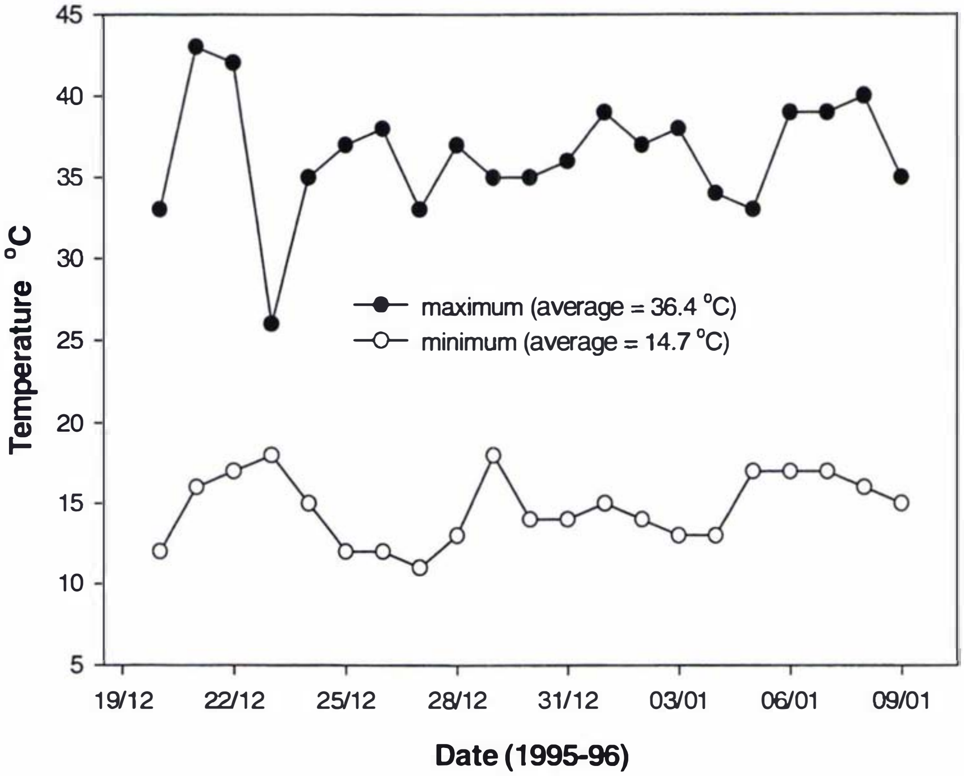

Figure 3.2. Daily maximum and minimum glasshouse temperatures during the application of the water treatments

3.3.3. Measurements

The following were measured at each of the four harvests.

- **Relative water content**

Pots were covered with plastic bags overnight and plants sampled at 9:00 am. The youngest fully expanded leaf of two tillers per pot was cut and weighed (fresh weight, FW), floated in distilled water, re-weighed (saturated weight, SW) and dried to constant weight. Relative water content (RWC) was determined as described in Appendix 2.1, Eq. *A*2.10.

- **Stomatal resistance and transpiration**

Stomatal resistance, stomatal conductance and transpiration on adaxial (upper) and abaxial (lower) surfaces of the youngest fully expanded leaf of two tillers per pot were measured with a steady state porometer (LI-1600, LI-COR, Lincoln, Nebraska) at midday.

- **Water, osmotic and turgor potentials**

Water potential (Ψ_w), osmotic potential (Ψ_o) and full turgor osmotic potential ($\Psi_{o(f)}$) were measured with a thermocouple psychrometer (SC10A, Decagon Devices Inc. Pullman, WA) on a sample comprising the combined youngest fully expanded leaves of two tillers per pot collected at midday. Conversion of thermocouple output (μV) to osmotic potential (MPa) was made from standard curves prepared from KCl each time the psychrometer was used. Samples for Ψ_w and Ψ_o were cut into small pieces, put in the psychrometer chambers and immediately sealed with Sellotape®. Samples for $\Psi_{o(f)}$ determination were floated in Petri dishes filled with distilled water overnight, dried with tissue paper, cut and kept in the psychrometer chamber as described above. Chambers with samples for Ψ_o and $\Psi_{o(f)}$ determinations were frozen with liquid air and stored in a freezer. They were later thawed and put in warm water until they reached room temperature, at which point readings were taken. Leaf pressure potential (Ψ_p) was estimated as the difference between leaf Ψ_w and leaf Ψ_o.

- **Dry weight**

Two plants of each pot were harvested and separated into stem base and leaf sheath (pseudostem), leaf blade and dead tissue. Each component was dried overnight at 60°C in a forced-air oven and weighed (DW).

After harvesting of above-ground material, pots were stored at 2°C until sequential harvests were completed. Roots were then washed and frozen. Coarse root length, total root length and organic matter were determined separately by soil depth for 0-75 mm, 75-150 mm, 150-300 mm and below 300 mm segments of the pots. Results were expressed on a per pot basis (i.e. four plants). Root length was measured by an experienced technician using the grid intersect method (Tennant, 1975). As a 'control' selected samples were recounted by a supervisor to ensure consistency. Samples were counted twice. In the first count, only the main axes of the roots in the sample were counted (i.e. about 1.0 mm diameter or more, coarse root length). In the second count all roots were counted (total length). To determine root mass (organic matter, OM), samples were dried overnight at 70 °C, weighed to determine DW, then ashed for four hours at 650 °C, and ash weight subtracted from DW. Mean root diameter and root surface area were calculated as shown in Appendix 3.1.

- **Carbon isotope discrimination**

Samples from the youngest fully expanded leaf of each of two tillers per pot were combined, dried, ground and submitted to Waikato University Stable Isotope Unit, for carbon isotope analysis. Carbon discrimination (Δ) was calculated according to Eq. *A* 2.15, Appendix 2.1.

3.4. Results and discussion

3.4.1. Plant structure

This section deals with plant morphology and the changes that took place with time, following the application of water treatments. It is divided in three subsections: shoot characteristics, root characteristics, and root : shoot ratio. Each subsection begins with a cultivar comparison of the variables measured before the water treatments were imposed. To have a better understanding of the treatment effects in relative terms, some variables are expressed as percentages of the total.

3.4.1.1. Shoot characteristics

- **Before water treatments were imposed**

The mean plant dry weight (DW) before the water treatments were imposed differed between cultivars ($P<0.05$). Maris Kasba (MK) plants were smaller than Grasslands Advance (GA) and El Palenque (EP) plants (Table 3.1). However, the proportions of sheath, leaf blade and dead tissue of the plants, and tiller number per plant (Table 3.1) were the same for all cultivars ($P>0.05$).

Table 3.1. Plant dry weight (DW), percentages of shoot DW as leaf blade, sheath and dead tissue, and mean tiller number per plant for the cultivars MK, GA and EP before the water treatments were imposed (Harvest 1).

Cultivar	Plant DW (g)	% DW as leaf blade	% DW as sheath	% DW as dead tissue	Tiller number
MK	3.41 **b**[1]	64.4 (53.4)	33.9 (35.6)	1.8 (7.2)	23.5
GA	6.77 **a**	62.9 (52.5)	34.7 (36.1)	2.4 (8.0)	18.2
EP	7.07 **a**	60.9 (51.3)	35.4 (36.5)	3.7 (10.0)	16.2
s.e.[2]	0.23	(0.6)	(0.6)	(1.5)	2.2

Data within brackets are arcsin square root transformed percentages.

[1] Letters indicate groupings of means that are significantly different at $P<0.05$.

[2] Standard error.

- **After water treatments were imposed**

After water treatments commenced, overall mean plant DW did not change between harvests (Table 3.2), although by Harvest 4, Control plants were 30 % heavier than Stressed plants (Table 3.3). The harvest x water treatment interaction was significant ($P<0.05$) because Control plants showed an increase in DW from Harvest 2 to Harvest 4 ($P<0.05$) while DW of Stressed plants tended to decrease ($P>0.05$) during the same period.

The differences between harvests indicate that while the percentage of DW as leaf blade decreased with time, the percentage of DW as sheath and dead tissue increased ($P<0.05$, Table 3.2). MK showed a higher percentage of DW as leaf blade than GA, and the latter cultivar higher than EP ($P<0.05$,Table 3.2). The percentage of DW as sheath was lower for MK than for EP ($P<0.05$), while GA had an intermediate percentage ($P>0.05$). Consequently, the percentage of DW as dead tissue was lower in MK compared with the other cultivars ($P<0.05$). Stressed plants had a higher percentage of dead tissue and a lower percentage of leaf blade than Control plants ($P<0.05$). Similarly, there was a significant harvest x water treatment interaction for the proportion of DW as dead tissue ($P<0.05$, Table 3.3) showing that while this shoot component did not change significantly with time in Control plants, it increased significantly in Stressed plants. This response was expected since the reduction in leaf area has been suggested as one of the most efficient means for the plant to conserve water (Passioura, 1982).

The duration of water treatments was insufficient to expect a large change in tiller number per plant between harvests ($P>0.05$, Table 3.2). However, the higher number of tillers per plant in MK seems to be characteristic of Mediterranean tall fescues. For instance, Morgan (1964) observed that Mediterranean fescues had more tillers than the British cultivar S170 under similar environmental conditions to those in the present experiment (high air temperature, high light intensity and long days). No statistically significant differences were found in tiller number between Control and Stressed treatments and none of the interactions was significant ($P>0.05$).

Table 3.2. Main effect means for plant dry weight (DW) and percentages of shoot DW as leaf blade, sheath and dead tissue, and mean tiller number per plant after imposition of water treatments.

Harvest	**Plant DW (g)**	**% DW as leaf blade**	**% DW as sheath**	**% DW as dead tissue**	**Tiller number**
2	6.69	60.7 (51.2) **a**[1]	37.1 (37.5) **b**	2.2 (7.5) **c**	19.7
3	7.92	55.5 (48.2) **b**	40.4 (39.4) **a**	4.1 (10.4) **b**	19.7
4	7.23	53.4 (46.9) **b**	40.7 (39.6) **a**	5.9 (13.1) **a**	19.9
s.e.[2]	0.42	(0.7)	(0.7)	(0.6)	1.1
Cultivar					
MK	4.72 **b**	60.4 (51.1) **a**	37.2 (37.5) **b**	2.4 (7.4) **b**	24.3 **a**
GA	8.69 **a**	56.7 (48.9) **b**	38.9 (38.6) **ab**	4.4 (11.2) **a**	18.8 **b**
EP	8.43 **a**	52.5 (46.5) **c**	42.1 (40.4) **a**	5.4 (12.4) **a**	16.1 **b**
s.e.	0.42	(0.7)	(0.7)	(0.6)	1.1
Water					
Control	8.29 **a**	58.9 (50.2) **a**	39.1 (38.7)	2.0 (7.5) **b**	20.8
Stressed	6.26 **b**	54.1 (47.4) **b**	39.7 (39.0)	6.2 (13.2) **a**	18.7
s.e.	0.34	(0.6)	(0.5)	(0.5)	0.9
Interactions (P=)					
Cult. * Water	0.795	0.350	0.797	0.232	0.798
Harvest * Cult.	0.320	0.757	0.932	0.637	0.196
Harvest * Water	**0.017**	0.121	0.236	**0.004**	0.845
Harv.*Cult.*Water	0.506	0.758	0.809	0.138	0.358

Data within brackets are arcsin square root transformed percentages.

[1] Letters indicate groupings of means that are significantly different at $P<0.05$ within harvests, cultivars or water treatment.

[2] Standard error.

Table 3.3. Plant dry weight (DW) and percentage of plant DW as dead tissue for harvests and water treatments.

	Plant DW (g)	
Harvest	**Control**	**Stressed**
2	6.68 **b**[1]	6.70 **b**
3	9.62 **a**	6.23 **b**
4	8.58 **a**	5.87 **b**
s.e.[2]	0.60	

	% DW as dead tissue	
Harvest	**Control**	**Stressed**
2	1.4 (6.4) **c**	2.9 (8.6) **c**
3	1.8 (7.2) **c**	6.4 (13.5) **b**
4	2.7 (8.8) **c**	9.1 (17.3) **a**
s.e.	(0.9)	

Data within brackets are arcsin square root transformed percentages.

[1] Letters indicate groupings of means that are significantly different at P<0.05.

[2] Standard error.

3.4.1.2. Root system characteristics

- **Before water treatments were imposed**

The salient feature of root measurements made prior to imposing water treatments was that, independently of the differences in shoot dry weight, the three cultivars had similar total root mass (Table 3.4). However, the amount and proportion of total root mass in the uppermost stratum (0-75 mm) was lower for MK (P<0.05, Table 3.4 and Appendix 3.2).

While no differences were found in root length between cultivars (P>0.05, Table 3.4), GA showed the lowest percentage of total root length (P<0.05, Appendix 3.2) in the lowest stratum (below 300 mm).

The coarse root length of MK was lower than for the other cultivars in the uppermost stratum (P<0.05, Table 3.4). However, MK showed a higher percentage of total root length as coarse root (P<0.05, Appendix 3.2) in all strata below 75 mm.

No differences were found between cultivars in mean root diameter for any of the strata, nor for the average mean root diameter (P>0.05, Table 3.4). However, in the uppermost stratum, the mean surface of GA was higher than that of MK (P<0.05, Table 3.4), while EP had an intermediate value

Table 3.4. Root mass, root length, coarse root length, mean root diameter and root surface area for the cultivars MK, GA and EP before water treatments were imposed (Harvest 1).

	Root mass (g OM)				
Cultivar	**0-75 mm**	**75-150 mm**	**150-300 mm**	**below 300 mm**	**Total**
MK	1.37 **b**[1]	0.87	1.15	2.11	5.50
GA	2.07 **a**	1.01	1.08	1.84	6.00
EP	1.90 **a**	0.97	1.10	2.34	6.31
s.e.[2]	0.06	0.04	0.12	0.24	0.36

	Root length (m)				
Cultivar	**0-75 mm**	**75-150 mm**	**150-300 mm**	**below 300 mm**	**Total**
MK	94.0	111.9	123.6	227.0	556.4
GA	218.9	165.4	162.2	229.0	775.5
EP	166.6	116.0	142.7	306.9	732.1
s.e.	33.7	21.4	23.6	42.8	109.7

	Coarse root length (m)				
Cultivar	**0-75 mm**	**75-150 mm**	**150-300 mm**	**below 300 mm**	**Total**
MK	10.4 **b**	7.0	7.6	13.1	38.3
GA	19.3 **a**	6.6	7.6	8.8	42.4
EP	14.0 **ab**	7.3	5.6	7.9	34.9
s.e.	1.6	1.2	0.7	1.9	2.7

	Mean root diameter (mm)				
Cultivar	**0-75 mm**	**75-150 mm**	**150-300 mm**	**below 300 mm**	**Entire profile**
MK	0.52	0.36	0.38	0.40	0.40
GA	0.40	0.32	0.33	0.36	0.35
EP	0.44	0.37	0.35	0.36	0.38
s.e.	0.05	0.02	0.02	0.02	0.02

	Root surface area (m^2)				
Cultivar	**0-75 mm**	**75-150 mm**	**150-300 mm**	**below 300 mm**	**Total**
MK	0.14 **b**	0.12	0.15	0.27	0.69
GA	0.26 **a**	0.16	0.17	0.26	0.85
EP	0.22 **ab**	0.13	0.16	0.33	0.85
s.e.	0.02	0.01	0.02	0.04	0.09

[1] Letters indicate groupings of means that are significantly different at $P<0.05$.

[2] Standard error.

- **After water treatments were imposed**

- Harvest effect

After water treatments were imposed, an increase in total root mass ($P<0.05$) was found between harvests, reflecting the increase measured in each of the three lower strata ($P<0.05$, Table 3.5). Consequently, the percentage of total root mass recovered in the uppermost stratum at Harvest 4 was significantly lower ($P<0.05$, Appendix 3.3). Conversely, total root length, root length of the four strata and their percentages showed no differences between harvests ($P>0.05$, Table 3.6 and Appendix 3.5).

The coarse root length increased significantly with time in the three lowest strata ($P<0.05$,Table 3.7). Similarly, the percentage of coarse root in each stratum also showed a trend to increase with time (Appendix 3.8). In addition, mean root diameter, in the strata between 75 and 300 mm (Table 3.8), and total root surface area (Table 3.11) increased significantly ($P<0.05$) with time.

- Cultivar effect

As for Harvest 1, no significant differences were found between cultivars in total root mass ($P>0.05$, Table 3.5). However, the total root length was the lowest for MK and the highest for EP, while GA showed an intermediate value ($P<0.05$, Table 3.6). It was found that the cultivars differed in the distribution and characteristics of their root systems. GA tended to have a shallow root system, concentrating a greater root weight (Table 3.5 and Appendix 3.3) and root length (Table 3.6 and Appendix 3.5) in the uppermost stratum (0-75 mm). For the same stratum, GA also showed higher coarse root length (Table 3.7) and root surface area (Table 3.11) than MK and EP. Another characteristic of GA was a higher coarse root length than the other cultivars below 300 mm ($P<0.05$, Table 3.7). Coincidentally, in the same stratum, mean root diameter of GA showed a trend to increase with time (Table 3.9).

In contrast to GA, EP had the highest root mass, length and surface area below 300 mm ($P<0.05$, Table 3.5 and 3.6, and Appendices 3.3 and 3.5). Though in the lower stratum, root length was not different from GA ($P>0.05$) but the percentage of total root length was ($P<0.05$, Appendix 3.5). Percentage of total root length in the uppermost stratum, was lower for EP than for the other two cultivars (Appendix 3.5). In contrast with MK and GA, mean root diameter tended to decrease with time in EP below 300 mm (Table 3.9).

MK had the greater proportion of its root mass and length in the strata between 75 and 300 mm (Appendices 3.3 and 3.5). Despite this, MK still had the lowest actual root length in the 75-150 mm stratum. Moreover, in the lowest three strata the Mediterranean cultivar had the highest percentage of coarse root length and the highest mean root diameter ($P<0.05$, Appendix 3.8 and Table 3.8). The total mean root diameter was also higher in MK than in GA or EP ($P<0.05$, Table 3.8), and consequently, MK had the lowest total root surface area ($P<0.05$, Table 3.11).

The percentage of total root length showed a significant harvest x cultivar interaction for two of the strata ($P<0.05$). In the 75-150 mm stratum EP had an unexpectedly high value in Harvest 3 (Appendix 3.6). The interaction reflected an increase in root length percentage below 300 mm, from Harvest 2 to Harvest 3 in MK, while the other cultivars did not show significant change in the root length percentage between harvests (Appendix 3.6).

- Water treatment effect

While total root mass and root mass in the two uppermost strata were higher in Control plants than in Stressed ones ($P<0.05$, Table 3.5), the percentage of total root mass in the lowest stratum (below 300 mm) was higher in Stressed plants (Appendix 3.3). In the latter stratum, it was found that the percentage of total root mass tended to increase in MK and AG, but the opposite took place in EP (Appendix 3.4). In agreement with the trend showed by root mass, the total root length and percentage of root length in the uppermost stratum was higher in Control Plants (Table 3.6 and Appendix 3.5). In the 0-75 mm and below 300 mm strata a significant cultivar x water treatment interaction was found for the root length percentage ($P<0.05$). For the uppermost stratum, the root length percentage of MK and EP plants tended to decrease under water deficit (Appendix 3.7). For the lowest stratum, GA plants showed a greater increase in the percentage of root length in the Stressed treatment, compared with Control, than the other cultivars (Appendix 3.7).

The coarse root length and surface area were higher in the two uppermost strata in Control plants ($P<0.05$, Tables 3.7 and 3.11). In the 150-300 mm stratum, the percentage of coarse root length tended to increase in MK plants, to decrease in EP, and remain unchanged in GA (Appendix 3.9). The mean root diameter was higher in Stressed plants in the uppermost stratum ($P<0.05$, Table 3.8). The average root diameter of the four strata, tended to decrease in EP, but did not change in the other cultivars (Table 3.10).

The higher root diameter of Stressed plants in the uppermost stratum was consistent with results of Evans (1973), who reported that high temperature and low soil water content, which usually occur near the soil surface, promote root aging. This process was characterised by a thickened endodermis and by deposition of suberin on the epidermis resulting in an increased root diameter. Although at high water availability water uptake occurs mainly in the youngest parts of the roots, the opposite occurs under low water availability (Evans, 1973). Moreover, the changes associated with root aging seem to limit the loss of water to the surrounding medium (Cruz *et al.*, 1992).

Table 3.5. Total root mass and root mass recovered from the four soil strata after water treatments were imposed.

	Root mass (g OM)				
Harvest	**0-75 mm**	**75-150 mm**	**150-300 mm**	**below 300 mm**	**Total**
2	2.00	1.01 **b**[1]	1.31 **b**	2.36 **b**	6.68 **b**
3	2.26	1.18 **ab**	1.50 **b**	2.56 **b**	7.50 **b**
4	2.47	1.38 **a**	1.85 **a**	3.29 **a**	8.99 **a**
s.e.[2]	0.13	0.07	0.11	0.13	0.38
Cultivar					
MK	1.84 **b**	1.14	1.56	2.46 **b**	7.00
GA	2.74 **a**	1.23	1.61	2.52 **b**	8.10
EP	2.16 **b**	1.19	1.49	3.22 **a**	8.06
s.e.	0.13	0.07	0.11	0.13	0.38
Water					
Control	2.40 **a**	1.31 **a**	1.67	2.86	8.25 **a**
Stressed	2.09 **b**	1.06 **b**	1.43	2.61	7.19 **b**
s.e.	0.11	0.06	0.09	0.11	0.31
Interactions (P=)					
Cult. * Water	0.126	0.305	0.153	0.720	0.352
Harvest * Cult.	0.937	0.916	0.675	0.289	0.695
Harvest * Water	0.359	0.081	0.147	0.088	0.111
Harvest*Cult.*Water	0.872	0.121	0.496	0.535	0.593

[1] Letters indicate groupings of means that are significantly different at $P<0.05$ within harvests, cultivars or water treatment.
[2] Standard error.

Table 3.6. Total root length and root length recovered from the four soil strata after water treatments were imposed.

	Root length (m)				
Harvest	**0-75 mm**	**75-150 mm**	**150-300 mm**	**below 300 mm**	**Total**
2	171.3	122.2	166.2	273.2	732.9
3	194.2	125.9	159.0	281.1	760.3
4	175.0	126.9	187.7	515.3	1004.9
s.e.[1]	13.9	9.3	16.1	123.4[§]	124.6[§]
Cultivar					
MK	143.3 **b**[2]	104.5 **b**	140.7	179.2 **b**	567.6 **b**
GA	229.4 **a**	136.0 **a**	191.3	319.0 **ab**	875.7 **ab**
EP	167.8 **b**	134.5 **a**	181.0	571.5 **a**	1054.8 **a**
s.e.	13.9	9.3	16.1	123.4	124.6
Water					
Control	203.2 **a**	135.6	175.9	299.4	814.2
Stressed	157.2 **b**	114.3	166.0	413.6	851.1
s.e.	11.4	7.6	13.1	100.7[§]	101.7[§]
Interactions (P=)					
Cult. * Water	0.324	0.088	0.204	0.711	0.653
Harvest * Cult.	0.701	0.883	0.915	0.241	0.996
Harvest * Water	0.444	0.837	0.746	0.292	0.609
Harvest*Cult.*Water	0.691	0.303	0.458	0.435	0.271

[1] Standard error.

[§] Mean standard error.

[2] Letters indicate groupings of means that are significantly different at $P<0.05$ within harvests, cultivars or water treatment.

Table 3.7. Total coarse root length and coarse root length recovered from the four soil strata after water treatments were imposed.

	Coarse root length (m)				
Harvest	**0-75 mm**	**75-150 mm**	**150-300 mm**	**below 300 mm**	**Total**
2	11.0	5.9 **b**[1]	7.2 **b**	9.3 **b**	33.5 **b**
3	11.7	6.8 **ab**	6.8 **b**	8.4 **b**	33.8 **b**
4	14.0	8.2 **a**	10.9 **a**	25.3 **a**	58.4 **a**
s.e.[2]	0.9	0.5	0.7	5.0	5.4[§]
Cultivar					
MK	10.2 **b**	7.3	9.1	13.9	40.6
GA	15.4 **a**	7.0	8.0	10.2	40.6
EP	11.2 **b**	6.6	7.8	18.9	44.4
s.e.	0.9	0.5	0.7	5.0	5.4
Water					
Control	13.4 **a**	7.8 **a**	8.6	11.4	41.2
Stressed	11.1 **b**	6.2 **b**	8.0	17.3	42.6
s.e.	0.8	0.4	0.6	4.1	4.4[§]
Interactions (P=)					
Cult. * Water	0.377	0.177	0.159	0.339	0.542
Harvest * Cult.	0.455	0.596	0.737	0.569	0.552
Harvest * Water	0.646	0.109	0.394	0.304	0.120
Harvest*Cult.*Water	0.199	0.155	0.617	0.240	0.217

[1] Letters indicate groupings of means that are significantly different at P<0.05 within harvests, cultivars or water treatment.

[2] Standard error.

[§] Mean standard error.

Table 3.8. Mean diameter of roots recovered from the four soil strata and for the entire profile after water treatments were imposed .

	Mean root diameter (mm)				
Harvest	**0-75 mm**	**75-150 mm**	**150-300 mm**	**below 300 mm**	**Entire profile**
2	0.44	0.37 **b**[1]	0.36 **b**	0.40	0.39
3	0.44	0.40 **ab**	0.39 **a**	0.38	0.40
4	0.49	0.42 **a**	0.40 **a**	0.41	0.42
s.e.[2]	0.01	0.01	0.01	0.01	0.01
Cultivar					
MK	0.47	0.43 **a**	0.42 **a**	0.48 **a**	0.45 **a**
GA	0.44	0.38 **b**	0.36 **b**	0.36 **b**	0.38 **b**
EP	0.46	0.38 **b**	0.37 **b**	0.36 **b**	0.38 **b**
s.e.	0.01	0.01	0.01	0.01	0.01
Water					
Control	0.44 **b**	0.40	0.39	0.40	0.40
Stressed	0.47 **a**	0.40	0.38	0.39	0.40
s.e.	0.01	0.01	0.01	0.01	0.01
Interactions (P =)					
Cult. * Water	0.700	0.063	0.056	0.144	**0.033**
Harvest * Cult.	0.321	0.705	0.423	**0.031**	0.053
Harvest * Water	0.708	0.426	0.451	0.549	0.256
Harv.*Cult.*Water	0.747	0.745	0.361	0.090	0.254

[1] Letters indicate groupings of means that are significantly different at $P<0.05$ within harvests, cultivars or water treatment.

[2] Standard error.

Table 3.9. Mean diameter of roots recovered below 300 mm stratum, averaged for cultivars and harvests, after the application of water treatments.

	Mean root diameter of the below 300 mm stratum		
Harvest	**MK**	**GA**	**EP**
2	0.50 **a**[1]	0.33 **c**	0.37 **bc**
3	0.42 **b**	0.36 **c**	0.37 **bc**
4	0.52 **a**	0.38 **bc**	0.34 **c**
s.e.[2]		0.02	

[1] Letters indicate groupings of means that are significantly different at P<0.05.
[2] Mean standard error.

Table 3.10. Mean diameter of roots recovered from the entire profile, averaged for cultivars and water treatments.

	Average mean root diameter	
Cultivar	**Control**	**Stressed**
MK	0.43 **a**[1]	0.47 **a**
GA	0.39 **c**	0.37 **c**
EP	0.40 **bc**	0.36 **c**
s.e.[2]	0.01	

[1] Letters indicate groupings of means that are significantly different at P<0.05.
[2] Mean standard error.

Table 3.11. Total root surface area and root surface area for the four soil strata after the imposition of water treatments.

	Root surface area (m^2)				
Harvest	**0-75 mm**	**75-150 mm**	**150-300 mm**	**below 300 mm**	**Total**
2	0.23	0.14	0.18	0.31	0.87 **b**[1]
3	0.26	0.15	0.19	0.33	0.94 **b**
4	0.26	0.16	0.23	0.45	1.13 **a**
s.e.[2]	0.01	0.01	0.02	0.04§	0.05
Cultivar					
MK	0.20 **b**	0.14	0.18	0.26 **b**	0.79 **b**
GA	0.31 **a**	0.16	0.22	0.35 **b**	1.05 **a**
EP	0.24 **b**	0.16	0.20	0.49 **a**	1.11 **a**
s.e.	0.01	0.01	0.02	0.04	0.05
Water					
Control	0.27 **a**	0.17 **a**	0.21	0.36	1.02
Stressed	0.22 **b**	0.14 **b**	0.19	0.37	0.94
s.e.	0.01	0.01	0.01	0.03§	0.04
Interactions (P=)					
Cult. * Water	0.172	0.186	0.202	0.686	0.072
Harvest * Cult.	0.874	0.915	0.880	0.363	0.707
Harvest * Water	0.298	0.344	0.380	0.133	0.668
Harvest*Cult.*Water	0.758	0.196	0.502	0.331	0.288

[1] Letters indicate groupings of means that are significantly different at P<0.05 within harvests, cultivars or water treatment.
[2] Standard error.
§ Mean standard error.

3.4.1.3. Root : shoot ratio

- Before water treatments were imposed

The root : shoot ratio before the water treatments were imposed (Harvest 1) was higher for MK than the other cultivars (P<0.05, Table 3.12).

Table 3.12. Root : shoot ratio (g OM/g DM) for the cultivars MK, GA and EP before water treatments were imposed (Harvest 1).

Cultivar	Root : shoot ratio
MK	0.40 **a**[1]
GA	0.22 **b**
EP	0.22 **b**
s.e.[2]	0.02

[1] Letters indicate groupings of means that are significantly different at P<0.05.
[2] Standard error.

- After water treatments were imposed

The root : shoot ratio after water treatments were applied was significantly higher for Harvest 4 than for Harvests 2 or 3 (P<0.05, Table 3.13). Similar to the trend observed in Harvest 1, MK had a significantly higher root : shoot ratio (P<0.05, Table 3.13). On the other hand, Stressed plants also had a higher ratio than Control plants (P<0.05). This response seemed to result from a greater reduction in shoot growth rate than in root growth rate in Stressed plants. This agreed with results of Brouwer (1983) and Passioura (1982) who found that under drought, leaf elongation was reduced more than photosynthesis, leaving an increased proportion of assimilate available for the roots.

Table 3.13. Mean root : shoot ratio (g OM/g DM) after water treatments were imposed .

Harvest	**Root : shoot ratio**
2	0.29 **b**[1]
3	0.27 **b**
4	0.33 **a**
s.e.[2]	0.02
Cultivar	
MK	0.40 **a**
GA	0.24 **b**
EP	0.25 **b**
s.e.	0.02
Water	
Control	0.27 **b**
Stressed	0.32 **a**
s.e.	0.01
Interactions (P=)	
Cult. * Water	0.231
Harvest * Cultivar	0.333
Harvest * Water	0.408
Harvest*Cultivar*Water	0.912

[1] Letters indicate groupings of means that are significantly different at P<0.05 within harvest, cultivars or water treatments.
[2] Standard error.

3.4.2. Plant water relations

At Harvest 1, before the water treatments were imposed, no differences were found between cultivars for RWC, Ψ_w, Ψ_o, $\Psi_{o(f)}$, and Ψ_p (P>0.05, Table 3.14).

Table 3.14. Relative water content (RWC), water potential (Ψ_w), osmotic potential (Ψ_o), osmotic potential at full turgor ($\Psi_{o(f)}$) and pressure potential (Ψ_p) for the cultivars MK, GA and EP before water treatments were imposed (Harvest 1).

Cultivar	**RWC (%)**	**Ψ_w (MPa)**	**Ψ_o (MPa)**	**$\Psi_{o(f)}$ (MPa)**	**Ψ_p (MPa)**
MK	96.6 (80.2)	-1.42	-2.04	-1.92	0.62
GA	95.7 (78.9)	-1.52	-2.21	-2.32	0.70
EP	90.9 (72.8)	-1.75	-2.23	-2.32	0.47
s.e.[1]	(1.9)	0.17	0.15	0.17	0.23

Data within brackets are arcsin square root transformed percentages.
[1] Standard error.

When the plants were exposed to the different water treatments, a significant decrease in RWC, Ψ_w and Ψ_o was observed from Harvest 2 to Harvest 4 ($P<0.05$, Table 3.15). MK showed a higher RWC, Ψ_w, Ψ_o and $\Psi_{o(f)}$ than GA and EP ($P<0.05$, Table 3.15). A significant harvest x cultivar interaction was found for Ψ_o ($P<0.05$, Table 3.16). The latter was because the decrease in Ψ_o with time was higher for GA and EP than for MK.

As expected, Stressed plants had a lower RWC, Ψ_w, and Ψ_o ($P<0.05$, Table 3.15), but no differences were found between water treatments for $\Psi_{o(f)}$ ($P>0.05$, Table 3.15). Turgor of Stressed plants was lower than that of Control plants ($P<0.05$, Table 3.15).

The harvest x water (Table 3.17) and cultivar x water (Table 3.18) interactions were significant for RWC, Ψ_w, Ψ_o and Ψ_p ($P<0.05$). The harvest x water interaction for RWC and Ψ_o was because these variables showed a slight decrease in Control plants from Harvest 2 to Harvest 4, and a sharp decrease in Stressed plants during this period (Table 3.17). A similar response was observed for Ψ_w in Stressed plants, but no differences between harvests were observed in Control plants (Table 3.17). As in Control plants Ψ_w did not change between harvests and Ψ_o decreased, Ψ_p increased between harvests, but in line with the rest of the variables mentioned above, Ψ_p decreased in Stressed plants (Table 3.17). These changes in Stressed plants were expected, given that they had not received water since the day prior to Harvest 1, and reflected decreased soil water availability with time. The decrease in RWC and Ψ_o of Control plants with time (Table 3.17) raised the possibility that the plants were receiving insufficient water by Harvest 4. However, as no differences were observed in Ψ_o, and Ψ_p indeed showed an increase, any stress on Control plants cannot have been very severe. The decrease in Ψ_o with time, in Stressed plants agreed with findings of West *et al.* (1990) and White *et al.* (1992b) in that leaf blades of tall fescue had adjusted osmotically in response to water deficits. However, as in the present experiment the $\Psi_{o(f)}$ did not decrease significantly in Stressed plants, the decrease in Ψ_o might have been due to a loss of water, and thus, to a concentration of solutes already present in the cell, more than to a genuine accumulation of solutes (i.e. osmotic adjustment).

The cultivar x water interaction (Table 3.18) observed for RWC, Ψ_w and Ψ_o was due to a greater decrease in the value of these variables in GA and EP plants than in MK between Control and Stressed water treatments. Interestingly, MK showed a higher value than the other cultivars for these variables in both water treatments, though in the Control treatment RWC and Ψ_w of MK were similar to GA plants. Moreover, in MK-Stressed plants, the value of Ψ_o was similar to MK-Control plants, the value of RWC was similar to GA- and EP-Control plants, and the value of Ψ_w was similar to EP-Control plants. The latter finding suggested that MK-Stressed plants had succeeded in postponing the water deficit in relation to the other cultivars due to MK having small plant size and a high root : shoot ratio. Both these factors, would tend to reduce the level of physiological stress on MK plants compared with GA or EP plants. In the case of Ψ_p, the cultivar x water treatment interaction was significant because GA showed a higher value ($P<0.05$) than MK and EP in Control plants, but a lower value similar to the latter cultivars in Stressed plants, consequently, the decrease in Ψ_p between Control and Stressed water treatments was higher for GA plants (Table 3.18).

The RWC measurements were taken in plants that had been watered the previous morning and had been exposed to temperatures of around 37 °C during the day. Therefore, at the time the samples were taken, the water content of the pots had reached the daily minimum. This partly explained why the RWC of Control plants was always below 100 % even though the plants had been covered overnight with a plastic bag and had been receiving amounts of water calculated to ensure that pots were as close as possible to soil field capacity.

Quadratic regressions were fitted for the response of Ψ_w, Ψ_o, $\Psi_{o(f)}$ and Ψ_p to RWC. Graphing the quadratic curves in this case has led to fits which had points of high leverage at lower values of RWC. Because of this, the curves relied heavily on the placement of only one or two points, were therefore difficult to interpret, and will not be presented here. Nevertheless, while, Ψ_w, Ψ_o, $\Psi_{o(f)}$ and Ψ_p, increased with increasing RWC, there was no indication of any difference between cultivars. This latter observation was confirmed in Chapter 5 for MK and EP cultivars (Fig. 5.14).

Table 3.15. Relative water content (RWC), water potential (Ψ_w), osmotic potential (Ψ_o), osmotic potential at full turgor ($\Psi_{o(f)}$) and pressure potential (Ψ_p) after water treatments were imposed.

Harvest	**RWC (%)**	**Ψ_w (MPa)**	**Ψ_o (MPa)**	**$\Psi_{o(f)}$ (MPa)**	**Ψ_p (MPa)**
2	91.6 (75.6) **a**[1]	-1.95 **a**	-2.47 **a**	-2.10	0.52
3	82.6 (68.2) **b**	-2.57 **b**	-3.19 **b**	-2.08	0.62
4	72.1 (60.8) **c**	-3.16 **c**	-3.72 **b**	-2.31	0.56
s.e.[2]	(1.3)	0.18[§]	0.21[§]	0.11	0.07
Cultivar					
MK	93.1 (77.3) **a**	-1.83 **a**	-2.31 **a**	-1.91 **a**	0.48
GA	79.0 (65.4) **b**	-2.77 **b**	-3.40 **b**	-2.27 **b**	0.62
EP	74.2 (61.8) **b**	-3.07 **b**	-3.67 **b**	-2.31 **b**	0.60
s.e.	(1.3)	0.18	0.21	0.11	0.07
Water					
Control	94.5 (77.6) **a**	-1.46 **a**	-2.33 **a**	-2.12	0.88 **a**
Stressed	69.7 (58.7) **b**	-3.66 **b**	-3.92 **b**	-2.21	0.26 **b**
s.e.	(1.1)	0.14[§]	0.17[§]	0.09	0.06
Interactions (P=)					
Cult. * Water	**0.001**	**0.000**	**0.011**	0.624	**0.003**
Harvest * Cult.	0.323	0.491	**0.042**	0.337	0.907
Harvest * Water	**0.001**	**0.000**	**0.005**	0.555	**0.000**
Harvest*Cult.*Wat.	0.675	0.599	0.774	0.884	0.882

Data within brackets are arcsin square root transformed percentages.

[1] Letters indicate groupings of means that are significantly different at P<0.05 within harvest, cultivars or water treatments.

[2] Standard error.

[§] Mean standard error.

Table 3.16. Osmotic potential (Ψ_o) averaged for cultivars and harvests after water treatments were imposed.

	Ψ_o (MPa)		
Harvest	**MK**	**GA**	**EP**
2	-1.93 **a**[1]	-2.65 **ab**	-2.82 **ab**
3	-2.21 **ab**	-3.31 **bc**	-4.05 **c**
4	-2.80 **b**	-4.23 **c**	-4.15 **c**
s.e.[2]		0.37	

[1] Letters indicate groupings of means that are significantly different at $P<0.05$.
[2] Mean standard error.

Table 3.17. Relative water content (RWC), water potential (Ψ_w), osmotic potential (Ψ_o) and pressure potential (Ψ_p) averaged for harvests and water treatments.

	RWC (%)	
Harvest	**Control**	**Stressed**
2	97.6 (81.6) **a**[1]	85.6 (69.7) **c**
3	94.0 (76.7) **ab**	71.2 (59.6) **d**
4	92.0 (74.6) **bc**	52.3 (47.0) **e**
s.e.[2]	(1.8)	

	Ψ_w (MPa)	
Harvest	**Control**	**Stressed**
2	-1.46 **a**	-2.44 **b**
3	-1.49 **a**	-3.64 **c**
4	-1.42 **a**	-4.90 **d**
s.e.	0.25[§]	

	Ψ_o (MPa)	
Harvest	**Control**	**Stressed**
2	-2.13 **a**	-2.81 **abc**
3	-2.31 **a**	-4.07 **cd**
4	-2.57 **b**	-4.88 **d**
s.e.	0.30[§]	

	Ψ_p (MPa)	
Harvest	**Control**	**Stressed**
2	0.67 **bc**	0.38 **c**
3	0.82 **b**	0.42 **c**
4	1.15 **a**	-0.02 **d**
s.e.	0.10	

Data within brackets are arcsin square root transformed percentages.
[1] Letters indicate groupings of means that are significantly different at $P<0.05$.
[2] Standard error.
[§] Mean standard error.

Table 3.18. Relative water content (RWC), water potential (Ψ_w), osmotic potential (Ψ_o) and pressure potential (Ψ_p) averaged for cultivars and water treatments.

	RWC (%)	
Cultivar	**Control**	**Stressed**
MK	97.5 (81.5) **a**[1]	88.72 (73.0) **b**
GA	94.5 (77.2) **ab**	63.61 (53.6) **c**
EP	91.6 (74.1) **b**	56.77 (49.6) **c**
s.e.[2]	(1.8)	

	Ψ_w (MPa)	
Cultivar	**Control**	**Stressed**
MK	-1.31 **a**	-2.35 **b**
GA	-1.38 **a**	-4.17 **c**
EP	-1.68 **b**	-4.46 **c**
s.e.	0.25[§]	

	Ψ_o (MPa)	
Cultivar	**Control**	**Stressed**
MK	-1.96 **a**	-2.66 **ab**
GA	-2.53 **b**	-4.26 **c**
EP	-2.51 **b**	-4.84 **c**
s.e.	0.30[§]	

	Ψ_p (MPa)	
Cultivar	**Control**	**Stressed**
MK	0.65 **b**	0.31 **c**
GA	1.15 **a**	0.09 **c**
EP	0.83 **b**	0.38 **c**
s.e.	0.10	

Data within brackets are arcsin square root transformed percentages.

[1] Letters indicate groupings of means that are significantly different at $P<0.05$.

[2] Standard error.

[§] Mean standard error.

3.4.3. Stomatal resistance and transpiration

- **Before water treatments were imposed**

Stomatal resistance and transpiration rate for both leaf blade surfaces at Harvest 1 are shown in Table 3.19. The abaxial leaf surface stomatal resistance was the lowest in MK and the highest in EP ($P<0.05$), while GA was intermediate. Stomatal resistance on the adaxial leaf surface was around half the values observed for the abaxial surface which also had a lower density of stomata. The abaxial leaf surface had 35-55 % the stomata number of the adaxial surface for the cultivars studied. Although the same trend was shown for both leaf surfaces, no significant differences in stomatal resistance ($P>0.05$) were found between cultivars on the adaxial surface.

The transpiration rate showed higher values on the adaxial surface consistent with the lower stomatal resistance (Table 3.19) and higher stomata density observed. Although for both leaf surfaces the transpiration rate of MK was higher than for the other cultivars, only for the abaxial surface this difference became significant ($P<0.05$). These results show that with high water availability, MK plants tended to have a lower stomatal resistance and, consequently, a higher transpiration rate than GA or EP.

Table 3.19. Stomatal resistance and transpiration for the cultivars MK, GA and EP before water treatments were imposed (Harvest 1).

	Stomatal resistance ($s\ cm^{-1}$)			Transpiration ($\mu g\ cm^{-2}\ s^{-1}$)	
Cultivar	**abaxial**	**adaxial**	**total**	**abaxial**	**adaxial**
MK	2.81 **c**[1]	1.70	1.03	6.63 **a**	9.50
GA	5.89 **b**	2.85	1.85	3.45 **b**	6.93
EP	8.79 **a**	3.44	2.34	2.30 **b**	6.66
s.e.	0.80	0.69	0.34	0.59	1.55

Abaxial: lower leaf surface, **adaxial**: upper leaf surface, **total**: $(abaxial^{-1}+adaxial^{-1})^{-1}$.

[1] Letters indicate groupings of means that are significantly different at $P<0.05$ within the same column.

[2] Standard error.

- **After water treatments were imposed**

After water treatments were imposed, the same ratio between stomatal resistance values of both sides of the leaf as for Harvest 1 was observed. The stomatal resistance increased from Harvest 2 to Harvest 4 ($P<0.05$, Table 3.20). MK stomatal resistance was lower than for EP and AG ($P<0.05$, Table 3.20). On the other hand, as expected (Turner, 1986; Farquhar *et al.*, 1989; Davies and Zhang, 1991) Stressed plants had a significantly higher stomatal resistance than Control plants ($P<0.05$, Table 3.20) on both leaf surfaces, and consequently the same response was found for the total leaf resistance.

The cultivar x water interaction was significant for the stomatal resistance of both leaf surfaces and, consequently for the total stomatal resistance ($P<0.05$, Table 3.21). The three interactions were due to a lower difference between the stomatal resistance of Control and Stressed plants of MK cultivar compared with the differences shown by the other cultivars. Moreover, the stomatal resistance of both surfaces in MK-Stressed plants was not statistically different from the stomatal resistance of Control plants of other cultivars.

The transpiration rate of both surfaces decreased with time because of the increased stomatal resistance ($P<0.05$, Table 3.20). MK transpiration rate was higher than GA or EP for both leaf surfaces ($P<0.05$, Table 3.20). Stressed plants showed lower transpiration rates on both leaf surfaces ($P<0.05$). The harvest x water treatment interaction was significant for the transpiration rate of both surfaces ($P<0.05$, Table 3.22). This was because, while no differences in the transpiration rate were found between harvests in Stressed plants, Control plants showed a decrease in the lower leaf surface from Harvest 3 to Harvest 4, and in the adaxial leaf surface from Harvest 2 to Harvest 3.

Table 3.20. Abaxial leaf surface, adaxial leaf surface and total stomatal resistance, and abaxial and adaxial leaf surfaces transpiration rate after water treatments were imposed.

	Stomatal resistance ($s\ cm^{-1}$)			**Transpiration ($\mu g\ cm^{-2}\ s^{-1}$)**	
Harvest	**abaxial**	**adaxial**	**total**	**abaxial**	**adaxial**
2	18.27 **b**[1]	8.05 **b**	5.14 **b**	2.80 **a**	4.77 **a**
3	22.57 **ab**	11.34 **ab**	6.98 **b**	2.30 **a**	3.10 **b**
4	31.62 **a**	15.44 **a**	9.30 **a**	1.32 **b**	2.47 **b**
s.e.[2]	3.44[§]	1.46[§]	0.75[§]	0.29[§]	0.41
MK	12.55 **b**	6.58 **b**	3.73 **b**	3.16 **a**	4.66 **a**
GA	34.61 **a**	15.22 **a**	9.67 **a**	1.57 **b**	2.46 **b**
EP	25.31 **a**	13.02 **a**	8.01 **a**	1.70 **b**	3.23 **b**
s.e.	3.44	1.46	0.75	0.29	0.41
Control	7.65 **b**	4.17 **b**	2.29 **b**	3.49 **a**	5.39 **a**
Stressed	40.66 **a**	19.05 **a**	11.98 **a**	0.79 **b**	1.51 **b**
s.e.	2.81[§]	1.19[§]	0.61[§]	0.24[§]	0.34
Interactions (P=)					
Cult. * Water	**0.016**	**0.010**	**0.000**	0.363	0.717
Harvest * Cult.	0.159	0.240	0.178	0.683	0.222
Harvest * Water	0.773	0.147	0.082	**0.005**	**0.039**
Harv.*Cult.*Wat.	0.482	0.101	0.071	0.939	0.384

Abaxial: lower leaf surface, **adaxial**: upper leaf surface, **total**: (abaxial^{-1}+adaxial^{-1})$^{-1}$.

[1] Letters indicate groupings of means that are significantly different at $P<0.05$ within harvest, cultivars or water treatments.

[2] Standard error

[§] Mean standard error.

Table 3.21. Abaxial leaf surface, adaxial leaf surface and total stomatal resistance averaged for cultivars and water treatments.

	Stomatal resistance (s cm^{-1})					
	abaxial		adaxial		total	
Cultivars	**Control**	**Stressed**	**Control**	**Stressed**	**Control**	**Stressed**
MK	3.73 **c**[1]	21.37 **b**	2.47 **c**	10.70 **b**	1.40 **d**	6.07 **b**
GA	10.74 **bc**	58.47 **a**	4.96 **bc**	25.47 **a**	2.68 **c**	16.66 **a**
EP	8.48 **bc**	42.14 **a**	5.07 **bc**	20.98 **a**	2.81 **c**	13.22 **a**
s.e.[2]	2.81		2.06		1.05	

Abaxial: lower leaf surface, **adaxial**: upper leaf surface, **total**: $(\text{abaxial}^{-1}+\text{adaxial}^{-1})^{-1}$.

[1] Letters indicate groupings of means that are significantly different at $P<0.05$.

[2] Mean standard error.

Table 3.22. Transpiration rate of both leaf surfaces averaged for harvests and water treatments.

	Transpiration (μg cm^{-2} s^{-1})			
	abaxial		adaxial	
Harvest	**Control**	**Stressed**	**Control**	**Stressed**
2	4.82 **a**[1]	0.79 **c**	7.59 **a**	1.94 **c**
3	3.61 **a**	0.98 **c**	4.71 **b**	1.50 **c**
4	2.04 **b**	0.60 **c**	3.86 **b**	1.08 **c**
s.e.[2]	0.40[§]		0.58	

[1] Letters indicate groupings of means that are significantly different at $P<0.05$.

[2] Standard error.

[§] Mean standard error.

3.4.4. Carbon isotope discrimination

The Δ at Harvest 1 was higher for MK than for the other cultivars (P<0.05, Table 3.23), consistent with the tendency for MK to have a lower stomatal resistance, that consequently, allowed a higher discrimination against the ^{13}C isotope (Farquhar *et al.*, 1989).

Table 3.23. Carbon isotope discrimination (Δ) for the cultivars MK, GA and EP before water treatments were imposed (Harvest 1).

Cultivar	Carbon isotope discrimination
MK	20.93 **a**[1]
GA	18.74 **b**
EP	18.44 **b**
s.e.[2]	0.40

[1] Letters indicate groupings of means that are significantly different at P<0.05.
[2] Standard error.

The difference between cultivars was maintained after water treatments were imposed (P<0.05, Table 3.24). No differences were found between harvests or between water treatments. The lack of response was because the treatments were imposed for a short time (Farquhar *et al.*, 1989). The harvest x water and harvest x cultivar x water treatment interactions were significant (P<0.05, Tables 3.25 and Appendix 3.10).

The harvest x water interaction was significant because, in Control plants, Δ decreased (P<0.05) from Harvest 3 to Harvests 4 to a value similar to that of Stressed plants in Harvest 2 (Table 3.25). On the other hand, Δ did not change significantly (P>0.05) with time in Stressed plants. The harvest x cultivar x water treatment interaction was due to a decrease in Δ of Control plants as the experiment progressed. Also, in Stressed plants the difference between MK and the other cultivars diminished because of a slight decrease in MK Δ and an unexpected increase in Δ of EP plants (Appendix 3.10).

Table 3.24. Carbon isotope discrimination (Δ) after water treatments were imposed.

Harvest	**Carbon isotope discrimination**
2	19.02
3	19.02
4	18.48
s.e.[1]	0.18
Cultivar	
MK	20.32 **a**[2]
GA	18.01 **b**
EP	18.19 **b**
s.e.	0.18
Water	
Control	18.81
Stressed	18.87
s.e.	0.15
Interactions (P=)	
Cult. * Water	0.151
Harvest * Cultivar	0.443
Harvest * Water	**0.048**
Harvest*Cultivar*Water	**0.043**

[1] Standard error.

[2] Letters indicate groupings of means that are significantly different at P<0.05 within harvest, cultivars or water treatments.

Table 3.25. Carbon isotope discrimination (Δ) averaged for harvest and water treatments.

	Carbon isotope discrimination	
Harvest	**Control**	**Stressed**
2	19.27 **a**[1]	18.76 **ab**
3	19.07 **a**	18.97 **a**
4	18.08 **b**	18.88 **a**
s.e.[2]	0.26	

[1] Letters indicate groupings of means that are significantly different at P<0.05.

[2] Standard error.

3.5. Summary

3.5.1. Water deficit effects

- Plants subjected to water deficit showed a decrease in shoot DW, and in green tissue percentage mainly due to a reduction in proportion of leaf blade. Stressed plants also had thicker roots in the uppermost stratum causing a reduction in root surface area at this level and a higher proportion of total root length in the lowest stratum of the soil profile.

- Stressed plants showed a higher root : shoot ratio than Control plants. The RWC, $\Psi_{w,}$ Ψ_o and Ψ_p decreased in the Stressed water treatment.

- As expected, the responses to water deficit were more pronounced with time.

3.5.2. Cultivar effects

- MK plants had a lower shoot DW, similar total root mass and higher root : shoot ratio and number of tillers per plant than cultivars GA and EP. MK roots tended to be thicker resulting in a lower root surface area than the other cultivars.

- Although MK plants showed higher ^{13}C discrimination, lower stomatal resistance and higher transpiration rate, they tended to maintain a higher water status than GA and EP under the Stressed treatment. The smaller shoot size and higher root : shoot ratio in MK plants would have helped the plant to delay dehydration. Conversely, the temperate cultivars with a bigger evaporative surface would have depleted the available water more rapidly.

CHAPTER 4. PLANT GROWTH AND RESPONSES TO WATER DEFICIT OF ENDOPHYTE-FREE MEDITERRANEAN AND TEMPERATE TALL FESCUES

4.1. Introduction

In the previous chapter, it was found that under the Stressed treatment, Maris Kasba (MK) tended to maintain a higher water status than the temperate cultivars. It was suggested that this response was due to the smaller shoot size and higher root : shoot ratio of the Mediterranean cultivar, and that these traits allowed it to delay dehydration. On the other hand, the temperate cultivars, with a higher evaporative surface, and consequently, a higher water demand, would be expected to more rapidly deplete the water available in the pot.

In order to have more information on the physiological and morphological responses of these contrasting tall fescue cultivars to water deficit, a second glasshouse experiment was carried out. To avoid differences in plant water status resulting from a higher pot water availability, because of a lower use of water, the cultivars were grown together in the same pot. Thus the water availability was as similar as possible for the contrasting cultivars. Unlike the previous experiment (Chapter 3), where water was completely withheld, water deficit treatments in this experiment comprised a proportional reduction in the amount of water added to the pots compared with the Control treatment. The reason for the change in methodology was to measure the plant water relations when the equilibrium at different water availabilities had been reached, rather than conduct measurements against a background of steady deterioration. Finally, as responses of temperate cultivars to water deficit were similar in the previous experiment (Chapter 3), only El Palenque (EP) and MK cultivars were studied here.

4.2. Objective

The objectives of this experiment were to compare morphological and physiological responses of a Mediterranean and a temperate tall fescue cultivar to controlled soil moisture deficit.

4.3. Materials and Methods

4.3.1. Experimental design and statistical analysis

The experiment included two tall fescue cultivars, Maris Kasba (MK) and El Palenque (EP), subjected to four watering treatments and three replicates as complete blocks. A split plot design was used with watering treatments as main units and cultivars as subunits. Variables measured at dawn and at midday were analysed individually and the difference between measuring times was analysed using the dawn measurement as a covariate, when the latter was significant. Consequently, the difference values shown in the tables are, when applicable, adjusted for the covariate and to simplify tables layout, the standard error shown was calculated as in Section 3.3.1. Percentages were arcsin square root transformed for statistical analysis. Means were separated using the LSD (Steel & Torrie, 1980) at 5 % significance level. Linear regressions were fitted using the SAS REG procedure.

The experimental management schedule, conditions of plant culture and periods of measurements are shown in Fig. 4.1. Photographs showing the layout of part of the trial at different stages are presented in Plate 4.1.

4.3.2. Plant culture

Seeds of both cultivars were incubated at 47 °C and 45 % relative humidity for 25 days to kill the endophyte *Neotyphodium coenophialum* (Bouton *et al.*, 1993; Dr. G. Latch, personal communication). They were then germinated and seedlings transferred to root-trainers filled with vermiculite. Plants were irrigated three times a week with 0.5 M Hoagland's solution (Hoagland and Arnon, 1938) and were grown in a glasshouse at Unidad Integrada INTA-FCA Balcarce, under natural photoperiod (37° 45' S). The glasshouse used in this experiment had limited temperature control. Heaters operated during winter. Maximum and minimum daily temperatures were monitored only from 10 October to 14 November (Fig. 4.2) during the water deficit experimental period. On 8 July, after a period of growth of approximately 70 days, plants were transplanted, after removal of all daughter tillers, to polystyrene pots of 305 x 375 x 323 mm, covered with a plastic bag with holes at the bottom and filled with soil. The soil characteristics were analysed at Soil Department, INTA Balcarce and are shown in Table 4.1. Each pot contained four rows of five plants (main tiller only) with the two cultivars arranged in alternate rows. EP tillers were defoliated to achieve similar leaf areas in both cultivars when transplanted. Plants were checked for infection by *N. coenophialum* by examining sheath (Latch & Christensen, 1982), to ensure that endophyte elimination had been successful. Pots were watered daily and once a week received 250 ml of 0.5 M Hoagland's solution.

From 17 to 19 October four water treatments were imposed on consecutive days in blocks 1 to 3, respectively. The treatments were: Control = 1,000 ml water day^{-1} pot^{-1}, S = 800 ml water day^{-1} pot^{-1}, S2 = 650 ml water day^{-1} pot^{-1} and S3 = 500 ml water day^{-1} pot^{-1}.

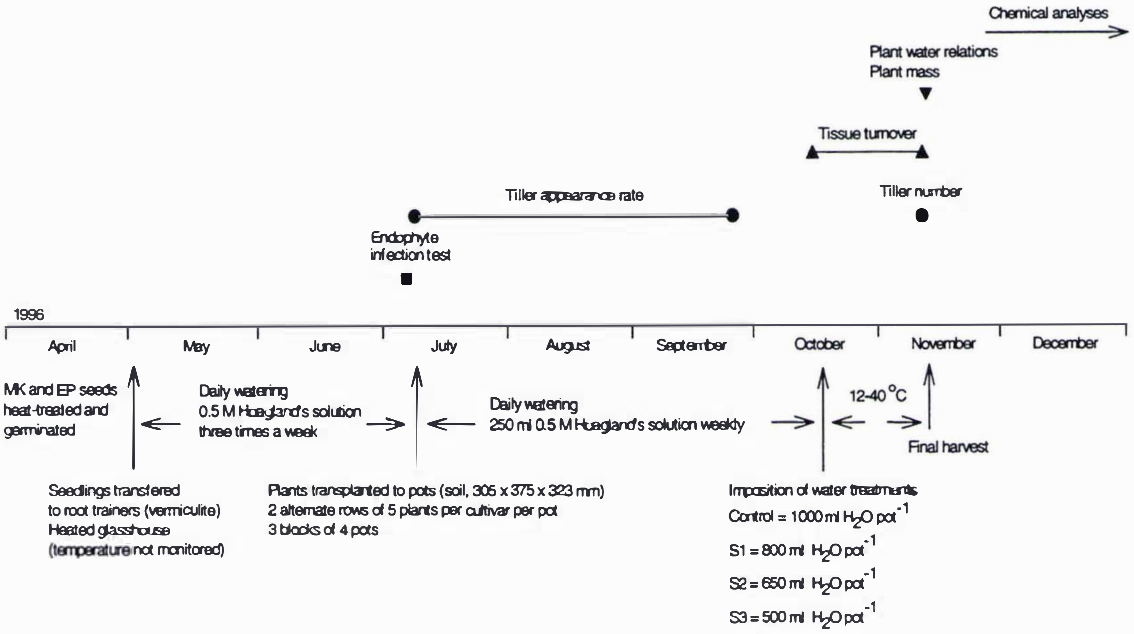

Figure 4.1. Experimental management schedule, conditions of plant culture and measurements for the second water deficit experiment. Unidad Integrada Balcarce INTA-FCA, Argentina.

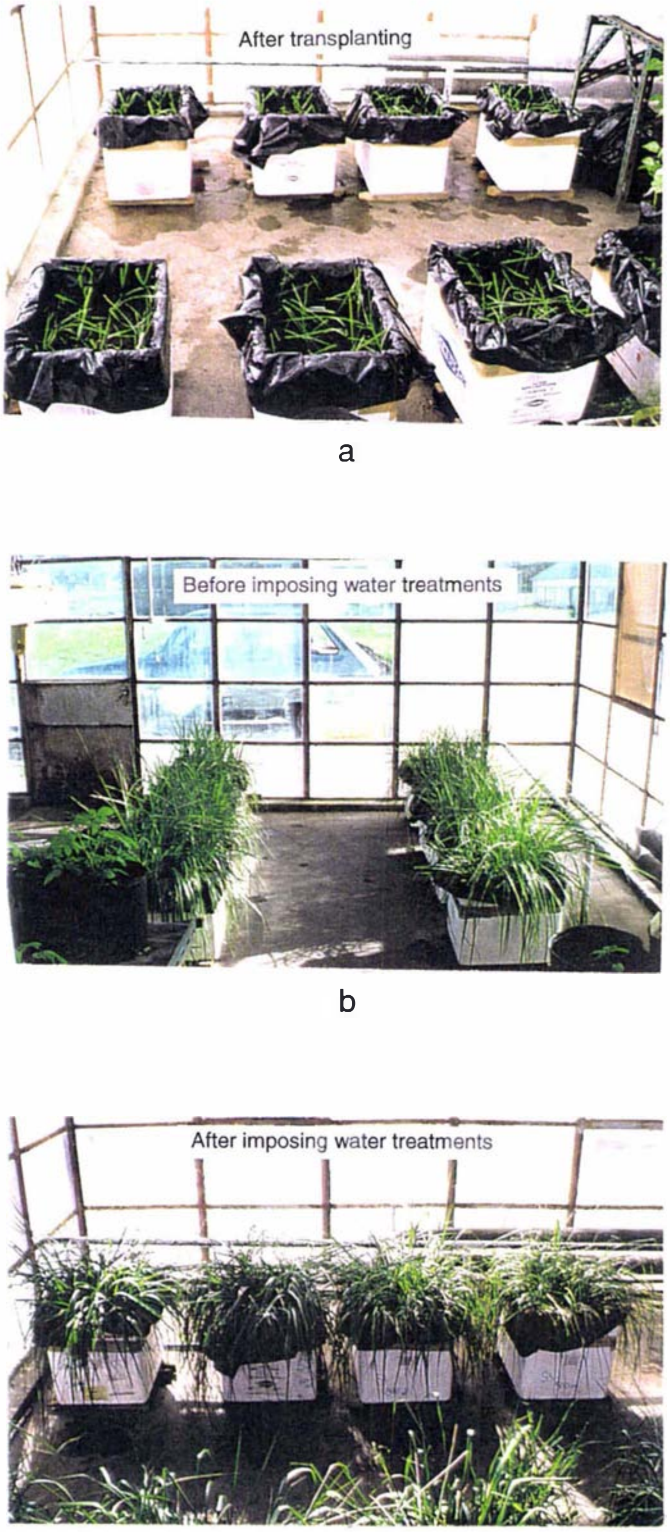

Plate 4.1. Experiment in progress. a: Blocks 1 and 2 after transplanting (8 July). b: Blocks 1 and 2 before the imposition of the water treatments (16 October). c: Block 1 after the imposition of the water treatments (11 November), from left to right Control, S2, S1, and S3 treatments.

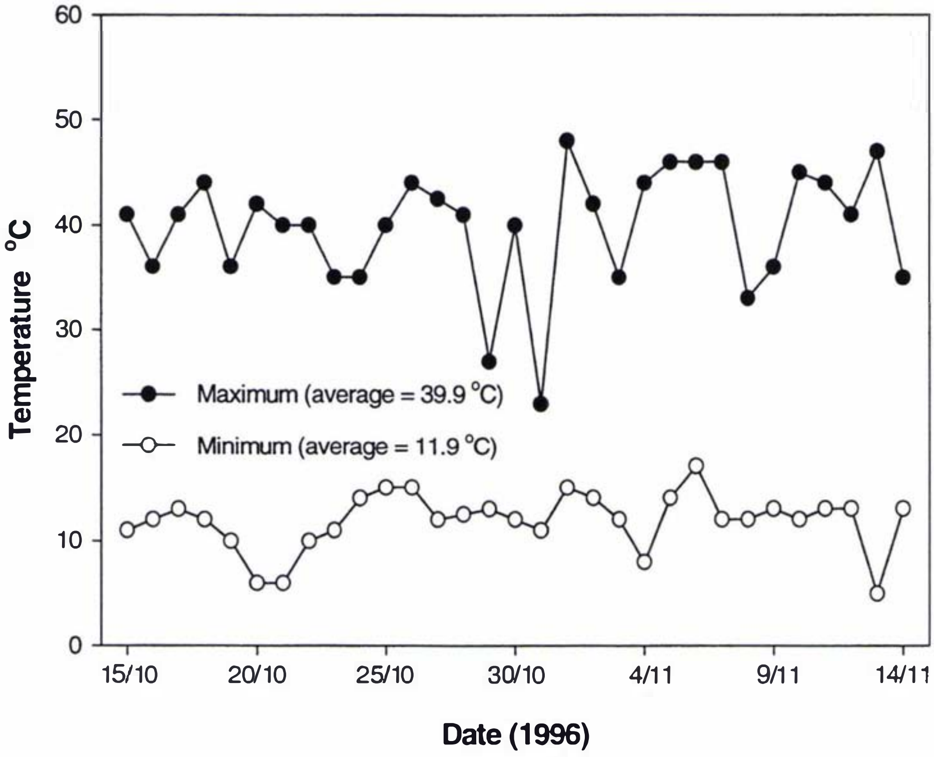

Figure 4.2. Daily maximum and minimum glasshouse temperature during the application of the water treatments.

Table 4.1. Soil characteristics

Clay	28.23 %
Fine silt	21.45 %
Coarse silt	17.25 %
Sand	33.07 %
Texture	Friable loam
Phosphate	29.90 ppm
Nitrate	47.30 ppm
Carbon	3.72 %
Organic matter	6.41 %

4.3.3. Measurements

Tiller appearance rate was measured before the application of water treatments, while tissue turnover was measured during the application of the water treatments. Other physiological and dry weight measurements were performed at harvest from 12 to 14 November, on consecutive days in blocks 1 to 3, respectively, consistent with the staggered introduction of the water deficit treatments (see Section 4.3.2).

- **Tiller appearance rate**

On three plants of each cultivar per pot, daughter tillers were labelled with coloured plastic wire at soil level fortnightly from 8 July to 25 September, before the water treatments were imposed, to calculate tiller appearance rate (TAR) and relative tiller appearance rate (RTAR, calculated on $\log_e$ transformed tiller number) by linear regression. Tillers were counted again on 11 November to determine average tiller number per plant during the period water treatments were applied.

- **Tissue turnover**

On one tiller of three plants of each cultivar per pot, tissue turnover measurements were carried out from 15 October to 11 November, during the application of the water treatments. The method was adapted from those described by Davies (1993), and Mazzanti and Lemaire (1994). Leaf extension rate and leaf senescence rate, expressed in mm $\text{tiller}^{-1}\text{d}^{-1}$, were converted to gross growth rate (GGR) and senescence (SR), respectively, and expressed in mg DM $\text{tiller}^{-1}\ \text{d}^{-1}$ by using the dry weight (DW) per unit of leaf length (i.e. specific leaf weight per unit length, mg mm^{-1}) estimated from the plants harvested to perform dry weight determinations (see below). Net growth rate (NGR) was calculated as the difference between GGR and SR. GGR, SR and NGR were also expressed as mg DM $\text{plant}^{-1}\ \text{d}^{-1}$ by using the average number of tillers per plant.

- **Relative water content**

Plants were sampled pre-dawn at 5:30 am. The youngest fully expanded leaf of two tillers per cultivar per pot was cut and weighed (fresh weight, FW), floated in distilled water, re-weighed (saturated weight, SW) and dried to constant weight (dry weight, DW). Relative water content (RWC) was determined as described in Apenddix 2.1, Eq. *A*2.10.

- **Water potential and its components**

Water potential (Ψ_w) was measured on the youngest fully expanded leaf of two tillers per cultivar per pot using a pressure chamber (Scholander, Hammel, Bradstreet & Hemmingsen, 1965) at dawn and midday. The leaves used to determine water potential were stored by freezing in individual 2.5 ml plastic syringes, the tip of which was sealed with a flame after introducing the leaf. The syringes were later thawed, the tip cut, and leaf sap squeezed out into a test tube. Osmotic potential (Ψ_o) was determined by vapor pressure osmometry (model 5500, Wescor, Logan, Utah) as described by Turner (1981). Osmolalities (mmol kg^{-1}) were converted into osmotic pressure (MPa) by multiplying by a factor of 2.48 x10^{-3} according to van't Hoff's equation (Hohl and Schopfer, 1991). Leaf turgor potential (Ψ_p) was estimated as the difference between leaf water potential and leaf osmotic potential.

- **Water soluble carbohydrates**

On two tillers per cultivar per pot, reducing and fructosyl sugar content at midday was determined. The extracts were prepared by boiling the fresh sample twice in alkaline water (2 drops of 0.1 M NH_3 solution per 125 ml water) for 10 minutes. The approximate ratio for each partial extraction was 1 ml water : 0.1 g sample fresh weight. Reducing sugars (glucose and fructose) were determined by Somogyi-Nelson's method (Somogyi, 1952) and fructosyl sugars (fructose, sucrose and fructans) by Kulka's method (Kulka, 1956). The methods are described in Appendix 4.1.

- **Dry weight**

One plant of each cultivar per pot, on which no destructive measurements had been carried out, was harvested at midday and separated into green leaf sheath (pseudostem), green leaf blade and dead tissue. The total length of green leaf blades of each plant was determined to calculate leaf weight per unit leaf length (g DW mm^{-1}). Each component was dried overnight at 60 °C in a forced-air oven and weighed.

- **Carbon isotope discrimination**

Combined samples of the youngest fully expanded leaf of the plants used for fresh and dry weight determinations were dried, ground and submitted to Waikato University Stable Isotope Unit, for carbon isotope analysis. Carbon discrimination (Δ) was calculated according to Eq. *A* 2.15, Appendix 2.1.

4.4. Results and discussion

4.4.1. Shoot characteristics

MK plants were smaller than EP plants (Table 4.2), as in the previous experiment (Chapter 3). However, in the present experiment, MK plants showed greater signs of water deficit, having a higher percentage of dead tissue and sheath, and a lower percentage of leaf blade than EP plants, and a lower number of tillers per plant ($P<0.05$, Table 4.2).

Table 4.2. Shoot dry weight (DW) and percentages of shoot DW as leaf blade, sheath and dead tissue, and mean tiller number per plant.

Cultivar	Shoot DW g	% DW as leaf blade	% DW as sheath	% DW as dead tissue	Tiller number
MK	4.02 **b**[1]	46.8 (43.1) **b**	39.7 (39.0) **a**	13.5 (21.2) **a**	8.8 **b**
EP	11.71 **a**	60.3 (51.0) **a**	32.0 (34.4) **b**	7.7 (15.9) **b**	14.3 **a**
s.e.[2]	0.70	(0.7)	(0.6)	(1.0)	1.1
Water					
Control	7.29	54.8 (47.8)	35.1 (36.3) **b**	10.1 (18.0)	12.0
S1	7.61	56.3 (48.6)	35.2 (36.3) **b**	8.5 (16.9)	11.8
S2	7.97	52.3 (46.3)	34.7 (36.1) **b**	13.1 (20.6)	11.7
S3	8.58	50.8 (45.5)	38.4 (38.3) **a**	10.8 (18.8)	10.7
s.e.	0.96	(0.7)	(0.4)	(1.4)	1.6
Interaction (P=)					
Cultivar* Water	0.219	0.753	0.419	0.324	0.705

Data within brackets are arcsin square root transformed percentages.

[1] Letters indicate groupings of means that are significantly different at $P<0.05$ within cultivars or water treatment.

[2] Standard error.

The fact that cultivars performed differently here, compared with the experiment reported in Chapter 3 probably results from the difference in experimental design. In the previous experiment cultivars were in individual pots, while in this experiment both cultivars were in the same pot. This would have negated the drought avoidance strategy of MK, seen when plants of this cultivar were grown in isolation. In addition, the water treatments were imposed differently. While in the experiment of Chapter 3, water was withheld for 12 days in the water deficit treatment, in the present experiment plants were watered daily, though under the most severe water treatment, pots were receiving only half the total amount of water of the Control treatment, for 26 days. Finally, there were differences in temperature and in daylength between experiments. Here maximum and minimum mean temperatures were more extreme and daylength was shorter than in the experiment of Chapter 3

There was less evidence of an effect of water treatments on the morphological characteristics of the plants than was observed in Chapter 3. This result was probably due to the fact that, even under the most stressful water treatment, pots were watered daily. However, the percentage of sheath increased under S3 water treatment ($P<0.05$). This result could have been due to the elongation of the pseudostem. Apparently, while stress delays flowering in perennial plants, conditions of extreme stress may promote flowering (Bazzaz *et al.*, 1987). However, despite the sheath biomass per plant showing a tendency to increase with water deficit, the differences were not significant (Control = 2.46 g DW, S1 = 2.55 g DW, S2 = 2.65 g DW and S4 = 3.17 g DW, s.e. = 0.34, $P>0.05$). Increased sheath percentage in S3 (Table 4.2) would partly reflect these increased sheath weights, but also the reduction in leaf lamina (Table 4.2). S3 sheath may also have had a higher dry matter content because of carbohydrate accumulation (Brouwer, 1966).

4.4.2. Tiller appearance rate

MK had lower TAR than EP (0.096 ± 0.007 tiller d^{-1} c.f. 0.166 ± 0.013 tiller d^{-1} for MK and EP, respectively. $P<0.05$). This result was opposite to that expected since MK is a cultivar characterised by having small plant size, but a high tiller number (Tables 3.2, 5.2 and 5.5). Consequently, a higher TAR in MK could have been expected. Indeed, by 8 July, when plants were transferred to the polystyrene pots, MK appeared to have more tillers per plant than EP, suggesting a higher TAR for MK prior to transplanting.

The reason for the unexpectedly low TAR in MK compared with EP after transplanting is unclear. It appeared that MK plants suffered a check to growth following removal of daughter tillers at transplanting and it is possible that such an effect resulted from pathogen invasion of wounds. Competitive effects due to increased stress on MK when the two cultivars were grown together would likely also have been partly responsible. A third possible explanation is that MK has greater reliance than EP on carbon from older daughter tillers and therefore was more affected by removal of these tillers at transplanting (Quinlan and Sagar, 1962; Marshall, 1967; Clifford *et al.*, 1973).

It is worth noting though, that observed differences in TAR arose from only small differences in RTAR (MK = 0.027 ± 0.004 tiller tiller^{-1} d^{-1} and EP = 0.032 ± 0.005 tiller tiller^{-1} d^{-1}, $P>0.05$). Also, during subsequent tissue turnover measurements, MK-Control plants had a lower leaf appearance rate than EP-Control plants (0.024 and 0.041 leaves tiller^{-1} d^{-1}, respectively) indicating that the site usage (Skinner and Nelson, 1992) would have been higher in MK than in EP.

4.4.3. Tissue turnover

The GGR and NGR, expressed per tiller or per plant, were higher for EP(Fig. 4.3). Conversely, the SR tended to be lower in MK plants, however this difference was significant only when the SR was expressed per plant (Fig. 4.3b). Lower growth rates for MK were expected because of the high temperatures recorded during the measuring period (Fig. 4.2). Although MK summer growth is considered acceptable (Delgado and Tanco, 1980), it is known that Mediterranean cultivars are relatively dormant during periods of high temperature, as observed during the present experiment, because they have evolved in environments where summer coincides with the dry season (Morgan, 1964). The relative growth rate (RGR) was estimated as the ratio between net growth rate (g plant^{-1} d^{-1}) and final plant size (g plant^{-1}) and was also consistently greater for EP than for the Mediterranean cultivar (EP = 0.53 % DW d^{-1} and MK = 0.40 % DW d^{-1}, s.e. = 0.03 % DW d^{-1}, $P<0.05$).

Water deficit decreased leaf growth rate in this experiment (Fig. 4.4). This decrease was significant ($P<0.05$) only for GGR when expressed on a per tiller basis, but it was significant for GGR and NGR when expressed on a per plant basis. However, the SR did not show any significant effect of water treatments when expressed on a per tiller, nor when expressed on a per plant basis. The lack of response of SR to water deficit was reported earlier by Barker (1983).

The above rates should be considered specific for the period and environmental conditions present at the time of the measurements. The calculation of the total plant DW, from the 8 July to the end of the experiment, using the estimated GGRs (assuming that the dead material remains attached to the plant and that the transference of material to new leaves or decomposition during the whole period is nil), resulted in average around 47 % and 55 % of the measured plant DW for MK and EP, respectively. This result suggests that both cultivars had higher growth rates at earlier stages of the experimental period.

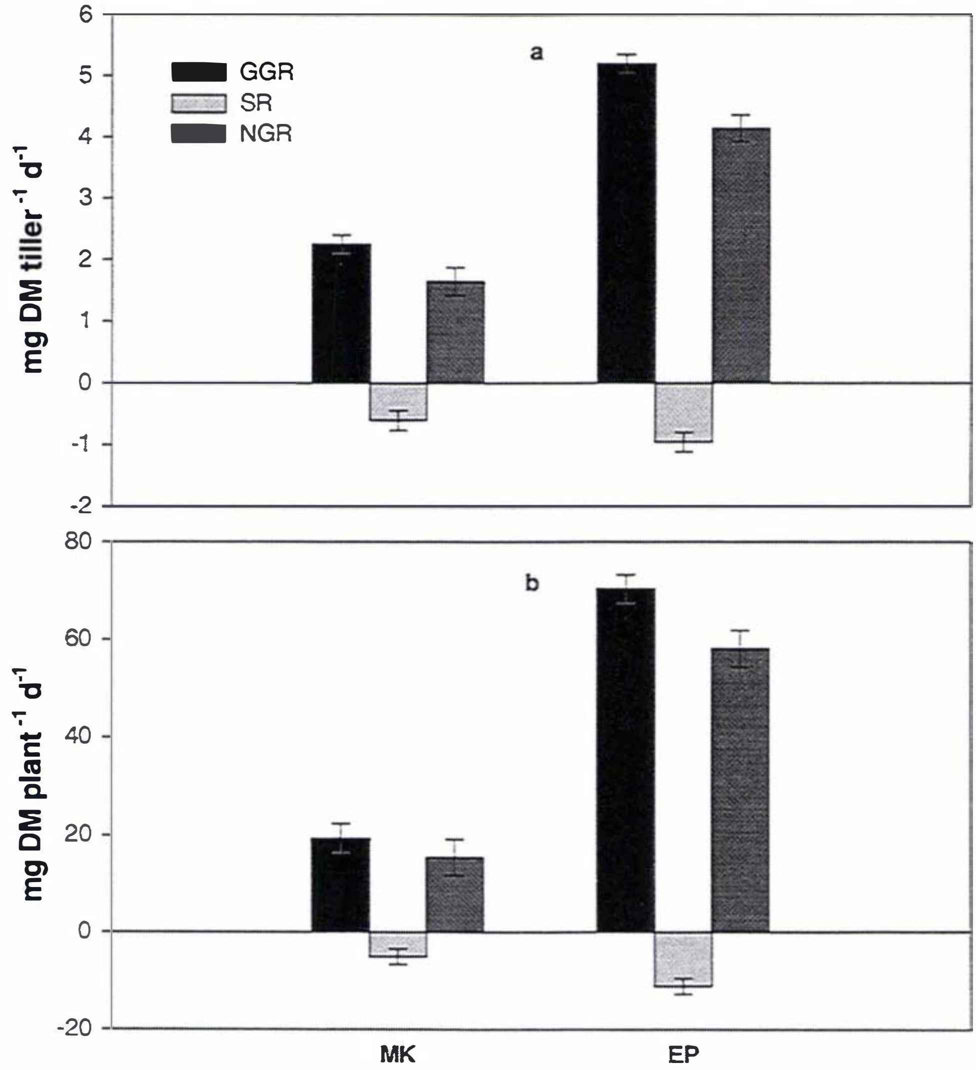

Figure 4.3. Gross growth rate (GGR), senescence rate (SR) and net growth rate (NGR) for MK and EP cultivars. a: Expressed per tiller (mg DM tiller^{-1} d^{-1}). b: Expressed per plant (mg DM plant^{-1} d^{-1}). Vertical lines represent standard errors.

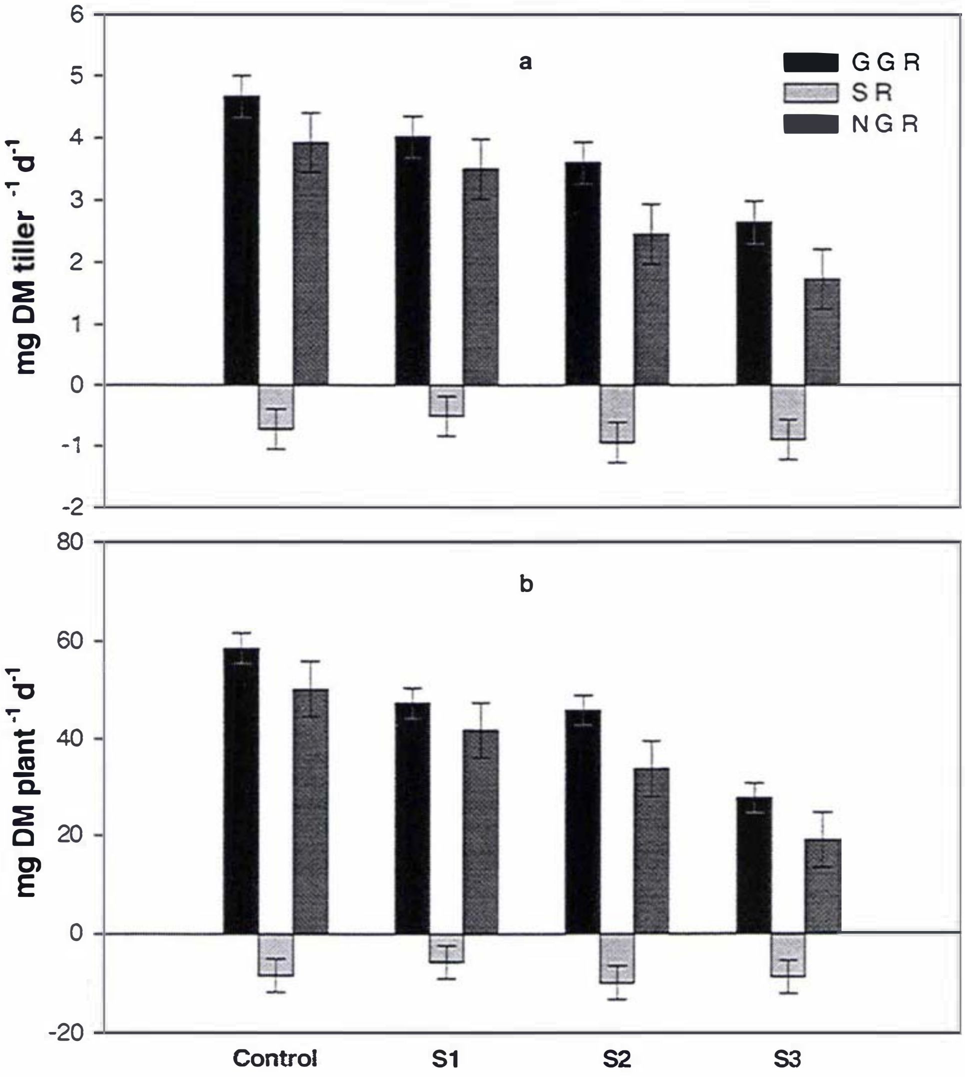

Figure 4.4. Gross growth rate (GGR), senescence rate (SR) and net growth rate (NGR) for the four water treatments. a: Expressed per tiller (mg DM tiller^{-1} d^{-1}). b: Expressed per plant (mg DM plant^{-1} d^{-1}). Vertical lines represent standard errors.

4.4.4. Plant water relations

No differences were found between MK and EP cultivars in RWC, Ψ_w, Ψ_o or Ψ_p, either for dawn, midday or for the difference between these sampling times ($P>0.05$, Table 4.3). These findings contrast with the results reported in Chapter 3, which showed that MK had a higher RWC, Ψ_w and Ψ_o than EP.

RWC along with the Ψ_w and its components, both for dawn and midday sampling times, decreased as the intensity of the water deficit increased from Control to S3 treatment ($P<0.05$, Table 4.3), as in Chapter 3. In contrast with the results of the first experiment (Chapter 3), here the cultivar x water treatment interaction was not significant, i.e. both cultivars responded similarly to the water treatments. This is consistent with Stressed-MK plants in the previous experiment being able to maintain a higher water status because of the morphological characteristics that reduce water use and delay water deficit, but not in the present experiment when growing in association with EP plants.

As in the first experiment, the RWC of Control plants was always below 100 % because RWC samples were taken in plants that had been watered the previous morning and had been exposed to temperatures of around 40°C during the day.

Ψ_w, Ψ_o and Ψ_p were always lower at midday than at dawn, as expected. However, no significant differences were found between cultivars or water treatments for Ψ_w-difference and Ψ_p-difference ($P>0.05$, Table 4.3), indicating the change was uniform for both cultivars and for the four water treatments. On the other hand, the Ψ_o-difference decreased with increasing water deficit ($P<0.05$, Table 4.3). Two possible reasons for the decrease in Ψ_o-difference are a higher concentration of the vacuolar sap as a consequence of the lower RWC of S3 or accumulation of solutes in response to the water deficit, i.e. osmotic adjustment, as has been observed in tall fescue by West *et al.* (1990). In order to investigate this further, the Ψ_o at midday was analysed using RWC as a covariate, and a significant difference between S3 and Control and S1 was found (Control = -1.97 ± 0.12, S1 = -2.13 ± 0.09, S2 = -2.37 ± 0.08 and S3 = -2.60 ± 0.13, $P<0.05$), indicating that osmotic adjustment took place. In addition, the cultivar x water treatment interaction for Ψ_o-difference was significant (Table 4.4). The latter result arose because while Ψ_o-difference decreased significantly from Control to S3 treatment in EP, there was no response to water treatments in MK, indicating a higher osmotic adjustment in EP.

Table 4.3. Relative water content (RWC) and water potential (Ψ_w), osmotic potential (Ψ_o) and pressure potential (Ψ_p) at dawn, at midday and the difference.

	RWC (%)		Ψ_w (MPa)			Ψ_o (MPa)			Ψ_p (MPa)		
Cultivar	dawn		dawn	midday	difference[1]	dawn	midday	difference	dawn	midday	difference
MK	90.5	(74.0)	-1.90	-2.61	-0.74	-1.99	-2.22	-0.23	0.09	-0.39	-0.53
EP	91.9	(74.3)	-1.82	-2.58	-0.73	-2.02	-2.32	-0.30	0.20	-0.26	-0.41
s.e.[2]		(0.9)	0.09	0.07	0.07	0.04	0.04	0.04	0.07	0.05	0.06
Water											
Control	95.7	(79.4) **a**[3]	-1.17 **a**	-1.91 **a**	-0.28	-1.78 **a**	-1.90 **a**	-0.12 **a**	0.61 **a**	-0.01 **a**	-0.19
S1	93.1	(76.9) **ab**	-1.54 ab	-2.31 **a**	-0.56	-1.87 **ab**	-2.09 **a**	-0.23 **a**	0.33 **ab**	-0.22 **ab**	-0.37
S2	89.5	(71.5) **bc**	-2.11 **bc**	-2.82 **b**	-0.88	-2.11 **bc**	-2.40 **b**	-0.29 **ab**	0.00 **bc**	-0.42 **bc**	-0.55
S3	86.5	(68.8) **c**	-2.62 **c**	-3.34 **c**	-1.24	-2.26 **c**	-2.68 **b**	-0.42 **b**	-0.37 **c**	-0.66 **c**	-0.77
s.e.		(2.2)	0.22	0.12	0.20[§]	0.09	0.08	0.05	0.13	0.10	0.16[§]
Interaction (P =)											
Cultivar*Water	0.312		0.514	0.464	0.602	0.344	0.162	**0.024**	0.576	0.622	0.733

Data within brackets are arcsin square root transformed percentages.

[1] Differences for Ψ_w and Ψ_p fail to sum due to covariate adjustment (p. 72).

[2] Standard error.

[3] Letters indicate groupings of means that are significantly different at P<0.05 within cultivars or water treatment.

[§] Mean standard error.

Table 4.4. Osmotic potential (Ψ_o) difference between dawn and midday for MK and EP and the four water treatments.

	Ψ_o-difference (MPa)	
Water	**MK**	**EP**
Control	-0.16 **ab**[1]	-0.08 **a**
S1	-0.31 **ab**	-0.14 **ab**
S2	-0.21 **ab**	-0.37 **ab**
S3	-0.24 **ab**	-0.61 **c**
s.e.[2]	0.07	

[1] Letters indicate groupings of means that are significantly different at $P<0.05$.

[2] Standard error.

Negative values of Ψ_p are presumed to be measurement artefacts since Ψ_p was calculated as the difference between Ψ_w and Ψ_o, and these potentials were measured using different instruments. Furthermore, in water stressed plants Ψ_w and Ψ_o tend to be very similar, so the experimental error involved in their measurement could easily give rise to negative values. In addition, no corrections were made in the Ψ_o to account for the possible dilution of the symplastic sap by apoplastic water. Because of this, measured values of Ψ_o were less negative than they should have been. Nevertheless, the negative Ψ_p values have been presented assuming that, in relative terms, they reflect the actual differences between treatments.

4.4.4.2. Carbon isotope discrimination

The results of this experiment (Table 4.5) were consistent with those of Chapter 3 in that MK showed a higher discrimination against ^{13}C than EP, indicating that the Mediterranean cultivar had a lower stomatal resistance (Farquhar *et al.*, 1989). In contrast to Chapter 3, in the present experiment a significant difference between water treatments was found. However, there was again no consistent response to water treatments with there being an unexpectedly high Δ value in S2. It has already been reported that this technique was not appropriate to detect responses arising from short term effects (Farquhar *et al.*, 1989).

Table 4.5. Main effects means for carbon isotope discrimination (Δ).

Cultivar	Δ
MK	18.86 **a**[1]
EP	18.31 **b**
s.e.[2]	0.15
Water	
Control	18.74 **ab**
S1	18.23 **b**
S2	19.17 **a**
S3	18.19 **b**
s.e.	0.20
Interaction (P=)	
Cultivar*Water	0.106

[1] Letters indicate groupings of means that are significantly different at P<0.05 within cultivars or water treatments.

[2] Standard error.

4.4.5. Reducing sugar and fructosyl sugar concentrations

The concentrations of reducing and fructosyl sugars were, respectively, 24 % and 127 % higher for EP than for the Mediterranean cultivar MK ($P<0.05$, Table 4.6). Eagles (1967b) compared the simple sugar and fructan concentrations of two populations of cocksfoot (*Dactylis glomerata* L.), one from a North European environment (Norway) and the other from a Mediterranean environment (Portugal) in response to different temperatures. In contrast with our results, he found no significant difference in simple sugar content between populations. However, for fructan content, the differences between populations and the interaction between temperature and population were significant.

The latter was a consequence of a more rapid reduction of fructan content in the Norwegian population at high temperatures and in the Mediterranean population at low temperatures, corresponding with their active period of growth (Morgan, 1964; Robson and Jewiss, 1968a). The lower content of fructans was attributed to the utilisation of simple sugars in processes such as leaf expansion, resulting in a lower concentration of sugars available for conversion to fructans. This behaviour was not observed in the cultivars of tall fescue compared here. Under the elevated temperatures measured in the glasshouse (Fig. 4.2), the temperate cultivar EP had a significantly greater net growth rate than the Mediterranean cultivar (Fig. 4.3), in agreement with Morgan (1964), although at the same time, EP had a higher fructosyl sugar concentration.

In the present study no systematic trend was observed in reducing sugar concentration in response to water stress (Table 4.6). The fructosyl sugar concentration in S1 tended to be lower than in Control treatment, while S2 and S3 tended to be higher, however, the variability was such that no definitive interpretation can be placed on these trends (Table 4.6). The tendency for a higher amount of sugars to be found in Control plants than in S1 is most likely the result of a higher photosynthetic rate in non-stressed plants.

The contribution of fructosyl sugars to the osmotic adjustment (calculated from the concentration of fructosyl sugars in the total volume of the tissue at 100 % relative water content and assuming that 40 µmol g^{-1} H_2O = -0.1 MPa, according to Munns and Weir, 1981) did not differ between water treatments (Table 4.7). This finding was consistent with that of Spollen and Nelson (1994) in that fructosyl sugars did not appear to be involved directly in osmotic adjustment. Moreover, it should be noted that the osmotic contribution has been overestimated because it was calculated without taking into account the differential contribution of fructose polymers of different molecular weight. As the osmotic contribution of the fructosyl sugars to Ψ_o did not differ between water treatments (Table 4.7), and the contribution of reducing sugars was small because of the lower concentration of these sugars, it is assumed that solutes other than the sugars measured were involved in the osmotic adjustment.

Table 4.6. Reducing sugar and fructosyl sugar concentrations.

Cultivar	Reducing sugars µmol glucose g^{-1} FW	Fructosyl sugars µmol fructose g^{-1} FW
MK	38.23 **b**[1]	98.43 **b**
EP	47.46 **a**	223.66 **a**
s.e.[2]	1.96	11.99
Water		
Control	43.36	161.59
S1	37.82	106.17
S2	45.55	174.51
S3	44.67	201.91
s.e.	6.35	22.12
Interaction (P=)		
Cultivar*Water	0.565	0.224

[1] Letters indicate groupings of means that are significantly different at $P<0.05$ within cultivars or water treatments.

[2] Standard error.

Table 4.7. Fructosyl sugar contribution to the osmotic potential at midday.

	Fructosyl sugar contribution	
Cultivar	**MPa**	**%**
MK	0.29 **b**[1]	12.84 (20.60) **b**
EP	0.67 **a**	29.28 (32.13) **a**
s.e.[2]	0.04	(1.27)
Water		
Control	0.49	25.71 (29.24)
S1	0.32	15.70 (22.40)
S2	0.52	20.85 (26.55)
S3	0.60	21.99 (27.27)
s.e.	0.07	(2.03)
Interaction		
Cultivar*Water	0.183	0.454

Data within brackets are arcsin square root transformed percentages.

[1] Letters indicate groupings of means that are significantly different at $P<0.05$ within cultivars or water treatments.

[2] Standard error.

4.5. Summary

4.5.1. Water deficit effects

- The morphological change observed in response to water deficit was an increase in sheath percentage.

- As expected , the growth rate, RWC, $\Psi_{w,}$ Ψ_o and Ψ_p decreased with reduced water availability.

- Reducing and fructosyl sugars do not appear to be directly related to the osmotic adjustment of these tall fescue cultivars.

4.5.2. Cultivar effects

- In this experiment, MK plants grown together with EP plants at high temperature had a lower shoot DW, leaf percentage and tiller number, and a higher sheath percentage and dead tissue percentage than EP plants.

- No differences were found between cultivars for the measures of plant water status. However, EP plants showed a decrease in the Ψ_o-difference from Control to S3 treatment, indicating a greater adaptation to water deficit than MK.

- MK plants showed a higher ^{13}C discrimination, which suggests a lower stomatal resistance.

- A higher concentration of reducing and fructosyl sugars was found in EP plants.

CHAPTER 5. PLANT GROWTH AND RESPONSES TO WATER DEFICIT OF EUROPEAN AND MEDITERRANEAN TALL FESCUES WITH AND WITHOUT ENDOPHYTE

5.1. Introduction

Recent experiments have aimed to understand the association between tall fescue and its endophyte, *Neotyphodium coenophialum* (Morgan-Jones & Gams) Glenn, Bacon & Hanlin. It has been postulated that the association gives both the plant and the fungus a higher ecological fitness (West and Gwinn, 1993). For instance, enhanced pest and drought resistance has been observed in infected tall fescue plants (Bacon, 1993). However, in most of the water stress experiments carried out (Belesky *et al.*, 1989; De Battista *et al.*, 1990; Hill *et al.*, 1990; Belesky and Fedders, 1996; Elbersen and West, 1996; Marks and Clay, 1996), the physiological and morphological responses have been inconsistent, with a strong interaction between the plant genotype and fungal strain (Bacon, 1993). Furthermore, no information seems to be available with respect to endophyte-infected Mediterranean tall fescues.

New *N. coenophialum* strains, that appear to have advantages over the most commonly studied Kentucky-31(KY31) wild type endophyte have been isolated recently. For example, a novel endophyte, designated AR501, is currently under study at AgReseach, New Zealand. This particular strain appears to be capable of conferring on the plant the ecological advantages of the association, without the alkaloids associated with 'tall fescue toxicosis' symptoms, often shown by cattle grazing E+ tall fescue.

For these reasons, a water stress experiment was carried out to compare the morphological and physiological responses of two tall fescue cultivars, one Mediterranean and one temperate, endophyte-free or artificially infected with a known strain of *N. coenophialum.* The cultivars were Maris Kasba and El Palenque, and the endophyte strains were isolates of KY31(wild type) and AR501. As in the previous experiment (Chapter 4) the water deficit treatments consisted of a proportional reduction in the amount of water added to the pots with respect to the Control treatment, rather than a complete withholding of water, but in this case small pots contained only one plant each.

5.2. Objectives

The objectives of this experiment were to compare morphological and physiological responses of one Mediterranean and one temperate tall fescue cultivar, infected with a known endophyte strain or endophyte-free, to a series of soil water availability conditions, and to compare KY31 and AR501 endophyte strains.

5.3. Materials and methods

5.3.1. Experimental design and statistical analysis

The experiment included two tall fescue cultivars, Maris Kasba (MK) and El Palenque (EP); three endophyte treatments, endophyte-free (E-) or endophyte-infected (E+) with one of two endophyte strains (AR501 or KY31); four watering treatments and four replicates. A split plot design in which watering treatments were main plots and cultivars were subplots was used (Steel and Torrie, 1980). This design was necessary to simplify watering, while different cultivar-endophyte combinations could be allocated easily within main plots. The SAS General Linear Models (GLM) procedure was used. Weighted ANOVAs were run for variables in which variance heterogeneity between water treatments was found. The reciprocal of the error variances of preliminary analysis carried out for each water treatment were used as the weight. Variables measured in two periods were analysed individually by period and the difference between periods was analysed using the first period measurement as covariate. Consequently, the difference values shown in the tables are, when applicable, adjusted for the covariate and to simplify tables layout, the standard error shown was calculated as in Section 3.3.1. Percentages were arcsin square root transformed for statistical analysis. Means were separated using the LSD (Steel & Torrie, 1980) at 5 % significance level. Quadratic regressions were fitted using SigmaPlot for Windows Version 4. Eight variables were analysed using SAS multivariate PRINCOMP procedure (i.e. principal component analysis - PCA) and SAS GLM procedure with the MANOVA statement and canonical option.

The experimental management schedule, conditions of plant culture and periods of measurements are shown in Fig. 5.1. Photographs showing the layout of part of the trial at different stages are presented in Plate 5.1.

5.3.2. Plant culture

Tall fescue seeds of cultivars EP and MK were treated to kill the endophyte as described in Chapter 3. From November 1995 to February 1996 several sets of seeds were surface-sterilized (Appendix 5.1), germinated and seedlings were inoculated with one of the two endophyte strains according to Method 1(iii) described by Latch and Christensen (1985). The endophytes used were KY31 wild type and a novel strain, AR501, both supplied by AgResearch, New Zealand. Plants were transferred to a glasshouse, and were grown first in root trainers and later in 100 mm diameter pots filled with a peat-sand mixture (3 : 2 by volume) supplemented with a mixture of 3 slow release fertilisers at a rate of approximately 7.2 kg m^{-3} of potting mix. Plants were periodically examined for endophyte infection. Six E+ plants of each cultivar-endophyte combination and six E- plants of each cultivar were split on 17 February 1997 to obtain several sets of two tillers from each plant. Each of these sets was transferred to a 100 mm pot filled with the peat-sand mixture as above. After seven weeks of growth, four plants of each cultivar-endophyte combination were transferred to individual 150x150x140 mm pots filled with the peat-sand mixture as above. On 13 May 1997 pots were sorted into their respective blocks and water treatments. Plants arising from the same mother plant were randomly assigned to one block and then randomly allocated to the water treatments. In this design the effect of cultivar variability was confounded with block effect. Because of the number of pots, two glasshouses had to be used. Blocks one and four were allocated to one glasshouse, and blocks two and three to the other.

Plants were sprayed to control aphids with 1.5 ml of Lannate® L (200 g l^{-1} methomyl in the form of a water soluble concentrate) per litre H_2O on 15 April and against mites with 4 ml of Yates Mite Killer® (80 g l^{-1} dicofol in the form of a suspension concentrate) per litre H_2O five times (on 5 May after cutting the plants to 200mm, and again on 25 May, 5 June, 15 June and 25 June) and with 4g of Omite® 30 W (300g kg^{-1} Propagite in the form of wettable power) per litre H_2O on 15 May. Plants were trimmed to 150 mm, beginning with blocks 1 on 31 May and concluding with block 4 on 3 June, following the staggering imposition of the water treatments (see below).

Maximum and minimum daily temperatures were registered in both glasshouses from 28 May to 17 July 1997 (Fig. 5.2). The glasshouse where blocks one and four were allocated tended to be colder and registered comparatively lower temperatures from 26 June to 28 June because of a failure in the heating system.

From 29 May to 1 June 1997 four different water treatments were imposed in blocks 1 to 4, respectively. The treatments were: Control=150 ml water $pot^{-1}d^{-1}$; S1=113 ml water $pot^{-1}d^{-1}$; S2=75 ml water $pot^{-1}d^{-1}$; and S3=38 ml water $pot^{-1}d^{-1}$.

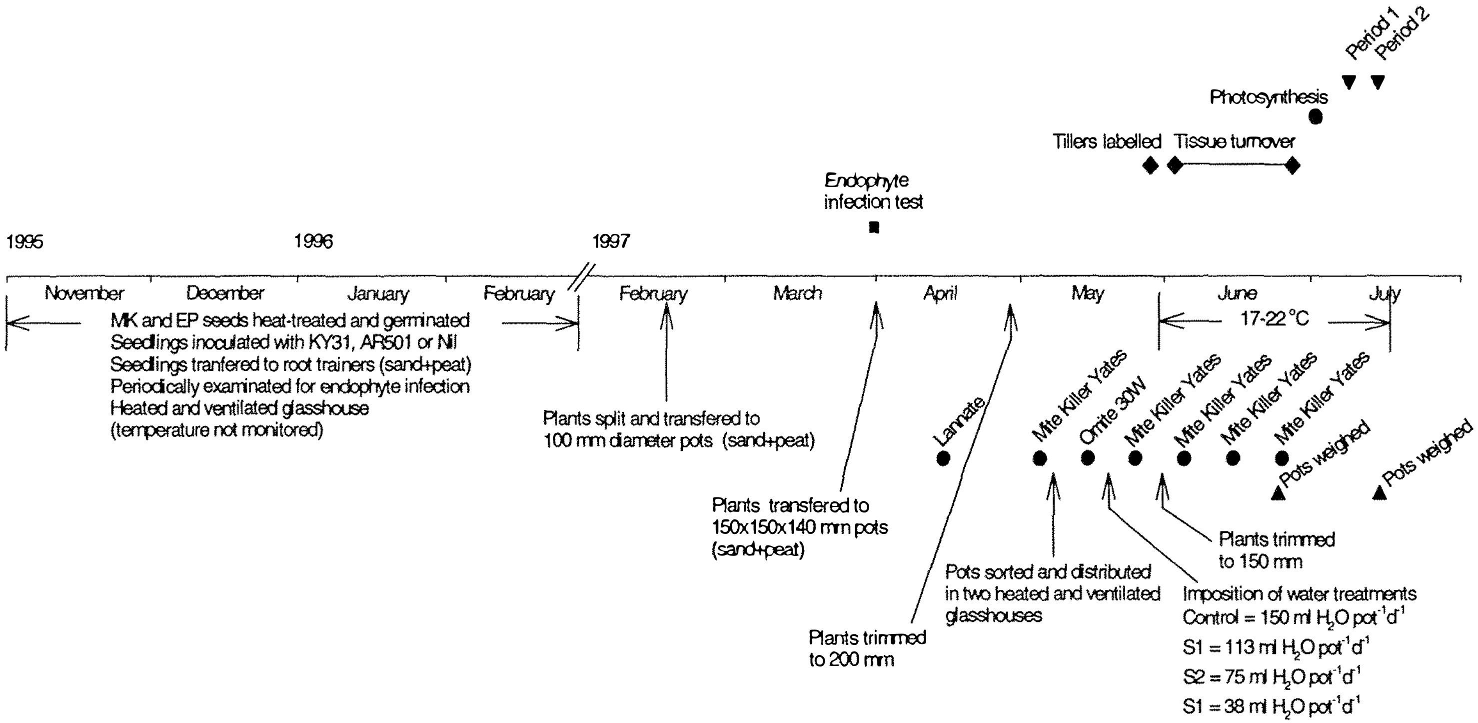

Figure 5.1. Experimental management schedule, conditions of plant culture and periods of measurements for the third water deficit experiment, AgResearch, New Zealand.

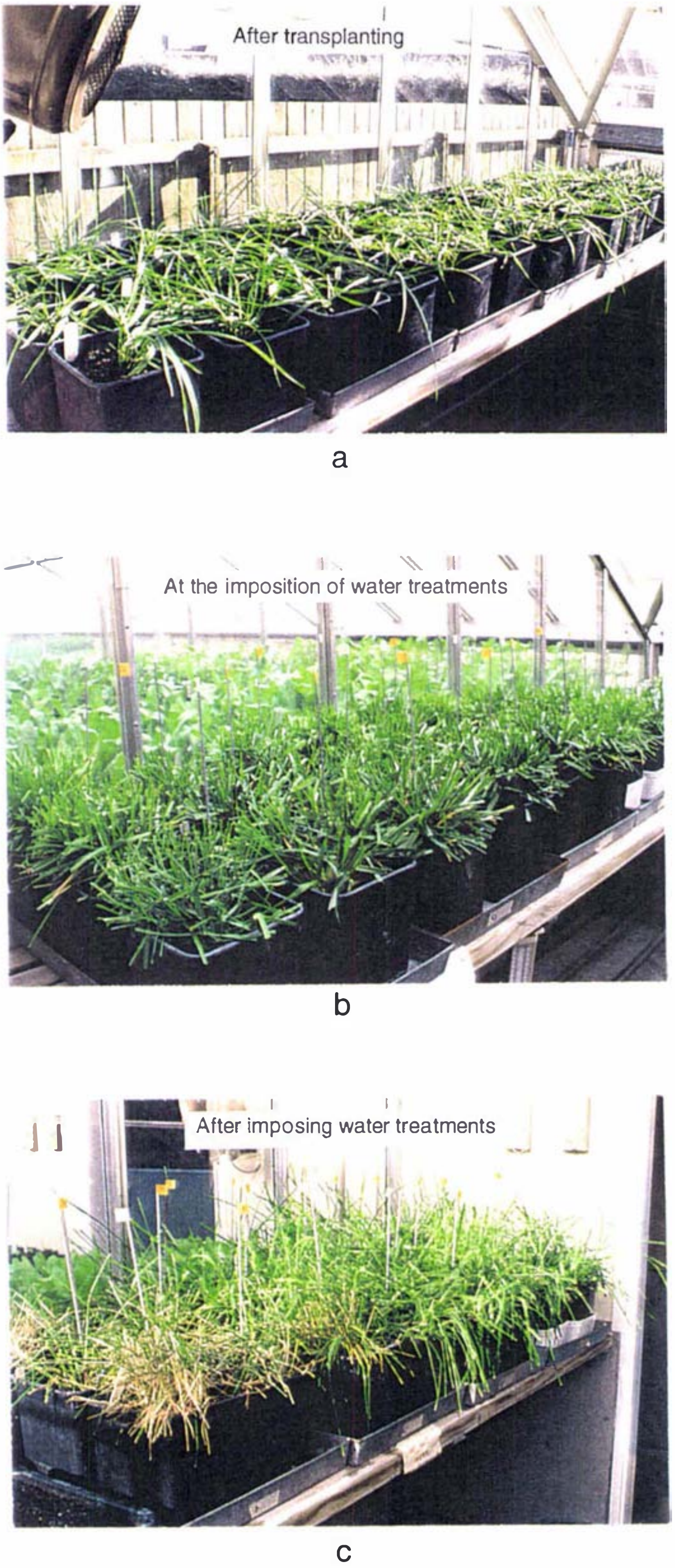

Plate 5.1. Plants after transplanting (a) and photographs showing the layout of part of the trial within the glasshouses (b and c). b: Block 2 at the imposition of the water treatments (1 June). c: Block 4 before Period 2 of measurements (13 July), fromleft to right S3, S2, S1, and Control treatments.

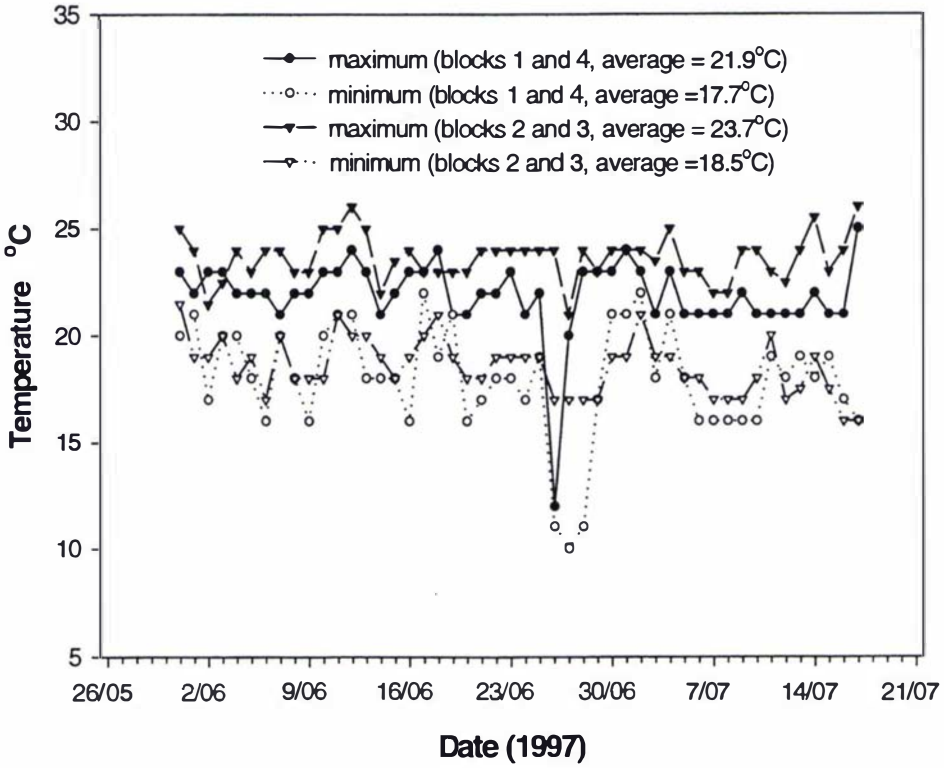

Figure 5.2. Daily maximum and minimum temperatures during the application of water treatments.

5.3.3. Measurements

All pots were weighed on 24 June and again from 14 July to 17 July for blocks 1 to 4, respectively. Gravimetric water content was calculated as g water per g dry rooting media, (soil plus roots) measured prior to daily watering (i.e. the lowest diurnal water content).

- **Tissue turnover**

On each pot two tillers were labelled on 28 May with coloured plastic wire and tissue turnover measurements were taken. The method was described in Chapter 4, Section 4.3.3. On each tiller, green length of individual leaves was recorded twice a week from 3 June to 27 June. GGR, SR and NGR were expressed as mg DM tiller^{-1}d^{-1} and as mg DM plant^{-1}d^{-1} by multiplying by the average number of tillers per plant. Tiller number was determined twice, first at the application of the water treatments and later when plants were harvested. Leaf appearance rate (LAR, leaf tiller^{-1} d^{-1}) was calculated from the number of leaves appearing in the period studied.

- **Photosynthesis rate and stomatal resistance**

Photosynthesis rate and stomatal resistance were measured on one youngest fully expanded leaf per plant at midday using a LI- 6200 portable photosynthesis meter (LI-COR, Lincoln, Nebraska). One block per day was measured from 1 July for four successive days. The portion of leaf that was held inside the chamber during the measurement was cut, placed in a labelled plastic bag and dried to constant weight in order to convert the photosynthesis rates calculated on a leaf area basis (μmol CO_2 m^{-2} s^{-1}) to leaf DW basis (μmol CO_2 g^{-1} s^{-1}).

The following measurements were carried out in two successive four-day periods (8-11 July and 14-17 July) in which one block per day was sampled.

- **Relative water content**

At 5:00 am one youngest fully expanded leaf per pot in treatments Control, S1 and S2, or one elongating leaf in S3 was cut and weighed (fresh weight, FW), floated in distilled water, re-weighed (saturated weight, SW) and dried to constant weight (dry weight, DW). Relative water content (RWC) was determined as described in Appendix 2.1, Eq. *A*2.10. In some plants in the S3 treatment, the elongating leaf was the only one alive.

- **Water, osmotic and turgor potentials**

Water potential (Ψ_w) was measured with a pressure chamber (Scholander, Hammel, Bradstreet & Hemmingsen, 1965) at predawn on the youngest fully expanded leaf of one tiller per pot in treatments Control, S1 and S2, and in the elongating leaf in S3. Preliminary data, obtained in spare control plants, showed that for both cultivars the Ψ_w measured at midday in elongating leaves was approximately 0.2 MPa lower ($P<0.05$) than in the youngest fully expanded leaves, no differences were found between cultivars ($P>0.05$) and the interaction between cultivar and leaf type was not significant ($P>0.05$). These results suggest that the measurements of S3 plants could have underestimated Ψ_w by around 0.2 MPa if the difference in Ψ_w between the expanding and the youngest fully expanded leaf was similar predawn to that measured at midday. The leaves used to measure Ψ_w were wrapped with cling-film and aluminium foil, immersed in dry ice and transferred to a -18°C freezer where they were kept until osmotic potential (Ψ_o) measurements were carried out using a thermocouple psychrometer (SCIOA, Decagon Devices Inc. Pullman, Washington). Leaf turgor pressure (Ψ_p) was estimated as the difference between Ψ_w and Ψ_o.

- **Dry weight**

In the second period plants were harvested and separated into leaf sheath (pseudostem), leaf blade of the elongating leaves and leaf blades of mature leaves and dead tissue. Additionally, the youngest fully expanded leaves of S1 plants in the second block were also separated. The total length of leaf blades of six plants per block (e.g. Control plants in block 1, S1 plants in block 2, S3 plants in block 3 and S2 plants in block 4) was determined to calculate specific leaf weight. Each component was dried at 60 °C in a forced-air oven overnight and weighed.

- **Carbon isotope discrimination**

The elongating leaf fraction of all plants and the fully elongated leaf fraction of S1 plants in block 2 dried, ground and submitted to Waikato University Stable Isotope Unit, for carbon isotope analysis. Carbon discrimination (Δ) was calculated according to Eq. *A* 2.15, Appendix 2.1.

5.4. Results and discussion

5.4.1. Water content

The gravimetric water content of the pots on 24 June and at harvest (measured immediately before the scheduled daily watering, Table 5.1) showed a significant ($P<0.05$) effect of water treatment. On 24 June the cultivar x water treatment interaction was significant ($P<0.05$, Fig. 5.3). Water content in MK tended to be higher than that of EP under less severe water deficit and similar to EP as water deficit increased. This trend was accentuated at harvest when MK pots showed a statistically significant increase in water content than EP pots ($P<0.05$, Table 5.1). Since both cultivars received the same amount of water per day, this probably was due to the smaller size of MK plants and their lower proportion of dry matter as leaf (Table 5.2) which contributed to a lower transpiration and consequently to a lower soil water demand. This finding was in agreement with the results of the first experiment described in Chapter 3.

The decline of water content between measurement dates was significantly greater ($P<0.05$) in Control water treatment. This suggested that the amount of water the Control plants were receiving was insufficient to meet their requirements during the last phase of the experiment. However, the difference in RWC between the two periods of measurement was not significant ($P>0.05$, Table 5.7) for water treatments and the mean of the Ψ_o-difference was positive, indicating a more positive osmotic potential in the Control plants during the second period (Table 5.9). This evidence suggested that Control plants were at a fairly constant water status despite decreasing soil moisture supply.

Table 5.1. Gravimetric water content on 24 June and at harvest, and water content difference.

	Water content 24 June		**Final water content 14-17 July**		**Water content difference**	
Cultivar	g H_2O g^{-1} dry soil	s.e.[1]	g H_2O g^{-1} dry soil	s.e.	g H_2O g^{-1} dry soil	s.e.
MK	0.199	0.015	0.111 **a**	0.004	-0.073	0.008
EP	0.151	0.010	0.104 **b**	0.004	-0.062	0.008
Water						
Control	0.303 **a**	0.043	0.121 **ab**	0.011	-0.105 **b**	0.012
S1	0.175 **b**	0.027	0.117 **a**	0.005	-0.057 **a**	0.005
S2	0.119 **bc**	0.011	0.101 **b**	0.003	-0.052 **a**	0.003
S3	0.102 **c**	0.004	0.091 **c**	0.003	-0.055 **a**	0.003
Endophyte						
Nil	0.154	0.015	0.106	0.005	-0.061	0.010
AR501	0.191	0.015	0.109	0.005	-0.073	0.010
KY31	0.179	0.015	0.107	0.005	-0.069	0.010
Interaction (P =)						
Cult.*Water	**0.041**		0.167		0.823	
Cult.* Endop.	0.729		0.909		0.102	
Water*Endop.	0.193		0.912		0.902	
Wat.*Cult.*Endop.	0.361		0.329		0.738	

[1] Standard error.

[2] Letters indicate groupings of means that are significantly different at $P<0.05$ within cultivars, water treatment or endophyte.

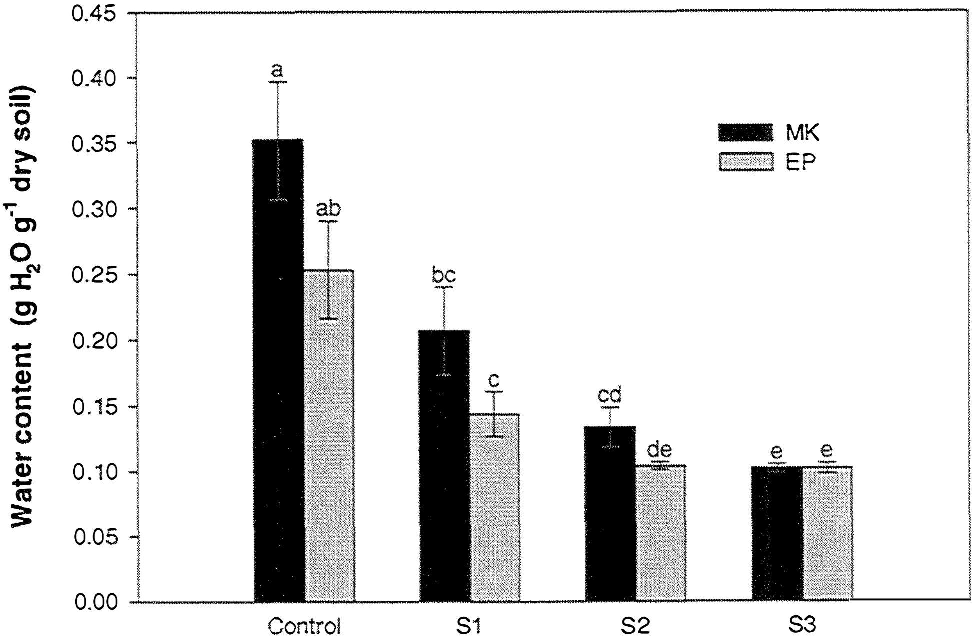

Figure 5.3. Gravimetric water content of the pots of MK and EP for the four water treatments studied. Letters indicate significant differences at $P<0.05$. Vertical lines represent standard errors.

5.4.2. Shoot characteristics

Plant dry weight and the percentages of the different plant components are shown in Table 5.2. These values should be considered as indicative only, because green leaf samples were taken prior to destructive sampling to determine plant weight. Consequently, small plants or plants with less green tissue were affected relatively more due to this procedure than big plants or plants with a higher proportion of green tissue. Even so, EP plants were clearly bigger than MK plants, in agreement with previous experiments (Chapters 3 and 4). That the proportion of dead material was lower and green leaf blade and sheath percentages higher for EP contrasted with the findings of the first experiment (Chapter 3), where EP plants had a higher percentage of dead tissue. No obvious reason for this difference was apparent.

Plant size was significantly reduced by increased water deficit ($P<0.05$, Table 5.2). As water deficit increased, the percentage of dead tissue increased, mainly because of a change in the proportion of green leaves, this diminishing from nearly 45 % in Control plants to around 26 % in S3 plants. A similar reduction of leaf area in response to water deficit was also observed in the first experiment (Chapter 3), and in general, it has been suggested as one of the most efficient means for the plant to conserve water (Passioura, 1982). A significant water x cultivar interaction for dead tissue percentage was found ($P<0.05$, Table 5.3). MK plants showed a more pronounced increase in dead tissue percentage, as water deficit increased, compared with EP plants suggesting that MK had greater stress in this third experiment.

Sheath percentage was significantly higher in EP than in MK ($P<0.05$, Table 5.2). For this variable a significant water x cultivar interaction ($P<0.05$, Table 5.4) was found. Both cultivars showed similar percentages of sheath in Control, S1 and S2 plants, but under S3 treatment the percentage of sheath increased in EP plants while decreasing in MK plants. This result reflected the higher dead tissue percentage in MK plants in relation to EP in S3 (Table 5.3). However, unlike the findings of the second experiment, in which the sheath biomass per plant tended to increase with increased water deficit, in this case the reverse occurred (Control = 6.11 g DW plant^{-1}, S = 5.76 g DW plant^{-1}, S2 = 4.86 g DW plant^{-1} and S3 = 3.66 g DW plant^{-1}, s.e. = 0.23 g DW plant^{-1}, $P<0.05$) indicating that the water deficit was not sufficiently extreme to promote flowering (Bazzas *et al.*, 1987).

The presence of the endophyte decreased plant dry weight ($P<0.05$, Table 5.2), which contrasts with the results of Cheplick *et al.* (1989) and Latch *et al.* (1985). Cheplick *et al.* (1989) found that adult E+ tall fescue plants had higher biomass than E- plants independently of the nutrient availability. Latch *et al.* (1985) observed an increased dry matter yield in Nui ryegrass E+ plants compared with E- plants. However, other experiments suggested that dry mass yield was affected by genotypic differences between individuals of both plant and fungus (De Battista *et al.*, 1990).

In the present experiment the endophyte AR501 decreased dead tissue percentage ($P<0.05$), and increased the percentage of green leaf blade ($P<0.05$, Table 5.2). Endophyte free plants had a sheath percentage similar to plants infected with either endophyte, however the proportion of sheath in AR501-E+ plants tended to be higher than in KY31-E+ plants ($P<0.10$).

As in the first experiment (Chapter 3), initial and final tiller number were approximately 60 % higher in MK plants (Table 5.5). The difference between both measurements showed that during the experimental period tiller number increased in

MK and decreased in EP ($P<0.05$, Table 5.5). Although final tiller number was not affected by the water treatments, the difference between tiller number at the beginning and end of the experiment showed that in Control and S1 treatments tiller number increased, while in S2 and S3 it decreased ($P<0.05$).

Endophyte-infected plants showed a reduced number of tillers, both at the start and end of the experiment, and the absence of any significant effect ($P>0.05$) of endophyte in the difference in tiller number between both dates, suggested that this tendency was not affected by the application of the water treatments. This result agrees with Maclean *et al.* (1993) who found that the endophyte decreased tillering rate in 'Grassland Roa' tall fescue plants. On the other hand, Belesky *et al.* (1989) observed that tiller production in response to endophyte varied among different accessions of KY31 tall fescue. Similarly, in the present experiment, a significant interaction between cultivar and endophyte was observed for initial tiller number ($P<0.05$). The presence of either of the endophytes studied diminished tiller number in MK plants but did not affect EP plants (Fig. 5.4). The same tendency was observed for final tiller number ($P<0.10$). This inconsistent effect of the endophyte on tiller production has also been observed by Hill *et al.* (1990).

Tillers of EP were significantly heavier than MK ($P<0.05$, Table 5.5), and tiller size diminished ($P<0.05$) as the water deficit increased. No differences in tiller size due to endophyte were found ($P>0.05$). Conversely, Hill *et al.* (1990) reported that yield per tiller was greater in E+ tall fescue plants than in E- plants.

Table 5.2. Plant dry weight (DW) and percentages of shoot DW as leaf blade, sheath and dead tissue for the cultivars MK, GA and EP, the four water treatments and three endophytes.

Cultivar	**Plant DW (g)**	**% DW as leaf blade**	**% DW as sheath**	**% DW as dead tissue**
MK	13.43 **b**[1]	35.2 (36.2) **b**	33.6 (35.3) **b**	31.2 (33.5) **a**
EP	15.73 **a**	37.2 (37.4) **a**	36.3 (37.0) **a**	26.5 (30.8) **b**
s.e.[2]	0.26	(0.47)	(0.5)	(0.7)
Water				
Control	17.92 **a**	44.4 (41.8) **a**	34.0 (35.6)	21.6 (27.5) **c**
S1	16.39 **b**	40.5 (39.5) **b**	35.1 (36.3)	24.4 (29.3) **c**
S2	13.59 **c**	33.7 (35.4) **c**	35.9 (36.7)	30.5 (33.4) **b**
S3	10.43 **d**	26.2 (30.6) **d**	34.8 (36.0)	39.0 (38.5) **a**
s.e.	0.40	(0.6)	(0.4)	(0.7)
Endophyte				
Nil	15.86 **a**	34.5 (35.8) **b**	35.8 (36.6)	29.8 (32.8) **a**
AR501	14.25 **b**	38.2 (38.0) **a**	36.1 (36.9)	25.7 (30.1) **b**
KY31	13.63 **b**	35.9 (36.7) **b**	33.0 (34.9)	31.1 (33.6) **a**
s.e.	0.32	(0.5)	(0.6)	(0.8)
Interactions (P=)				
Cult.*Wat.	0.100	0.384	**0.007**	**0.007**
Cult.*Endop.	0.309	0.719	0.181	0.592
Wat.*Endop.	0.771	0.899	0.976	0.999
Wat.*Cult.*Endop.	0.540	0.662	0.856	0.899

Data within brackets are arcsin square root transformed percentages.

[1] Letters indicate groupings of means that are significantly different at $P<0.05$ within cultivars, water treatment or endophyte.

[2] Standard error.

Table 5.3. Percentage of shoot DW as dead tissue (%) for cultivars and water treatments.

Water	**MK**	**EP**
Control	21.0 (27.0) **d**[1]	22.2 (27.9) **cd**
S1	24.8 (29.3) **cd**	24.1 (29.3) **cd**
S2	33.3 (35.2) **b**	27.6 (31.6) **bc**
S3	45.8 (42.6) **a**	32.2 (34.4) **b**
s.e.[2]	(1.38)	

Data within brackets are arcsin square root transformed percentages.

[1] Letters indicate groupings of means that are significantly different at $P<0.05$.

[2] Standard error.

Table 5.4. Percentage of shoot DW as sheath (%) for cultivars and water treatments.

Water	**MK**	**EP**
Control	34.68 (35.98) **bc**[1]	33.29 (35.17) **bc**
S1	35.45 (36.48) **ab**	34.78 (36.09) **ab**
S2	34.23 (35.75) **bc**	37.52 (37.73) **ab**
S3	29.98 (33.08) **c**	39.67 (38.98) **a**
s.e.[2]	(1.04)	

Data within brackets are arcsin square root transformed percentages.
[1] Letters indicate groupings of means that are significantly different at P<0.05.
[2] Standard error.

Table 5.5. Initial tiller number, final tiller number, tiller number difference and tiller size for the cultivars MK, GA and EP, the four water treatments and three endophytes.

Cultivar	**Initial tiller number**	**Final tiller number**	**Tiller difference**	**Tiller size (mg tiller^{-1})**
MK	93.0 **a**[1]	93.2 **a**	4.82 **a**	0.148 **b**
EP	57.1 **b**	58.8 **b**	-2.98 **b**	0.274 **a**
s.e[2]	2.9	2.5	1.67	0.007
Water				
Control	71.5	77.8	5.33 **a**	0.251 **a**
S1	77.1	80.2	3.57 **a**	0.228 **b**
S2	78.3	76.5	-0.93 **b**	0.198 **c**
S3	73.5	69.6	-4.29 **b**	0.167 **d**
s.e.	3.2	2.9	1.17[§]	0.004
Endophyte				
Nil	85.5 **a**	84.2 **a**	1.34	0.208
AR501	72.9 **b**	74.9 **b**	1.43	0.208
KY31	66.9 **b**	69.0 **b**	-0.02	0.217
s.e.	3.5	3.0	1.65[§]	0.008
Interactions (P=)				
Cult.*Water	0.588	0.927	0.475	0.320
Cult.*Endop.	**0.023**	0.073	0.796	0.727
Water*Endop.	0.769	0.933	0.636	0.983
Water*Cult.*Endop.	0.954	0.976	0.826	0.973

[1] Letters indicate groupings of means that are significantly different at P<0.05 within cultivars, water treatment or endophyte.
[2] Standard error.
[§] Mean standard error.

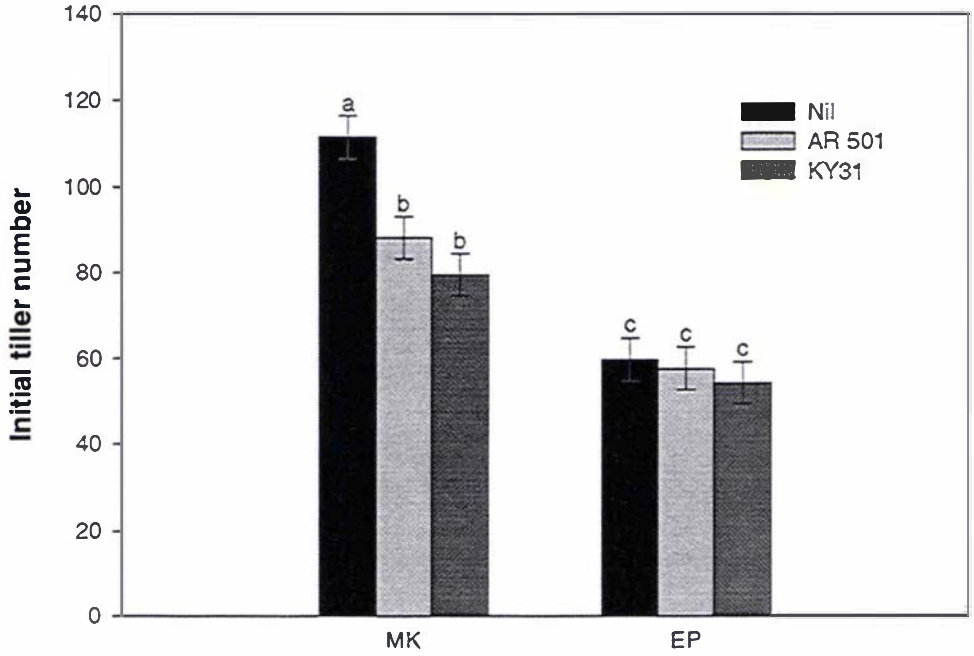

Figure 5.4. Effect of the endophyte on MK and EP initial tiller number. Letters indicate significant differences at P<0.05. Vertical lines represent standard errors.

5.4.3. Tissue turnover

During the period studied EP plants showed a higher GGR per tiller than MK plants ($P<0.05$), with no difference in SR per tiller ($P>0.05$) and consequently, NGR was greater in EP tillers ($P<0.05$, Fig. 5.5a). Correcting for difference in number of tillers per plant did not remove the yield differences between the two cultivars and in addition, SR in MK plants was significantly greater than in EP plants ($P<0.05$, Fig. 5.5b). The relative growth rate (RGR), estimated as the ratio between NGR (g plant^{-1} d^{-1}) and final plant size (g plant^{-1}), consistently, was also greater for EP than for the Mediterranean cultivar (EP = 0.71 % DW d^{-1} and MK = 0.36 % DW d^{-1}, s.e. = 0.05 % DW d^{-1}, $P<0.05$).

Direct comparison between growth rates obtained for MK and EP in this experiment and the previous one (Chapter 4) is not valid for three reasons. First, as the cultivar means result from the average through all water treatments, lower mean rates should be expected for the present experiment. This is because in the previous experiment under the most severe water treatment, S3, plants received 50 % of the amount of water of the Control treatment, while here the proportion was only 25 %. Second, the glasshouse maximum daily temperatures, during the period when the tissue turnover measurements were taken, were higher in the experiment of Chapter 4 (about 40°C) than in the current experiment (about 23°C). Third, in the experiment of Chapter 4 MK and EP plants were grown together in the same pots, while here isolated plants of each cultivar were grown in smaller pots.

The GGR per tiller decreased significantly ($P<0.05$) with increasing water deficit (Fig. 5.6a). This rate showed a significant ($P<0.05$) water x cultivar interaction. While EP differed significantly ($P<0.05$) between treatments, MK presented similar values of GGR in Control and S1 treatments and also in S2 and S3 treatments (Fig. 5.7). However, in relative terms, MK showed a higher reduction in GGR from Control to S3 than EP (70 % for MK and 61 % for EP). As shown in Fig. 5.7, maximum GGR in MK was similar to minimum GGR in EP. GGR per plant (Fig. 5.6b) followed the same pattern as GGR per tiller (Fig. 5.5a) but all water treatments were significantly different ($P<0.05$) and no water x cultivar interaction was found ($P>0.05$).

SR expressed either on a per tiller (Fig. 5.6a) or per plant (Fig. 5.6b) basis, increased ($P<0.05$) with the severity of the water deficit, but no differences ($P>0.05$) were found between Control and S1 treatments. A significant ($P<0.05$) water x cultivar interaction in SR per plant was found (Fig. 5.8). SR per plant was lower in MK plants than in EP plants under the Control water treatment, but tended to be higher in S1 and S2, difference that became significant ($P<0.05$) in S3.

NGR per tiller decreased significantly ($P<0.05$) with increasing water deficit (Fig. 5.6a). However, while NGR mean was positive in S3 when expressed on a per tiller basis it was negative on a per plant basis. This was because NGR per tiller was influenced more by the positive accumulation in the larger EP tillers whereas NGR per plant reflected more the larger tiller number per plant of MK (Table 5.5) and the high senescence rate of that cultivar. Similarly, the RGR per plant was significantly different between treatments (Control = 1.06 % DW d^{-1}, S1 = 0.80 % DW d^{-1}, S2 = 0.35 % DW d^{-1} and S3 = -0.09 % DW d^{-1}, s.e. = 0.08 % DW d^{-1}, $P<0.05$). However, for this variable the interaction cultivar x water treatment was significant because while both cultivars showed a similar RGR in Control and S1 water treatments, MK plants showed a lower RGR than EP plants in S2 and S3. This result is further evidence that the growth rate of the Mediterranean cultivar was more affected than the temperate cultivar by the soil water deficit.

The GGR per tiller was lower ($P<0.05$) in E- plants than in AR501-E+ or KY31-E+ plants (Fig. 5.9a). No significant differences between endophyte treatments were found for GGR per plant ($P>0.05$, Fig. 5.9b). GGR either on a per tiller or per plant basis showed a significant ($P<0.05$) cultivar x endophyte interaction (Fig. 5.10). EP GGR was lower ($P<0.05$) in E- than in E+ EP tillers, while no endophyte effect was observed in MK tillers (Fig. 5.10a). The same pattern was observed for GGR per plant interaction, however, E- plants of EP did not differ from E+ plants and, on the other hand, MK plants tended to have a lower GGR when they were infected by KY31 endophyte than either when they were E- or infected by AR501 endophyte ($P>0.05$, Fig. 5.10b). Other workers, including Richardson *et al.* (1990), Eerens *et al.* (1993) and Belesky *et al.* (1989) have also found that endophyte influence on biomass production or leaf extension rate varied depending on plant genotype.

SR per tiller of E- plants was higher ($P<0.05$) than for AR501-E+ plants and KY31-E+ plants were intermediate ($P>0.05$, Fig. 5.9a). On the other hand, SR per plant was greater in E- plants ($P<0.05$, Fig. 5.9b). A clear advantage of E+ plants in NGR per tiller was observed ($P<0.05$, Fig. 5.9a). However, when NGR per plant was calculated, KY31-E+ plants were intermediate between AR501-E+ and E- plants (Fig. 5.9b). The RGR, on the other hand, was higher for the E+ plants and similar for both endophytes (E- = 0.34 % DW d^{-1}, AR501 = 0.66 % DW d^{-1} and KY3 = 0.59 % DW d^{-1}, s.e. = 0.06 % DW d^{-1}, $P<0.05$).

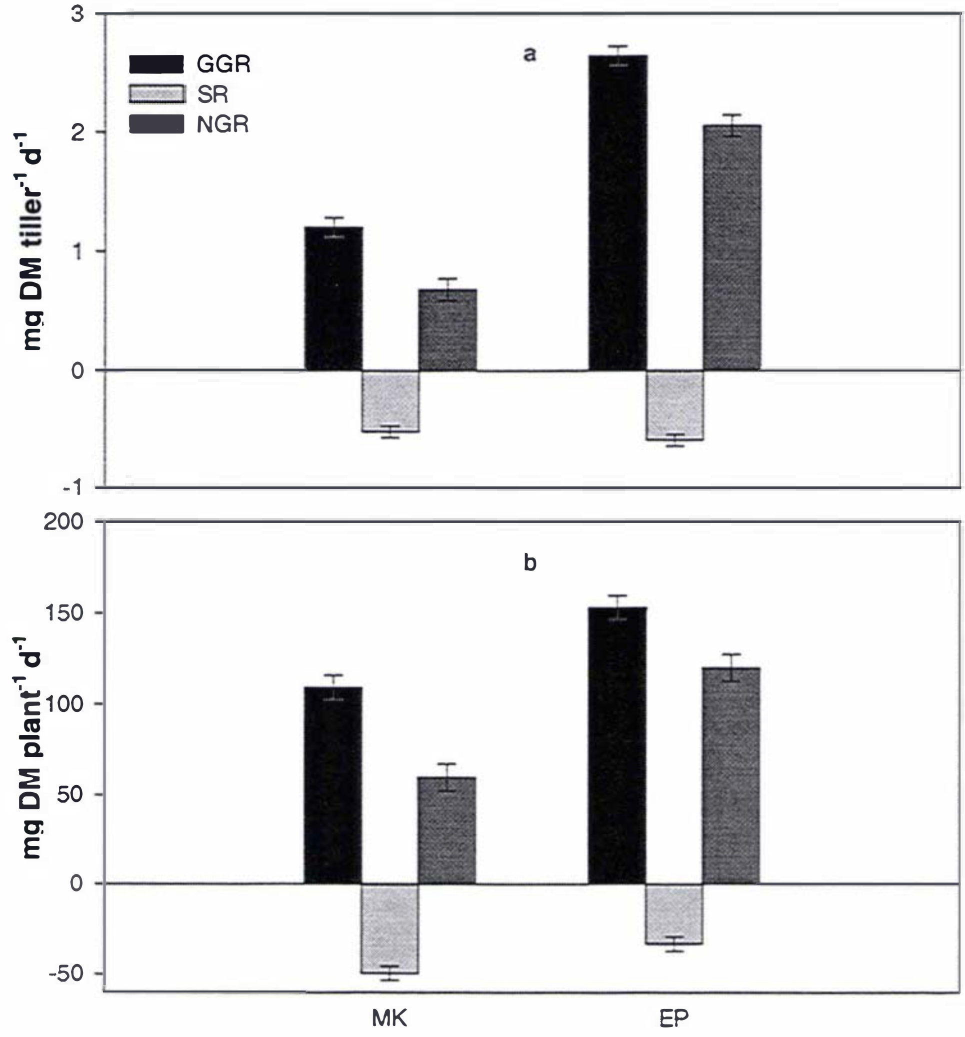

Figure 5.5. Gross growth rate (GGR), senescence rate (SR) and net growth rate (NGR) for MK and EP cultivars. a: Expressed per tiller (mg DW tiller^{-1} d^{-1}). b: Expressed per plant (mg DW plant^{-1} d^{-1}). Vertical lines represent standard errors.

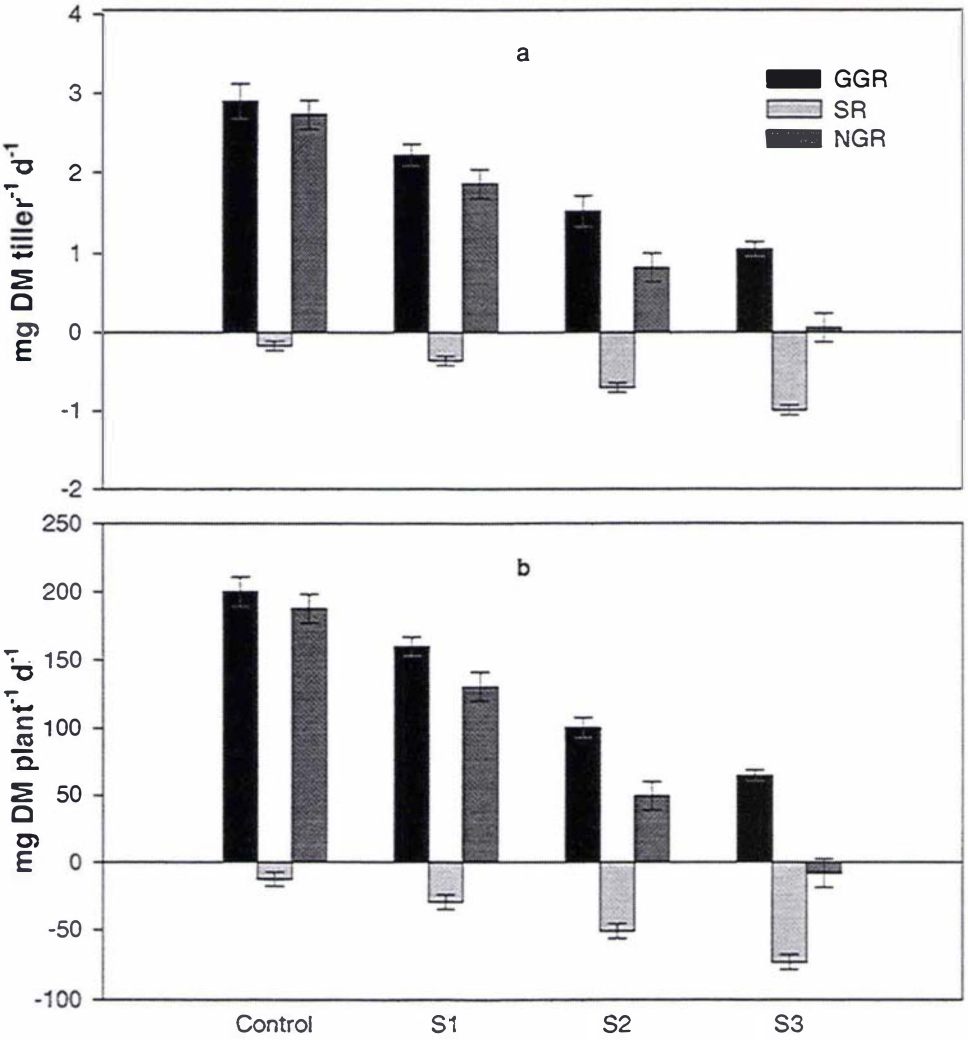

Figure 5.6. Gross growth rate (GGR), senescence rate (SR) and net growth rate (NGR) for the four water treatments. a: Expressed per tiller (mg DW tiller^{-1} d^{-1}). b: Expressed per plant (mg DW plant^{-1} d^{-1}). Vertical lines represent standard errors.

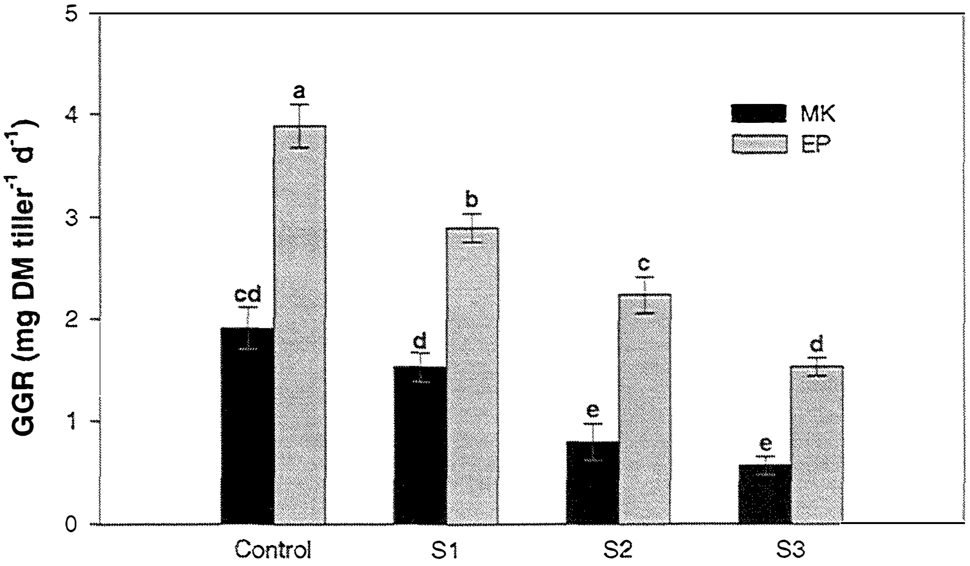

Figure 5.7. Gross growth rate (GGR) per tiller of MK and EP cultivars for the four water treatments. Letters indicate significant differences at P<0.05. Vertical lines represent standard errors.

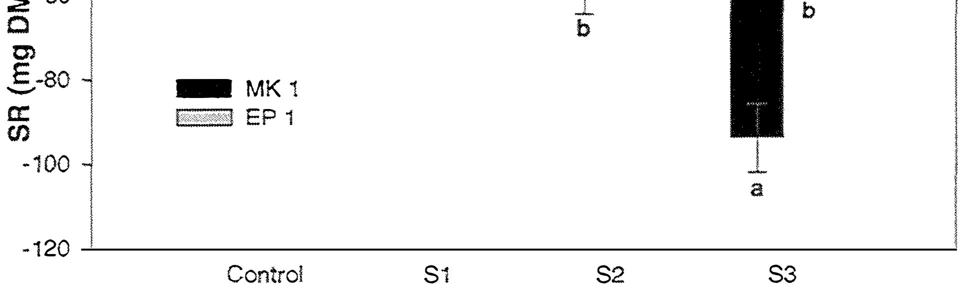

Figure 5.8. Senescence rate (SR) per plant of MK and EP cultivars for the four water treatments. Letters indicate significant differences at P<0.05. Vertical lines represent standard errors.

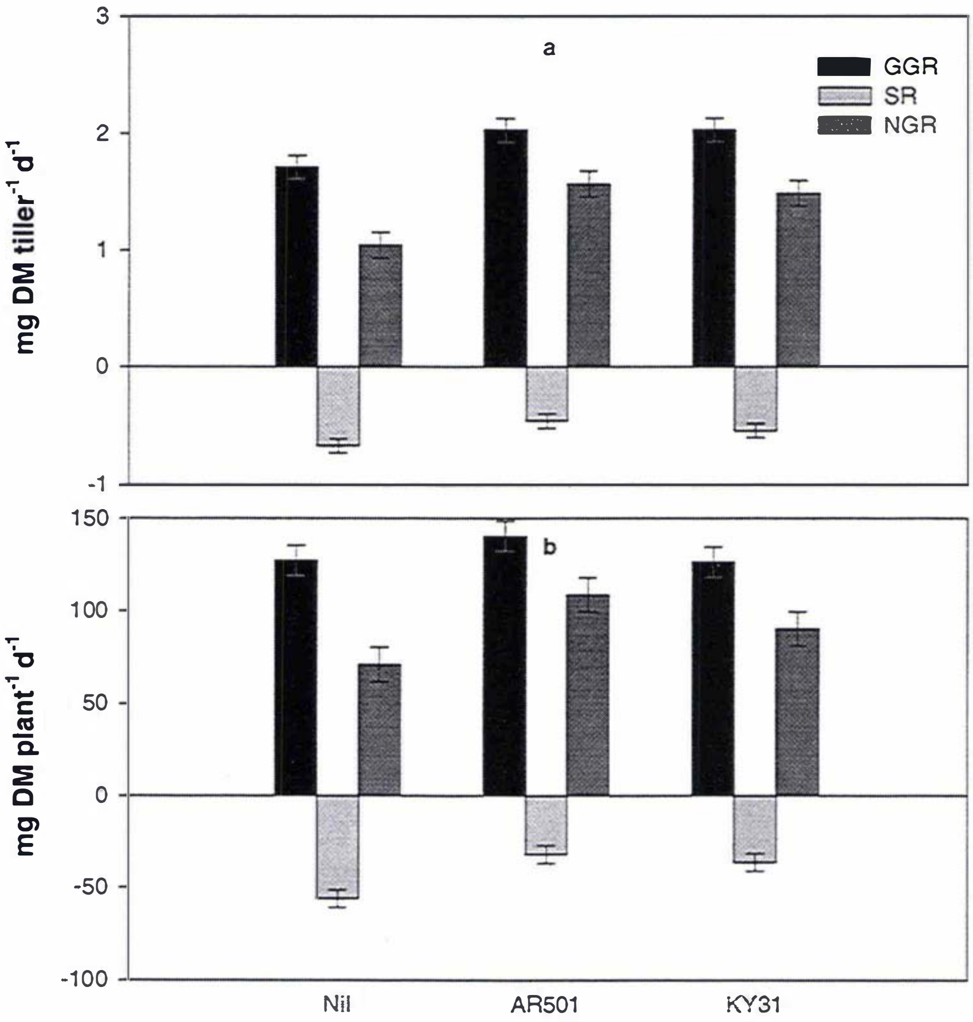

Figure 5.9. Gross growth rate (GGR), senescence rate (SR) and net growth rate (NGR) for the endophyte treatments. a: Expressed per tiller (mg DM tiller^{-1} d^{-1}). b: Expressed per plant (mg DM plant^{-1} d^{-1}). Vertical lines represent standard errors.

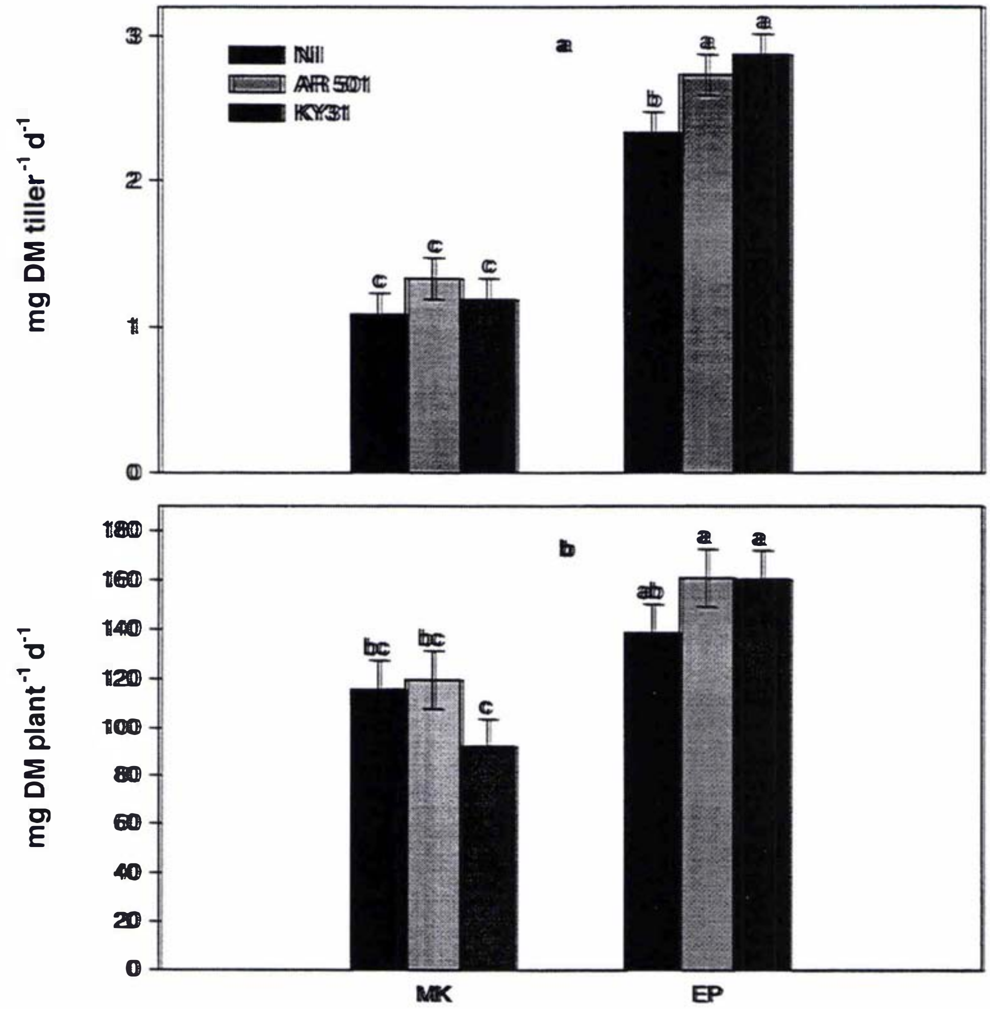

Figure 5.10. Gross growth rate (GGR) of MK and EP cultivars for the three endophyte treatments. a: Expressed per tiller (mg DM tiller^{-1} d $^{-1}$). b: Expressed per plant (mg DM plant^{-1} d^{-1}). Letters indicate significant differences at $P<0.05$. Vertical lines represent standard errors.

Since LAR data were derived from counts rather than measurement of a continuous variable, maximum likelihood analysis techniques are often recommended as more appropriate (McCullagh and Nelder, 1989). However, the ANOVA produced such small standard errors (relative to the differences between cultivars and some of the water regimes) that the distributional assumptions could make no difference to the substantive conclusions. EP plants had a higher ($P<0.05$) LAR than MK plants (Table 5.6). LAR was reduced ($P<0.05$) by water deficit, although LAR for S3 was not significantly less than for S2. Endophyte effect and the interactions were not significant ($P>0.05$).

Table 5.6. Leaf appearance rate (LAR).

Cultivar	LAR (leaf tiller^{-1} d^{-1})
MK	0.022 **b**[1]
EP	0.031 **a**
s.e.[2]	0.002
Water	
Control	0.049 **a**
S1	0.030 **b**
S2	0.015 **c**
S3	0.012 **c**
s.e.	0.004
Endophyte	
Nil	0.022
AR501	0.031
KY31	0.026
s.e.	0.003
Interactions (P=)	
Cult.r*Water	0.978
Cult.*Endop.	0.933
Water*Endop.	0.443
Water*Cult.*Endop.	0.152

[1] Letters indicate groupings of means that are significantly different at $P<0.05$ within cultivars, water treatment or endophyte.
[2] Standard error.

5.4.4. Plant water relations

MK plants showed a higher ($P<0.05$) RWC than EP plants in both periods (Table 5.7). As expected, RWC diminished with increases in soil water deficit ($P<0.05$). In this experiment, no significant effect of endophyte on RWC was observed ($P>0.05$). Conversely, Elbersen and West (1996) found that the fractional water content (e.g. (FW-DW)/FW), was higher in E+ plants, but in their experiment the sheath tissue was measured rather than leaf blade tissue as was measured here. The RWC-difference between periods indicated that while RWC increased in MK plants, the opposite occurred in EP plants ($P<0.05$).

Table 5.7. Relative water content (RWC) of the two periods (1 and 2), and the adjusted difference between them.

Cultivar	RWC 1 (%)	RWC 2 (%)	RWC-difference (%)
MK	82.4 (67.0) **a**[1]	86.0 (69.3) **a**	4.60 **a**
EP	78.7 (63.9) **b**	78.1 (63.2) **b**	-1.57 **b**
s.e.[2]	(1.0)	(0.8)	1.09
Water			
Control	93.0 (75.4) **a**	90.8 (73.3) **a**	4.32
S1	86.3 (69.8) **ab**	85.3 (68.9) **ab**	2.06
S2	79.2 (63.5) **b**	79.7 (64.1) **bc**	-0.20
S3	63.6 (53.2) **c**	72.3 (58.7) **c**	-0.10
s.e.	(2.0)	(1.8)	3.48§
Endophyte			
Nil	78.5 (63.9)	81.7 (65.9)	2.16
AR501	81.8 (66.6)	81.7 (66.1)	0.61
KY31	81.3 (65.9)	82.7 (66.8)	1.79
s.e.	(1.2)	(0.9)	1.32§
Interactions (P =)			
Cult.*Water	0.855	0.216	0.189
Cult.*Endop.	0.364	0.569	0.556
Water*Endop.	0.596	0.830	0.969
Water*Cult.*Endop.	0.673	0.627	0.926

Data within brackets are arcsin square root transformed percentages.

[1] Letters indicate groupings of means that are significantly different at $P<0.05$ within cultivars, water treatment or endophyte.

[2] Standard error.

§ Mean standard error.

The Ψ_w tended to be significantly lower in EP than in MK ($P<0.10$) in the first period, but not in the second (Table 5.8). As in the previous experiments (Chapters 3 and 4), the Ψ_w decreased as the soil water deficit increased. In both periods Ψ_w of Control and S1 treatments differed from Ψ_w of S2 and S3 treatments ($P<0.05$). While Hill *et al.* (1996) found that tall fescue Ψ_w responded differently to water deficit depending upon endophyte isolate, White *et al.* (1992a) found, as in this experiment, that endophyte presence did not affect Ψ_w ($P>0.05$). The Ψ_w-difference between periods indicated that Ψ_w tended to increase in Control and S1 treatment and to decrease in S2 and S3 treatments ($P<0.10$).

Table 5.8. Water potential (Ψ_w) of the two periods (1 and 2), and the adjusted difference between them.

Cultivar	Ψ_w 1 (MPa)	Ψ_w 2 (MPa)	Ψ_w-difference (MPa)
MK	-1.69	-1.74	0.011
EP	-1.87	-1.82	-0.015
s.e.[1]	0.07	0.05	0.048
Water			
Control	-1.03 **a**[2]	-1.19 **a**	0.363
S1	-1.37 **a**	-1.55 **a**	0.108
S2	-2.12 **b**	-2.09 **b**	-0.204
S3	-2.59 **b**	-2.31 **b**	-0.274
s.e.	0.16	0.13	0.176§
Endophyte			
Nil	-1.78	-1.83	-0.045
AR501	-1.76	-1.75	0.025
KY31	-1.81	-1.77	0.015
s.e.	0.09	0.06	0.058§
Interactions (P =)			
Cult.*Water	0.266	0.498	0.608
Cult.*Endop.	0.717	0.253	0.244
Water*Endop.	0.169	0.634	0.858
Water*Cult.*Endop.	0.211	0.934	0.830

[1] Standard error.

[2] Letter indicate groupings of means that are significantly different at $P<0.05$ within cultivars, water treatment or endophyte.

§ Mean standard error.

The Ψ_o was higher in MK plants, however this difference was significant ($P<0.05$) only in the second period (Table 5.9). Consequently, Ψ_o-difference between periods suggested that Ψ_o increased in MK while remaining unchanged in EP ($P<0.05$). The water treatments affected Ψ_o ($P<0.05$) in both periods. The Ψ_o decreased as water deficit increased ($P<0.05$). When the Ψ_o was analysed using RWC as covariate, a significant difference between water treatments was found for both periods ($P<0.05$, Appendix 5.2) indicating that osmotic adjustment took place. The Ψ_o-difference showed an increase in Ψ_o of the Control treatment in relation to Ψ_o of other water treatments. For Ψ_o in the second period and the Ψ_o-difference between periods, a significant water x cultivar interaction was found ($P<0.05$, Figs. 5.11a and 5.11b). This arose because in the S3 treatment, Ψ_o was significantly lower for EP than MK, while in the other three water treatments both cultivars had a similar Ψ_o ($P>0.05$).

As for Ψ_w, no endophyte effect on Ψ_o was observed ($P>0.05$). However, the cultivar x endophyte interaction was significant at both measurement dates ($P<0.05$, Fig. 5.12). This was because in MK the endophyte AR501 tended to increase Ψ_o and the KY31 endophyte tended to decrease Ψ_o compared to the E- plants, while in EP, the opposite was observed. The experimental results reported in the literature are variable with respect to the effect of endophyte on Ψ_o. Hill *et al.* (1996) and White *et al.* (1992a) found no effect, whereas, the endophyte decreased the Ψ_o of KY31 population plants (Elmi *et al.*, 1990; Elmi and West, 1995) and the cultivar Grasslands Roa (Maclean *et al.*, 1993). This adds to a growing body of evidence that plant responses to endophyte can vary between experiments, or even between plant genotypes or endophyte strains within an experiment.

The Ψ_p calculated as the difference between Ψ_w and Ψ_o (Table 5.10) was significantly different between water treatments. In the first period, the Ψ_p of S2 plants was lower ($P<0.05$) than the Ψ_p of plants subjected to Control and S1 water treatments. In the second period, plants subjected to both S2 and S3 water treatments differed from plants under Control and S1 treatments ($P<0.05$). The water x cultivar interaction was significant for Ψ_p in the second period (Fig. 5.13a) and for the Ψ_p-difference (Fig. 5.13b). For EP Ψ_p and Ψ_p-difference under S3 were similar to under S2, whereas for MK both variables showed a continuous decrease. These results suggest that EP was more responsive to water deficit and was able to maintain Ψ_p by decreasing its Ψ_o under more intense water deficit. The absence of an endophyte effect on the Ψ_p contrasts with the results reported by Elmi (1992; quoted by West and Gwinn, 1993), who observed an increase in the turgor pressure of water stressed E+ plants. However, that increase was larger in the basal 20 mm growing zone that in the leaf blade, which was the tissue measured in the present experiment. Moreover, the short term nature of the present experiment could have been another factor contributing to the lack of a significant endophyte effect on Ψ_p.

Table 5.9. Osmotic potential (Ψ_o) of the two periods (1 and 2), and the adjusted difference between them.

Cultivar	**Ψ_o 1 (MPa)**	**Ψ_o 2 (MPa)**	**Ψ_o-difference (MPa)**
MK	-2.11	-2.04 **a**[1]	0.112 **a**
EP	-2.21	-2.19 **b**	-0.029 **b**
s.e.[2]	0.07	0.04	0.039
Water			
Control	-1.58 **a**	-1.75 **a**	0.322 **a**
S1	-1.99 **b**	-2.06 **b**	0.072 **b**
S2	-2.23 **b**	-2.22 **b**	-0.047 **b**
S3	-2.83 **c**	-2.44 **c**	-0.182 **b**
s.e.	0.09§	0.07	0.078§
Endophyte			
Nil	-2.13	-2.11	0.047
AR501	-2.18	-2.11	0.052
KY31	-2.17	-2.14	0.024
s.e.	0.09	0.05	0.047
Interactions (P =)			
Cult.*Water	0.843	**0.040**	**0.051**
Cult.*Endop.	**0.005**	**0.033**	0.272
Water*Endop.	0.718	0.790	0.810
Water*Cult.*Endop.	0.628	0.300	0.380

[1] Letters indicate groupings of means that are significantly different at $P<0.05$ within cultivars, water treatment or endophyte.

[2] Standard error.

§ Mean standard error.

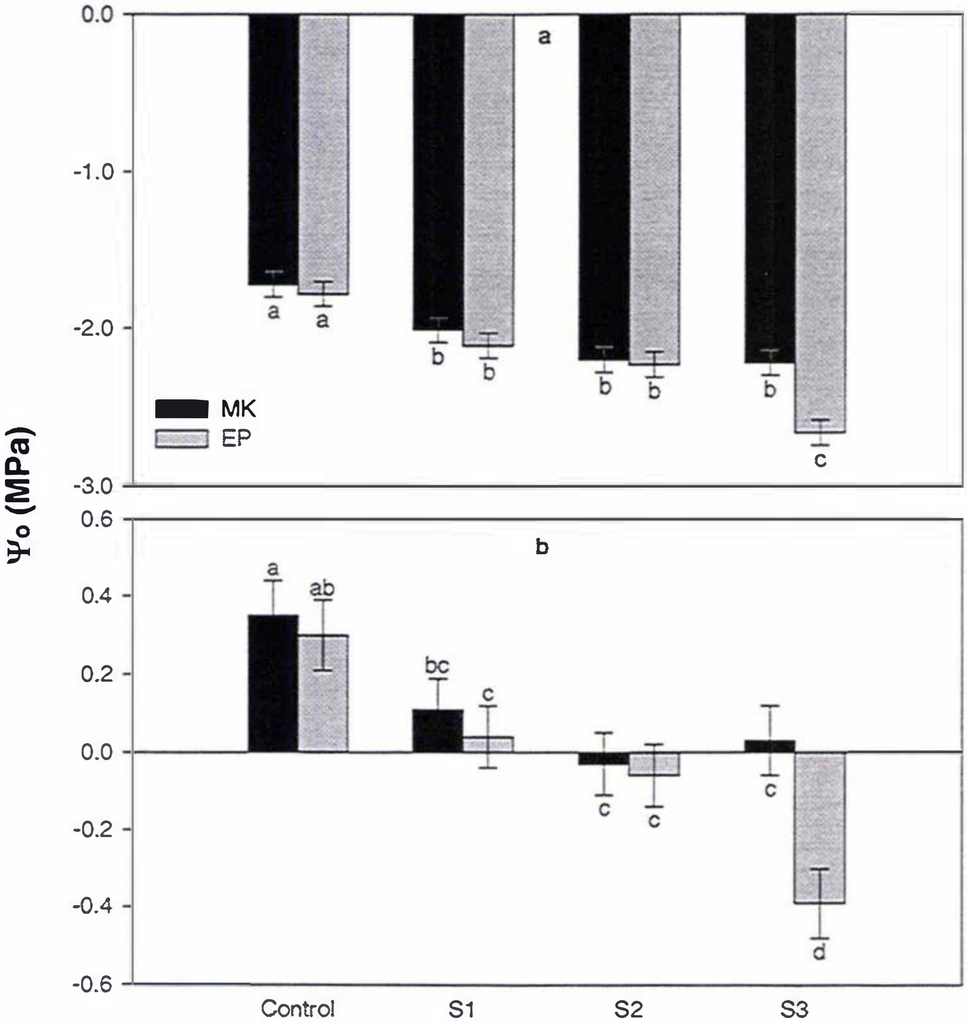

Figure 5.11. Osmotic potential for the second period (a) and osmotic potential difference between periods (b) for MK and EP cultivars under the four water treatments. Letters indicate significant differences at $P<0.05$. Vertical lines represent standard errors.

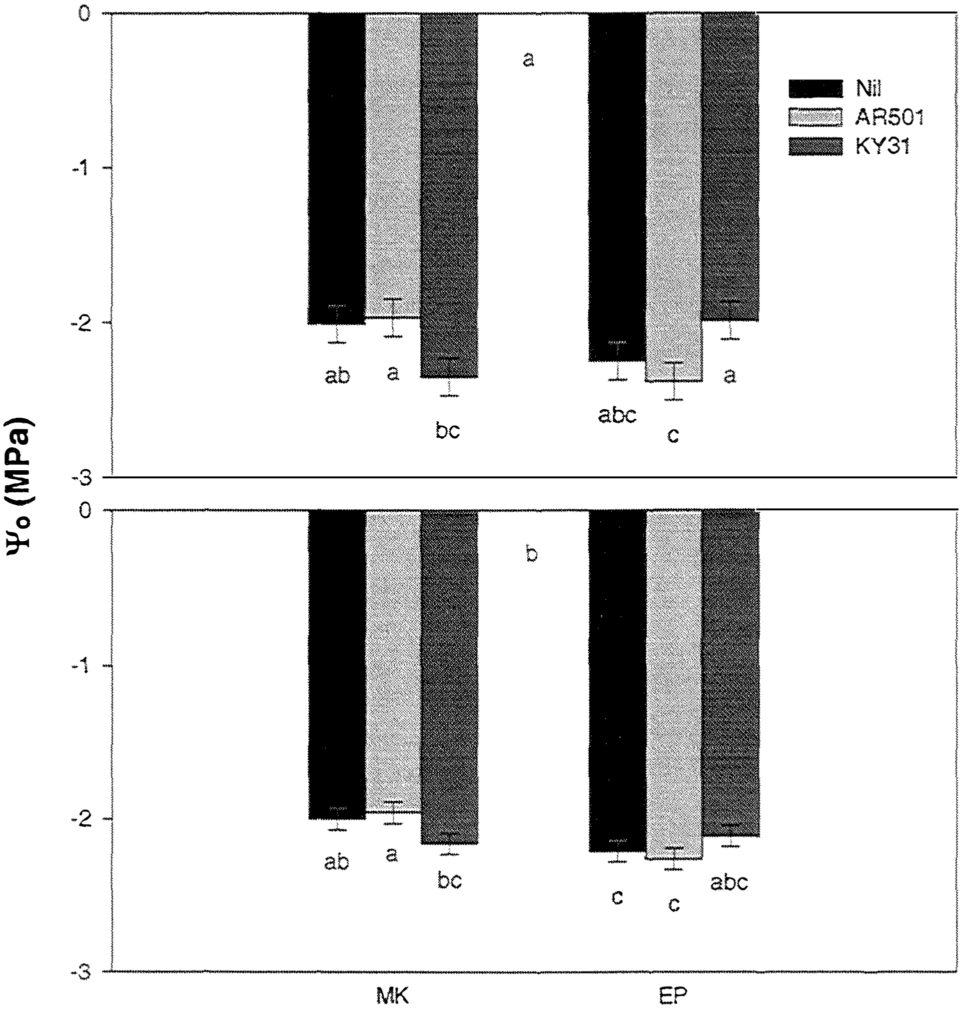

Figure 5.12. Osmotic potential of MK and EP cultivars for the three endophyte treatments. a: Period 1. b: Period 2. Letters indicate significant differences at $P<0.05$. Vertical lines represent standard errors.

Table 5.10. Pressure potential (Ψ_p) of the two periods (1 and 2), and the adjusted difference between them.

Cultivar	**Ψ_p 1 (MPa)**	**Ψ_p 2 (MPa)**	**Ψ_p-difference (MPa)**
MK	0.42	0.30	-0.081
EP	0.33	0.37	-0.005
s.e.[1]	0.07	0.06	0.057
Water			
Control	0.55 **a**[2]	0.56 **a**	0.178
S1	0.62 **a**	0.51 **a**	0.126
S2	0.11 **b**	0.13 **b**	-0.240
S3	0.24 **ab**	0.14 **b**	-0.236
s.e.	0.12[§]	0.12	0.122[§]
Endophyte			
Nil	0.35	0.28	-0.095
AR501	0.42	0.36	-0.019
KY31	0.36	0.36	-0.015
s.e.	0.09	0.07	0.069
Interactions (P=)			
Cult.*Water	0.399	**0.023**	**0.027**
Cult.*Endop.	0.080	0.272	0.306
Water*Endop.	0.262	0.657	0.739
Water*Cult.*Endop.	0.497	0.926	0.915

[1] Standard error.

[2] Letters indicate groupings of means that are significantly different at $P<0.05$ within cultivars, water treatment or endophyte.

[§] Mean standard error.

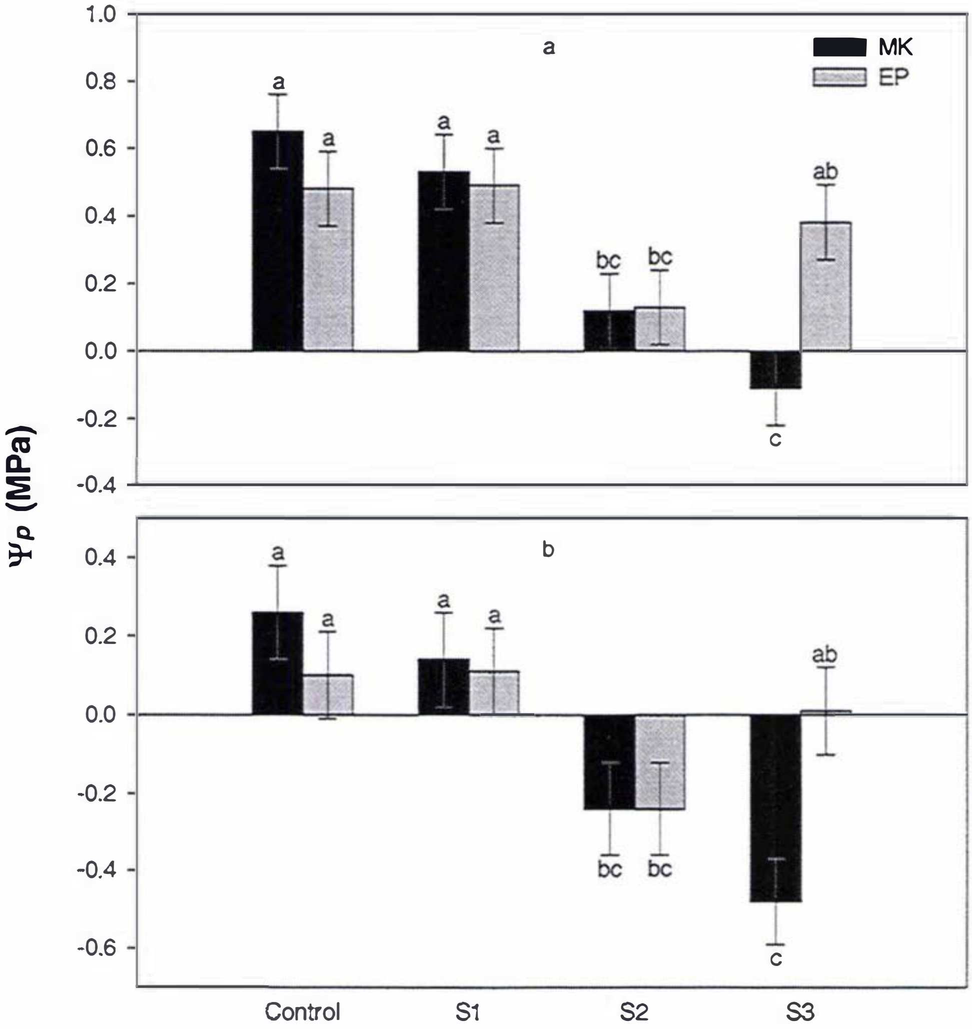

Figure 5.13. Pressure potential for the second period (a) and pressure potential difference between periods (b) for MK and EP under the four water treatments. Letters indicate significant differences at P<0.05. Vertical lines represent standard errors.

The relationships between Ψ_w, Ψ_o and Ψ_p and RWC were investigated to check for cultivar differences and were found to follow quadratic responses as shown in Fig. 5.14. There was no advantage in fitting individual curves for each cultivar ($P>0.05$), suggesting that both cultivars responded similarly to RWC. Each point is the average of the three endophyte treatments. It should be noted, however, that three EP points were omitted because their RWC value was outside the range of MK RWC values (i.e. the three EP RWC values were lower than 60 %).

The NGR overall means for cultivar and water treatment, expressed per tiller or per plant, showed a linear response to the overall mean average RWC (Figs. 5.15a and 5.15b). This response was different for each cultivar ($P<0.05$) but the cultivar curves were parallel ($P>0.05$). EP showed a greater NGR at any RWC, both expressed per tiller or per plant. In addition, the evidence of curvature for EP would suggest that EP maintained its NGR better than MK at lower RWC.

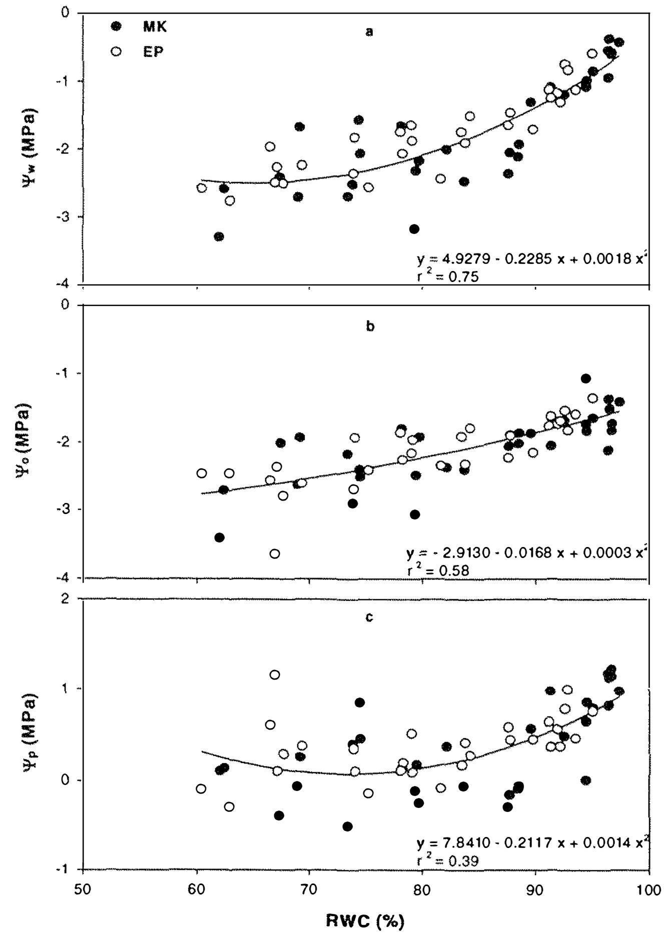

Figure 5.14. Water potential (a), osmotic potential (b) and pressure potential (c) in relation to the relative water content (RWC) for MK and EP cultivars. Each point is the average of three endophyte treatments.

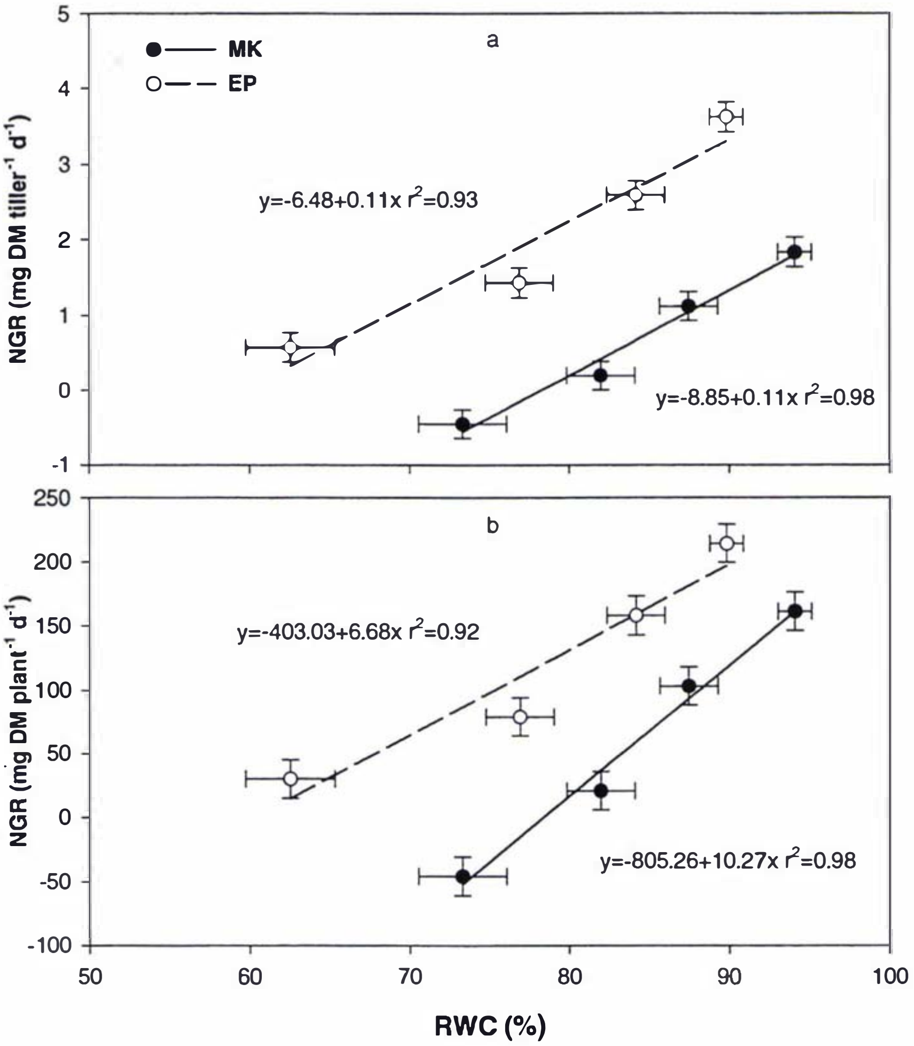

Figure 5.15. Tiller (a) and plant (b) net growth rate (NGR) in relation to the relative water content (RWC) for MK and EP cultivars. Each point is the average of 3 endophytes, 4 blocks and 2 periods. Vertical and horizontal lines represent standard errors.

5.4.5. Photosynthesis and stomatal resistance

MK showed a higher photosynthesis rate than EP, however the difference was significant only when expressed per unit leaf area ($P<0.05$). This could be explained by the lower stomatal resistance of MK ($P<0.05$, Table 5.11). The lack of significant differences between cultivars when expressed on a weight basis could be related to the slighter higher specific leaf weight (SLW) of MK than EP (MK = 4.46 mg cm^{-2} and EP = 4.29 mg cm^{-2}, s.e. = 0.08 mg cm^{-2}, $P>0.05$). Water treatment effects on photosynthesis rate were only evident when photosynthesis was expressed on a weight basis ($P<0.05$) and reflected the increase in SLW in response to water deficit (Control = 4.03 mg cm^{-2}, S1 = 4.16 mg cm^{-2}, S2 = 4.56 mg cm^2 and S3 = 4.74 mg cm^2, s.e. = 0.14 mg cm^{-2}, $P<0.05$). Interestingly, no effects of water treatment on stomatal resistance were found ($P>0.05$). None of these variables was affected by the endophyte presence ($P>0.05$). Both the cultivar x endophyte interaction, for the photosynthesis rate expressed on a leaf area basis, and the cultivar x water treatment x endophyte interaction, for stomatal resistance, were significant ($P<0.05$, Appendices 5.3 and 5.4, respectively), but no biological interpretation for these interactions was evident.

Table 5.11. Photosynthesis on leaf area basis (Pa) and on leaf weight basis (Pb) and stomatal resistance.

	Pa	**Pb**	**Resistance**
Cultivar	$\mu mol\ CO_2 m^{-2} s^{-1}$	$\mu mol\ CO_2 g^{-1} s^{-1}$	$s\ cm^{-1}$
MK	8.16 **a**[1]	0.191	0.153 **b**
EP	6.42 **b**	0.154	0.288 **a**
s.e.[2]	0.44	0.011	0.011
Water			
Control	9.81	0.245 **a**	0.212
S1	8.35	0.207 **ab**	0.198
S2	6.12	0.134 **bc**	0.240
S3	4.87	0.105 **c**	0.232
s.e.	1.28	0.031[§]	0.029
Endophyte			
Nil	7.09	0.163	0.221
AR501	7.13	0.175	0.205
KY31	7.65	0.181	0.236
s.e.	0.54	0.014	0.013
Interactions (P=)			
Cult.*Water	0.236	0.285	0.566
Cult.*Endop.	**0.023**	0.110	0.110
Water*Endop.	0.656	0.815	0.779
Water*Cult.*Endop.	0.678	0.652	**0.050**

[1] Letters indicate groupings of means that are significantly different at $P<0.05$ within cultivars, water treatment or endophyte.

[2] Standard error.

[§] Mean standard error.

5.4.6. Carbon isotope discrimination

MK plants showed a higher Δ than EP plants ($P<0.05$, Table 5.12). This result was expected because of the lower stomatal resistance of the Mediterranean cultivar (Table 5.11). As in the previous experiments, the differences between water treatments were not statistically significant ($P>0.05$, Table 5.12), although there was a trend with Δ values being lower at higher moisture deficit. In the current experiment newly formed leaf tissue was analysed for Δ with the expectation that differences between water treatments were more pronounced. To check the latter assumption Δ of the youngest mature leaves of S1 plants in block 2 were analysed and compared with Δ of the elongating leaves in the same plants. The paired t-test between the Δ of the two leaf categories confirmed the hypothesis ($t = 5.85$, degrees of freedom = 5, $P<0.05$). This indicated that those leaves that have grown during the water deficit period reflected this effect better than those that have completed their growth before they were applied. However, in S3 plants, it is likely that even the elongating leaves had been formed before the water deficit started. This is because their leaf appearance interval (i.e. LAR inverse) was 83.3 days per leaf. Consequently, very few leaves appeared over the 26 days that the plants were subjected to water deficit and, it is likely that the proportion of turnover of chemical constituents of the leaf tissue, that took place during the experimental period, was not enough for detection of the higher proportion of ^{13}C carbon fixed under water deficit. Therefore, significant differences could only have been detected if the carbohydrate fractions within leaves had been analysed (Brugnoli *et al.*, 1988; Farquhar *et al.*, 1989).

While West and Gwinn (1993) did not find any endophyte effect on Δ, in this experiment AR501-E+ plants showed an increased Δ compared with E- plants or KY31-E+ plants ($P<0.05$). This finding reflected the comparatively lower, though not statistically different ($P>0.05$, Table 5.11), stomatal resistance of AR501-E+ plants. This was interesting because the maintenance of a low stomatal resistance has been proposed as an alternative mechanism of adjustment to water stress. One hypothesis is that the increased photosynthesis could promote root growth or osmotic adjustment to counterbalance the effects of the water deficit (Richardson *et al.*,1990). The increase in photosynthesis in response to this endophyte has not been observed here. However, the interactions between cultivars and endophyte suggested that the endophyte AR501 tended to increase photosynthesis (Appendix 5.3) and decrease Ψ_o (Fig. 5.12) in EP plants.

Table 5.12. Main effects means for carbon isotope discrimination (Δ)

Cultivar	**Δ**
MK	20.94 **a**[1]
EP	20.43 **b**
s.e.[2]	0.07
Water	
Control	20.89
S1	20.78
S2	20.57
S3	20.50
s.e.	0.15
Endophyte	
Nil	20.60 **b**
AR501	20.91 **a**
KY31	20.55 **b**
s.e.	0.08
Interactions (P=)	
Cult.*Water	0.523
Cult.*Endop.	0.483
Water*Endop.	0.931
Water*Cult.*Endop.	0.872

[1] Letters indicate groupings of means that are significantly different at $P<0.05$ within cultivars, water treatment or endophyte.
[2] Standard error.

5.4.7. Multivariate analyses

In order to find some information about the structure of interrelationships between some of the variables studied, multivariate analyses were carried out. The variables analysed were: 1) the average of the water content on 24 June and at the end of the experiment; 2) the percentage of green tissue in the plants; 3) the average of initial and final tiller number; 4) photosynthesis rate expressed on a leaf area basis; 5) stomatal resistance; 6) the average of RWC measured in periods 1 and 2; 7) carbon isotope discrimination (Δ); and 8) net growth rate (NGR) per plant. Two multivariate analyses were performed, first, an exploratory principal component analysis (PCA), then a multivariate analysis of variance (MANOVA).

The PCA was performed using the correlation matrix. This analysis was exploratory because it discriminates only between observations without taking account of treatments. Only the first two principal components (PCs) were considered because the other six PCs together only explained 34 % of the variance of the data. Results are summarised in Table 5.13. The first PC explained around 42 % of the total variation in the data. The coefficient for average tiller number was nearly zero and can be ignored. On the other hand, the coefficient of stomatal resistance was the only negative one while the rest of the coefficients were all positive and of a similar magnitude (between 0.32 and 0.47). This showed that most of the variation in PC 1 reflected a contrast between stomatal resistance and the other variables. From a biological perspective PC 1 separated water treatments. The increase in the availability of water in the pots caused an increase in percentage of green tissue, photosynthesis rate, RWC, Δ and net growth, and decreased stomatal resistance.

The second PC explained 24 % of the total variation in the data. Here, the coefficients of average water content and RWC were nearly zero and can be ignored. Most of the variation associated with PC 2 arose from a contrast between average number of tillers, photosynthesis rate and carbon discrimination on one hand, and green tissue, stomatal resistance and NGR per plant on the other hand. Biologically PC 2 represented the contrast between the cultivars studied. EP had lower tiller number, photosynthesis rate and Δ, and higher green tissue percentage, stomatal resistance and net growth per plant than MK.

Table 5.13. Principal component (PC) coefficients, variance (eigenvalues) and percentage of total variance explained.

Variable	PC 1	PC 2
Average water content (g H_2O g^{-1} dry soil)	0.473	-0.004
Green tissue (%)	0.383	0.379
Average tiller number	0.024	-0.453
Photosynthesis (μmol CO_2 m^{-2} s^{-1})	0.414	-0.154
Stomatal resistance (cm s^{-1})	-0.176	0.563
Average RWC (%)	0.439	0.021
Δ	0.325	-0.381
NGR (mg DW plant $^{-1}$)	0.359	0.405
Variances (eigenvalues)	3.354	1.901
% of total variation explained	41.920	23.760

The MANOVA with canonical option was performed to maximise differentiation between treatment effects. Only cultivar, endophyte and cultivar x endophyte effects were significant ($P<0.05$). Unexpectedly, water treatment was not significant ($P>0.05$). However, the standardised mean scores for water treatments showed a tendency to decrease as water deficit increased. The reason for the lack of significance was probably due to the large number of variables involved in the MANOVA (i.e. 8) and the low number of degrees of freedom of the error term (i.e. 9). For instance, removing any one of the variables from the model caused the water effect to be significant. The standardised mean scores are shown in Table 5.14. The total canonical structure and the standardised canonical coefficients for the significant effects are shown in Table 5.15.

The total canonical structure showed the correlation between the canonical variates (CVs) and the original variables. The canonical structure only showed the importance of each variable independent of the other variables. Conversely, the standardised canonical coefficients indicated the mutual influence of the variables on each other.

The cultivar canonical structure showed that the variables more correlated to the CV were average tiller number and, to a lesser extent, Δ, both positively correlated, and stomatal resistance, negatively correlated (Table 5.15). The standardised canonical coefficients, on the other hand, showed that carbon discrimination was less important, while NGR and RWC were important. This CV indicated, as was observed in the univariate analysis, that MK, compared with EP had higher tiller number (Table 5.5), RWC (Table 5.7) and Δ (Table 5.12), and lower stomatal resistance (Table 5.11) and NGR per plant (Fig. 5.5b). The standardised mean scores for cultivars are shown in Table 5.14.

Two CVs were significant ($P<0.05$, Table 5.14) for the endophyte effect. The first CV separated the three endophyte treatments. The canonical structure for CV 1 indicated that the variables that were positively correlated with CV 1 were, mainly, Δ, average tiller number, green tissue and RWC, while stomatal resistance was negatively correlated (Table 5.15). The standardised canonical coefficients for CV 1 showed a contrast between green tissue percentage, average tiller number and Δ with photosynthesis rate and stomatal resistance. Plants infected with AR501 and KY31 endophytes showed contrasting effects, each different from endophyte-free plants in different ways. While AR50-infected plants had a higher percentage of green tissue (Table 5.2), a higher tiller number (Table 5.5), and higher Δ (Table 5.12), KY31-infected plants were characterised by higher photosynthesis and stomatal resistance (Table 5.11), although, the differences were not always statistically significant in the univariate analyses. Usually, photosynthesis and stomatal resistance tend to be negatively correlated, though in a non-linear way or with a non-zero intercept (Farquhar *et al.*, 1989). Because of this effect of KY31 of positive correlation between both photosynthesis and stomatal resistance, an increase the plant water use efficiency it is expected in KY31-infected plants, and this result is interesting in its own right, as is the evidence of AR501-infected plants behaving as if they were less stressed.

The second CV separated E- plants from E+ plants (Table 5.14). The canonical structure for CV 2 showed that NGR per plant and green tissue percentage were positively correlated, and average tiller number was negatively correlated, with CV 2. The standardised canonical coefficients for CV 2 showed a contrast between average tiller number and NGR per plant. This was because while E+ plants had fewer tillers per plant (Table 5.5) they showed a higher NGR (Figure 5.8). The standardised mean scores for endophyte for both CVs are shown in Table 5.14.

Finally, one CV was significant ($P<0.05$) for the cultivar x endophyte interaction. The total canonical structure showed that the variables most correlated to this CV were RWC and average tiller number (Table 5.15). The higher standardised coefficients corresponded to average tiller number, followed by green tissue percentage and RWC, and photosynthesis, the latter being negative. The standardised mean scores for cultivar x endophyte are shown in Table 5.14. It can be observed that MK E- plants or MK AR501 plants had a higher mean score than MK-KY31 plants or any EP-endophyte combination. This indicated that MK-E- and MK-AR501 plants had a higher RWC (Appendix 5.5) and average tiller number (Fig. 5.3), but it is difficult to interpret because these plants did not have a greater percentage of green tissue (Appendix 5.5) nor a lower photosynthesis rate than any endophyte-EP plant combination (Appendix 5.3), as was indicated by the coefficients. Probably this is partially explained by the lower correlations between these two variables and the CV, compared with the correlations shown by RWC and average tiller number.

Table 5.14. Standardised mean scores for cultivar, endophyte, water and cultivar x endophyte interaction.

	Standardised mean scores	
Cultivar	**CV 1**	
MK	1.68	
EP	-1.68	
Endophyte	**CV 1**	**CV 2**
Nil	0.14	-0.74
AR501	0.69	0.47
KY31	-0.82	0.27
Water	**CV 1**	
Control	4.02	
S1	2.50	
S2	-0.92	
S3	-5.60	
Cultivar * endophyte	**CV 1**	
MK-Nil	1.19	
MK-AR501	1.25	
MK-KY31	-0.63	
EP-Nil	-0.74	
EP-AR501	-0.55	
EP-KY31	-0.52	

Table 5.15. Total canonical structure and standardised canonical coefficients of the first canonical variate (CV) for cultivar and cultivar x endophyte interaction, and of the first and second canonical variate for endophyte.

	Total canonical structure		Standardised canonical coefficients	
Cultivar	**CV 1**		**CV 1**	
Average water content (g H_2O g^{-1} soil)	0.2692		0.1796	
Green tissue (%)	-0.1574		0.1615	
Average tiller number	0.7566		1.1487	
Photosynthesis (μmol CO_2 m^{-2} s^{-1})	0.3013		-0.0809	
Stomatal resistance (cm s^{-1})	-0.7111		-0.5464	
Average RWC (%)	0.3937		0.8059	
Δ	0.5767		0.4079	
NGR (mg DW plant $^{-1}$)	-0.2838		-0.9865	
Endophyte	**CV 1**	**CV 2**	**CV 1**	**CV 2**
Average water content (g H_2O g^{-1} soil)	0.3210	0.3228	-0.3486	-0.0624
Green tissue (%)	0.4543	0.4562	1.3393	-0.2852
Average tiller number	0.4679	-0.7195	0.7477	-1.3605
Photosynthesis (μmol CO_2 m^{-2} s^{-1})	0.2010	0.1409	-0.6337	-0.3335
Stomatal resistance (cm s^{-1})	-0.5752	-0.0113	-0.8638	-0.5943
Average RWC (%)	0.4169	0.2729	-0.0220	-0.0680
Δ	0.6357	0.2087	0.7168	0.4843
NGR (mg DW plant $^{-1}$)	0.2179	0.6111	0.0402	1.2879
Cultivar * Endophyte	**CV 1**		**CV 1**	
Average water content (g H_2O g^{-1} soil)	0.2936		-0.1148	
Green tissue (%)	0.4532		0.9595	
Average tiller number	0.5429		0.9808	
Photosynthesis (μmol CO_2 m^{-2} s^{-1})	-0.0047		-0.7977	
Stomatal resistance (cm s^{-1})	-0.0571		0.2662	
Average RWC (%)	0.6523		0.9214	
Δ	0.3690		0.4555	
NGR (mg DW plant $^{-1}$)	0.3607		-0.2776	

5.5. Summary

5.5.1. Water deficit effects

- The water deficit treatments induced a reduction in plant dry weight and leaf blade percentage, tiller number and tiller size, and produced an increase in dead tissue percentage. There was also a decrease in plant growth and in LAR.

- The RWC, Ψ_w and its components, and photosynthesis rate were reduced as water deficit increased.

5.5.2. Cultivar effects

- MK plants were smaller, with a higher number of tillers and a higher percentage of dead tissue than EP plants. MK plants showed a higher increase of dead tissue percentage than EP in response to water deficit.

- Plant growth rate for MK was lower than for EP and was relatively more reduced with increase in water deficit, because of a higher SR. MK plants also showed a lower LAR.

- The RWC was higher and the Ψ_o tended to be higher in MK than in EP plants. The Ψ_p of EP plants was maintained along the water gradient because of a decrease in Ψ_o from Control to S3 water treatment. No differences were found between cultivars for the relationship between RWC and Ψ_w and its components. At the same RWC, MK plants showed lower NGRs than EP plants. Photosynthesis rate was higher and stomatal resistance lower in MK plants. Consequently, MK plants showed a higher Δ than EP plants.

5.5.3. Endophyte effects

- Endophyte presence reduced plant dry weight and tiller number per plant, while the percentages of leaf blade and dead tissue were affected differently depending on the endophyte strain.

- NGR per tiller was higher in E+ plants.

- The results related to plant water status did not show any endophyte effect. The cultivar x endophyte interaction was significant for Ψ_o and photosynthesis rate.

- Plants infected with AR501 showed a higher Δ.

CHAPTER 6. GENERAL DISCUSSION OF WATER DEFICIT EXPERIMENTS

This chapter provides a general discussion that relates the main findings of the three water deficit experiments carried out as part of this study. It is divided into three sections. The first section deals with the responses to water deficit, the second compares the cultivars evaluated and the third refers to the endophyte effects and compares the strains studied. For brevity, experiments reported in Chapter 3, Chapter 4 and Chapter 5 are referred to as Experiment 1, Experiment 2 and Experiment 3, respectively. The following table summarises the main characteristics of each experiment.

Table 6.1. Summary of details of the three water deficit experiments carried out.

	Experiment 1	**Experiment 2**	**Experiment 3**
Site	Massey University, Palmerston North, New Zealand	Unidad Integrada Balcarce INTA-FCA, Argentina	AgResearch, Palmerston North, New Zealand
Experimental period	12 days	26 days	46 days
Pots	150mm diameter x 1m	305 x 375 x 323 mm	150 x150 x 140mm
Number of plants per pot	4 plants of the same cultivar	20 plants, 2 rows of 5 plants of each cultivar	1plant
Cultivars:			
• **Mediterranean**			
Maris Kasba (MK)	✓	✓	✓
• **Temperate**			
El Palenque (EP)	✓	✓	✓
Grasslands Advance (GA)	✓		
Water treatments			
• **Water added daily** (**100% = field capacity**)	Control: watered (100%) Stressed: unwatered (0%)	Control: 100% S1: 80% S2: 65% S3: 50%	Control: 100% S1: 75% S2: 50% S3: 25%
Endophyte	Nil	Nil	Nil, AR501, KY31
Measurements			
• **Plant mass**	✓	✓	✓
• **Root mass and length**	✓		
• **Tiller number**	✓	✓	✓
• **RWC**	✓	✓	✓
• **Ψ_w,Ψ_o and Ψ_p**	✓	✓	✓
• **Stomatal resistance**	✓		✓
• **Photosynthesis**			✓
• **Transpiration**	✓		
• **^{13}C discrimination (Δ)**	✓	✓	✓
• **Tissue turnover**		✓	✓
• **Sugars**		✓	

6.1. Water deficit effects

Morphological and physiological changes occur under water deficit that allow plants to diminish water loss and improve their water uptake. In this respect, morphological changes have been considered the most efficient means of conserving water (Passioura, 1982). The morphological changes observed here resulted in a decrease in the evaporative surface, primarily by the death of leaves (Tables 3.2 and 5.2) and a slight decrease in tiller number (Tables 3.2, 4.2 and 5.5). These changes resulted in an increased root : shoot ratio (Table 3.13), and indicated a greater negative effect of drought on shoot growth than on root growth, as has been reported earlier (Sharp and Davies, 1979; Ismail *et al.*, 1994). Also leaf rolling was observed, which is a common phenomenon in tall fescue plants (Arachevaleta *et al.*,1989; White *et al.*,1992b; Elmi and West;1995) and results in the exposure of the abaxial side of the leaf with its lower stomatal density.

Morphological changes in response to water limitation were less pronounced in Experiment 2 than in Experiments 1 and 3 (Sections 3.4.1, 4.4.1 and 5.4.2). The only statistically significant difference ($P<0.05$) in Experiment 2 was a higher percentage of sheath for the S3 treatment. Even so, it was possible visually to distinguish the gradient among the four levels of water availability (Plate 4.1). The limited morphological changes in this study could have been due to the different method of water deficit application. For instance, it has been reported (Socias *et al.*, 1997) that subterranean clover (*Trifolium subterraneum* L.) leaves showed a higher water content and water potential under gradual soil water depletion (replacing only a fraction of the water evaporated) than under rapid soil water depletion (withholding water completely) for a similar soil water availability. However, the method of reduced daily water application resulted in more severe stress than in Experiment 2 when, in Experiment 3, the experimental period was prolonged, the daily amount of water given under the most severe treatment was reduced further and the pots were smaller (Table 6.1).

It has been reported that reductions in pot size promoted a decrease in plant growth independently of the soil water availability (Ismail *et al.*, 1994). While in water deficit treatments this finding was related to the lower volume of available water in the smaller pots, it was suggested that in wet conditions root confinement would affect both root and shoot growth through a root signal, which appears to be independent of plant nutrition, carbon or water status (Ismail *et al.*, 1994). However, although ABA has been suggested as a potential root signal (Tumer, 1986; Quarrie, 1989; Davies and Zhang, 1991), Ismail *et al.* (1994) observed that changes in xylem ABA concentration were not related to pot size and they suggested that a reduction in the amount of cytokinins transported from roots to shoots might have been involved. The effect of pot size also influenced the Δ and water use efficiency (WUE) of different genotypes in response to water deficit and the results differed from those under natural soil conditions. Differences in soil bulk density were discarded as the cause of the disparity (Ismail *et al.*, 1994). These findings emphasise the necessity of identifying experimental conditions that represent better the natural growing conditions if it is hoped to obtain results that will be reproduced in the field.

In Experiment 1 it was observed that although total root mass was lower in Stressed plants (Table 3.5), their total root length was not different from Control plants (Table 3.6). In comparison with Control plants, Stressed plants tended to have a higher proportion of root mass and root length in the lowest stratum of the pots (Appendices 3.3 and 3.5). This indicates that water deficit promoted the production of roots deep in the profile. Stressed plants also showed a higher mean root diameter and, consequently, a lower root surface area in the uppermost soil strata (Tables 3.8 and 3.11). Thicker roots can indicate lignification and suberization (Cruz *et al.*, 1992). These two processes have been observed to take place under drought and high temperature conditions (Evans, 1973) and it has been suggested that they are an important means to restrict the leakage of water from the roots as soil dries and to help the maintenance of the root turgor so that the root can reinitiate growth after the relief of the water deficit (Cruz *et al.*, 1992).

In agreement with other studies (Hsiao, 1976; Davies and Zhang, 1991), plant growth decreased as the water deficit increased (Figures 4.4 and 5.5). In the three experiments Stressed plants showed lower RWC, Ψ_w, Ψ_o, Ψ_p and photosynthesis rate than Control plants (Tables 3.15, 4.3 to 4.6, 5.7 to 5.11). The differences in RWC measured between experiments indicate again that in Experiment 2 plants were subjected to a less severe stress than in the other experiments. Osmotic adjustment, which is necessary to maintain the physiological plant processes (Turner, 1986; Thomas, 1987) and delay plant dehydration (Kramer and Boyer, 1995), was observed in Experiments 2 and 3, but was not observed in Stressed plants in Experiment 1. In Experiment 1, since the $\Psi_{o(f)}$ did not diminish in the Stressed treatment, the decrease in Ψ_o instead of being by solute accumulation, could have been due merely to an increase of the solute concentration of the vacuolar sap because of the lower RWC.

As already mentioned, the carbon isotope composition of plant tissue estimates the long term integration of a complex series of biochemical and physiological events (Brugnoli *et al.*, 1988). However, in the short term, when new growth has been limited as a consequence of decreased soil moisture levels, the impact of the stress, which cannot be detected in the leaf tissue as a whole, would be detected in carbohydrate fractions within leaves (Brugnoli *et al.*, 1988; Farquhar *et al.*, 1989). This explains why in the present study the responses of Δ to the short term water deficits imposed were inconsistent when mature leaves were analysed (Tables 3.23 and 4.5), and showed only a non-significant trend consistent with the water deficit treatments applied, but still did not attain statistical significance when elongating leaves were analysed (Table 5.12).

Because fructosyl sugars are present in species that tolerate desiccation and can alter vacuolar Ψ_o through polymerization or breakdown, it has been suggested that they can contribute to osmotic adjustment (Pontis and del Campilllo, 1985). In this study, however, neither the fructosyl sugar concentration nor the reducing sugar concentration contributed greatly to the osmotic adjustment of MK or EP (Tables 4.6 and 4.7). This suggests that these sugars were not directly involved in the decrease of the Ψ_o under lower availabilities of soil water. Spollen and Nelson (1994) also concluded that fructosyl sugars were not involved directly in osmotic adjustment of tall fescue.

6.2. Mediterranean and temperate cultivars

The Mediterranean cultivar MK differed from the temperate cultivars studied morphologically and physiologically. MK plants were smaller (Tables 3.1, 3.2, 4.2 and 5.2), tended to have a higher tiller number (Tables 3.2 and 5.5) and had a higher root : shoot ratio (Table 3.13) than EP. The latter characteristic would give the plant an advantage under water deficit conditions considering that a relatively high proportion of roots could assist the plant in the uptake of water to satisfy the evaporative requirement (Passioura, 1982). A higher root : shoot ratio for Mediterranean grasses than north European ones, under high temperatures, has been observed previously and it has been linked with carbohydrate accumulation and the summer drought survival strategy of Mediterranean populations (Eagles, 1967a). However, this advantage was evident only when MK plants were growing in a situation where the volume of soil available was not limiting and no other plants were depleting the water available. For instance, in Experiment 1, where MK plants were grown in isolation in long pipes simulating a deep soil profile, they showed a lower percentage of dead tissue and a higher percentage of leaf blade (Table 3.2) than GA and EP plants. But, under the conditions of Experiment 2, where MK plants and EP plants were grown together, MK plants showed a lower proportion of leaf and higher percentages of sheath and dead tissue than EP plants (Table 4.2) indicating the adverse competitive effect , not only for water but also for nutrients and light, of the bigger EP plants on MK. Thomas and Evans (1990) also observed a competitive disadvantage for a hybrid ryegrass when growing in a similar alternating pattern with its parental lines, compared with when growing in monoculture in pots or field plots.

In Experiment 3, although single MK plants were grown, the small pot size limited the amount of water available and MK showed a lower percentage of leaf (Table 5.2) and a proportionally higher increase in dead tissue percentage than EP as water deficit increased (Table 5.3). In Experiments 2 and 3 it appears that the physiological adaptations of EP are more able to confer competitive advantage than the morphological adaptations of MK.

The higher root : shoot ratio in MK, probably involves a high cost of maintenance because of the associated higher root respiration and, consequently, a lower water use efficiency. This attribute could therefore be a negative trait under conditions of ample water supply. MK roots were also thicker than GA or EP roots (Table 3.8). This characteristic could give an advantage to MK plants under water deficit because, according to Evans (1973) the plant water uptake under drought appears to occur primarily in the thickest roots. In addition, the turnover rate of thick roots may be lower than the turnover rate of thin roots (Fitter, 1996), and consequently, MK plants might counterbalance the higher cost of maintenance respiration of their relatively large root system with a lower cost of root turnover.

Overall, EP plants had a higher root weight and length in the lowest stratum than the other cultivars (Tables 3.5 and 3.6). However, under water deficit it was observed that the percentage of total root OM below 300 mm tended to increase in MK and GA plants, and tended to diminish in EP plants (Appendix 3.4). On the other hand, the percentage of root length in the stratum considered did not change in Stressed-EP plants (Appendix 3.7), suggesting that under water deficit the deepest roots of EP plants became thinner and, consequently would have had a higher surface area per unit mass than those of the other cultivars.

In general, the water relations of both the Mediterranean cultivar, MK, and the temperate cultivar, EP, were similarly affected by water deficit. This was shown by the relationships between Ψ_w and its components with RWC, for which no differences between cultivars were found within the common RWC range (Fig. 5.14). However, MK was able to maintain a better water status than EP and GA when growing alone (Experiments 1 and 3), probably because its smaller plant size contributed to a slower depletion of available soil moisture (Table 5.1).

On the other hand, EP had greater osmotic adjustment than MK (Table 4.4 and Fig. 5.11). Although some doubts have been expressed in relation to the correlations between bulk tissue osmotic potential and growth and survival during drought (Thomas and Evans, 1991), the greater osmotic adjustment showed by EP is a valuable characteristic, considering that the maintenance of a high Ψ_p has been proposed as vital for short-term plant productivity (Passioura, 1982). For instance, in Experiment 3, while the RGR was similar for both cultivars under Control and S1 water treatments, EP plants were able to maintain a higher RGR than MK plants under S2 and S3 water treatments (Section 5.4.3). The lower RGR for MK plants was due to a greater decrease in GGR and a higher increase in SR compared with EP plants in response to increasing water deficit.

In the three experiments MK stomatal resistance was lower than for the temperate cultivars. This was found both when stomatal resistance measured directly in Experiments 1 and 3 (Tables 3.20 and 5.11) and when estimated indirectly from ^{13}C discrimination (Tables 3.24, 4.5 and 5.12). The intraspecific genetic variation in Δ has been suggested as a criterion for selection in C_3 breeding programmes (Brugnoli *et al.*, 1988). For example, a positive relationship between yield and Δ has been reported for well-watered wheat genotypes (Condon *et al.*, 1987). However, in this study it was also observed that the extent of MK stomatal adjustment in response to changes in soil water availability was less than for the other cultivars (Table 3.21). If the latter is a characteristic of the Mediterranean cultivar, then the lack of stomatal closure could be advantageous under short periods of drought, because the plant would be able to maintain high rates of photosynthesis even under water deficit (Passioura, 1982). However, in prolonged periods of drought, such a plant will be unable to control water loss, with risk of dehydration and death.

A distinctive difference found between cultivars was the greater reducing and fructosyl sugar concentrations shown by EP plants compared with MK plants (Table 4.6). This finding is even more interesting since EP plants also showed a higher growth rate. Under these circumstances, a lower proportion of sugars available for fructosyl sugar synthesis would have been expected (Eagles, 1967b; Thomas, 1990).

The results of this study agree with Morgan (1964) in that the cultivars have adapted to the climatic conditions of their respective regions of origin. In this way, under the high temperatures registered in these experiments (Figures 4.2 and 5.2), the cultivar of temperate origin, EP, has shown higher growth rates than the Mediterranean cultivar, MK (Figures 4.3 and 5.5). This is because populations from climates characterised by cold winters have been adapted to resist frost in a relatively less active form, while Mediterranean populations, on the other hand, have been adapted to grow vigorously in the winter rainy season and to become relatively dormant in the dry summer. Results obtained in Canberra in the field

(Neal-Smith and Wright, 1969) suggested that favourable soil moisture content was needed for Mediterranean tall fescues to express their winter growth potential, but also that despite their growth being highly depressed during drought in comparison with a temperate line, Mediterranean lines showed a lower tiller loss. The results of the present experiments indicate that MK has a morphology that would allow the plant to postpone water deficit. However, when the soil moisture is low, its stomata do not respond to the same extent as the temperate cultivars and, it appears that ability for osmotic adjustment is also less in MK. Because of these features, while MK can be expected to withstand short periods of drought successfully, it seems doubtful that it will persist under prolonged periods of water shortage. However, it is worth noting that in Balcarce, MK swards affected by a severe drought in 1995 (e.g. total rainfall from May to September less than 50 mm), did not visually appear to have been more affected than EP swards. This seems consistent with results of Experiment 1. For these reasons, it would be desirable that future experiments measure the osmotic adjustment of meristematic tissues, include a recovery phase after drought, be carried out under field conditions or at least in pipes, simulating a deeper soil profile than that available in pots and at lower temperatures than those recorded in the glasshouses employed here.

In France, a hybrid between European and Mediterranean tall fescues has been successfully obtained[1]. This hybrid has the increased winter production of the Mediterranean parent line and the summer production of the European line. Consequently, there is likely to be interest in attempting to develop a hybrid between MK and EP for the Pampa region. It is known that these cultivars are well adapted to the prevailing environmental conditions of the area (Mazzanti and Arosteguy, 1985). A hybrid plant is likely to have a more stable seasonal production and an improved drought tolerance. However, the present findings that both tall fescue cultivars have different drought strategies raises questions of how these two strategies might be best combined in a hybrid, and whether selection for a particular strategy would be feasible.

6.3. Endophyte effects

The results of this study confirmed previous experimental evidence that the endophyte effect is highly variable and depends on both, the plant genotype (Belesky *et al.*, 1989, De Battista *et al.*, 1990, Malinowski *et al.*, 1998), and the endophyte strain (De Battista *et al.*, 1990; Richardson *et al.*, 1990). In contrast with the results of Latch *et al.* (1985) in ryegrass, in this experiment E- plants showed

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[1] Personal communication Dr L. Hazard, INRA, Lusignan, France.

a higher dry weight and tiller number per plant than E+ plants (Tables 5.2 and 5.5). A decrease in tillering rate in E+ tall fescue plants has also been reported by Maclean *et al.* (1993). Furthermore, Hill *et al.* (1990) suggested that the tillering rate would be stimulated or inhibited depending on the amount of IAA produced by the endophyte and that this effect could also be modified by the plant genotype. The results of this experiment however, contrast with findings of Hume *et al.* (1993). In that experiment there was no endophyte effect on tillering rate (Table 5.5.). So the suggestion by Hume *et al.* (1993) that artificial infection may be the factor modifying plant-endophyte interaction does not appear to be supported.

Although E+ plants showed a lower plant dry weight (Table 5.2), their growth rate during the application of the water treatments was higher than the growth rate of E- plants (Fig. 5.9). This finding suggests that E- plants had a higher growth rate during the pre-experimental period, this effect being reversed once the water deficit treatments were applied. However, a different result was obtained by West *et al.* (1993) who found that although E+ plants had a better recovery after drought stress was alleviated, they ceased growth at the same time as E- plants at the onset of drought conditions. The maintenance of growth under stress shown by E+ plants in this experiment could result in an advantage to short-term productivity, but it could equally have adverse consequences for plant persistence in prolonged drought because a plant with a greater evaporative surface will be more susceptible under a long-term stress situation, or a succession of soil water deficit events (Belesky *et al.,*1989).

Interestingly, the interactions between the endophytes and cultivars, that were observed both in the univariate and in the multivariate analysis, indicate that endophyte effects on plant physiology appeared to differ between cultivars. For instance, KY31 tended to decrease plant GGR in MK and to increase it in EP, while AR501 tended to increase GGR in both cultivars (Fig. 5.10). However, no significant differences were found within each cultivar for the endophyte effect on GGR per plant (Fig. 5.10). Similarly, Eerens *et al.* (1993), for ryegrass, observed that the effect of the endophyte on leaf extension was variable. In addition, KY31 tended to decrease Ψ_o for MK but increase it for EP, while AR501 had the opposite effect. Decreased Ψ_o (MK-KY31and EP-AR501, Fig. 5.12) is probably due to increase in photosynthesis rate in these combinations (Appendix 5.3). Endophyte-free plants always showed an intermediate value in comparison with E+ plants for both Ψ_o and photosynthesis rate. Because of the previous interaction these results contrast with the findings of Hill *et al.* (1996) who found no endophyte effect on Ψ_o in tall fescue, but they agree with Elmi *et al.* (1990), Elmi and West (1995) and Maclean *et al.* (1993). In addition, the finding of Richardson *et al.*

(1990), that the endophyte effect on photosynthesis was grass genotype specific, was confirmed.

Because of the distinctive response of each endophyte-grass genotype combination, these results confirm the earlier suggestion that the endophyte presence in a mixed population increases the phenotypic expression without increasing genetic variability (Hill *et al.*, 1990), and would confer the tall fescue-endophyte association an increased plasticity and adaptability (Bacon, 1994). Both endophytes studied tended to increase the GGR of EP plants, and thus increased short-term productivity, while at the same time, possibly jeopardising persistence, if the water deficit period were prolonged (Belesky *et al.*,1989). However, the MK-KY31 and EP-AR501 combinations have shown a trend to increase photosynthesis rate (Appendix 5.3) and to promote osmotic adjustment (Fig. 5.12), which could be of vital importance for tiller survival during water deficit (Elmi and West, 1995), specially when osmotic adjustment takes place in the meristematic and elongating leaf tissues (West *et al.*,1990; West and Gwinn, 1993).

CHAPTER 7. WINTER PASTURE GROWTH AND ANIMAL PRODUCTION FROM MARIS KASBA AND EL PALENQUE TALL FESCUES IN RESPONSE TO NITROGEN FERTILISATION UNDER GRAZING

7.1. Introduction

As noted in Section 2.2, tall fescue is the most important perennial cool season grass in Argentina (Gonzáles and Gardner, 1977; Mazzanti and Arosteguy, 1985). Because of seasonal differences in growth patterns of Mediterranean and Temperate tall fescues, their complementary use in temperate animal production systems might improve continuity of seasonal forage supply (Mazzanti and Arosteguy, 1985; Mazzanti *et al.*, 1985). Mediterranean cultivars would compensate the low winter productivity of the temperate cultivars, while the opposite would occur in summer.

Pasture growth in winter is of particular interest since alternative strategies for producing additional feed are expensive and involve risk. In the SE of Buenos Aires Province, Argentina, water availability during autumn and winter is not normally limiting, however, low soil nitrate levels have been found (Navarro, 1966). These N levels have resulted in reduced forage production (Femández Grecco *et al.*, 1995; Marino *et al.*, 1995). Consequently, there is industry interest in evaluating the extent to which both winter pasture production and animal production could be improved through the use of a Mediterranean cultivar such as Maris Kasba instead of temperate cultivars such as El Palenque, and also in knowing the response to N if a Mediterranean cultivar were used in this way.

7.2. Objective

The objective was to compare the herbage growth and animal production of two endophyte-free tall fescue swards with contrasting seasonal growth, maintained at the same leaf area index (LAI), and their response to N fertilisation under grazing conditions during the winter-early spring.

7.3. Materials and Methods

7.3.1. Experimental site

The experiment was carried out from the end of April to October 1996 at Unidad Integrada Balcarce INTA-FCA, Argentina (37° 45' S : 58° 18' W), altitude approximately 130 m. Mean maximum/minimum temperatures (1968-1995) were 27.1 °C/13.2 °C in January and 12.1 °C/2.9 °C in July. Lowest recorded temperature (1968-1995) is -5.7 °C. The mean frost free period (1971-1990) was 231 days and the minimum 166 days. The soil at the site was a Typic Natraquoll (Buol *et al.*, 1989; Soriano *et al.*, 1992).

7.3.2. Experimental design

The experimental design was a completely randomised factorial arrangement of two cultivars and two levels of N with two replicates. Swards of Maris Kasba (MK) and El Palenque (EP) tall fescue were established in 1982. The two levels of N fertilisation were: 0 kg N ha^{-1} (N0) and 100 kg N ha^{-1} (N100) applied as urea. The SAS General Linear Models (GLM) procedure was used for statistical analysis and the SAS REG procedure used to fit linear regressions. Repeated measures analyses were performed for variables measured repeatedly on the same experimental unit. Percentages were arcsin square root transformed for statistical analysis. Means were separated using the LSD (Steel and Torrie, 1980) at 5 % significance level.

7.3.3. Experimental procedure

The experimental management schedule and periods of measurements are shown in Fig. 7.1. The swards were mown at a uniform height in March 1996. Half of the N fertiliser was applied on 6 May and the remaining N on 4 July. Thirty kilograms P per hectare were applied as superphosphate on 25 April. Weeds were controlled with methyl metsulfuron mixed with Dicamba (Misil; 60 % d.f./48 % s.c.; Sandoz-Agar Cross) applied in doses of 100 ml of commercial product per ha on 10 and 29 April. Radiation and mean air temperature at 1.2 m height and at ground level were recorded from 10 May to 14 October using a data logger (LI-1200 Minimum Data Set, LI-COR, Lincoln, Nebraska) placed at Balcarce Unidad Integrada INTA-FCA at approximately 5 km from the experimental site (Fig. 7.2). Monthly rainfall, from April of October 1996, was lower than the average (1986-95) only in May, and was particularly high during August and October (Fig. 7.3). Views of the experiment on 4 July and 8 October are shown in Plate 7.1a and 7.1b, respectively.

The paddocks, of approximately 1.5 ha each, were continuously grazed by growing Aberdeen Angus steers (161±16 kg initial weight) and maintained at a uniform LAI by 'put and take' grazing. Because of the greater sward height of MK, plots were grazed from16 July while EP plots were grazed from 12 August.

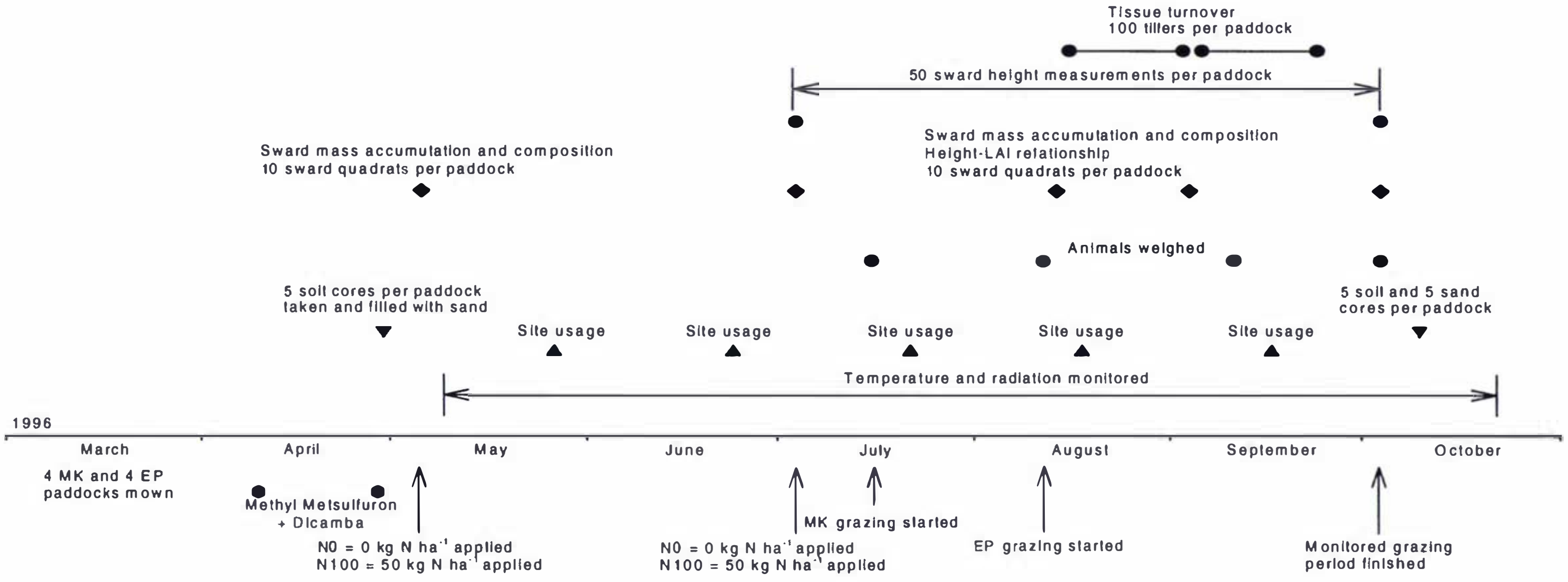


Figure 7.1. Experimental management schedule and measurements for the grazing experiment, Unidad Integrada Balcarce INTA-FCA, Argentina.

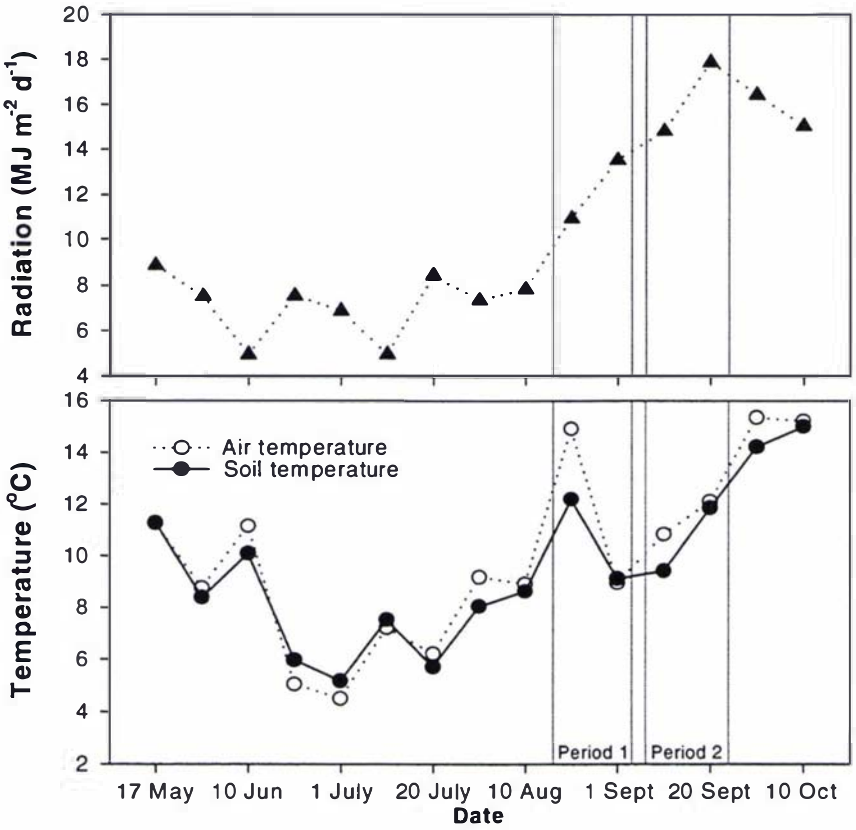


Figure 7.2. Radiation and mean soil and air temperatures recorded from 10 May to 14 October 1996 at INTA Balcarce. Reference lines indicate tissue turnover measurement periods.

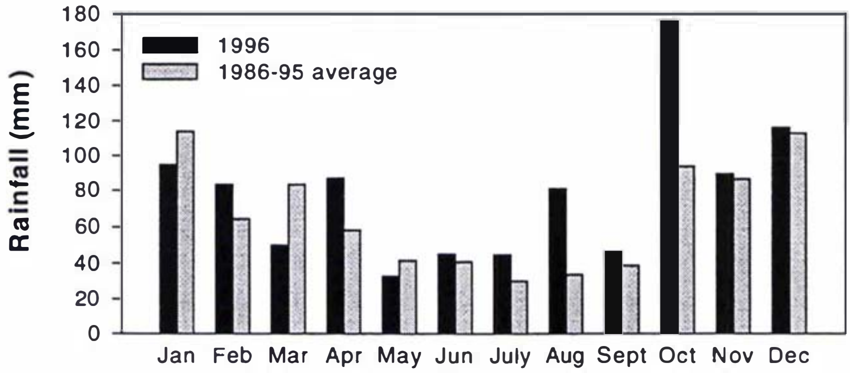
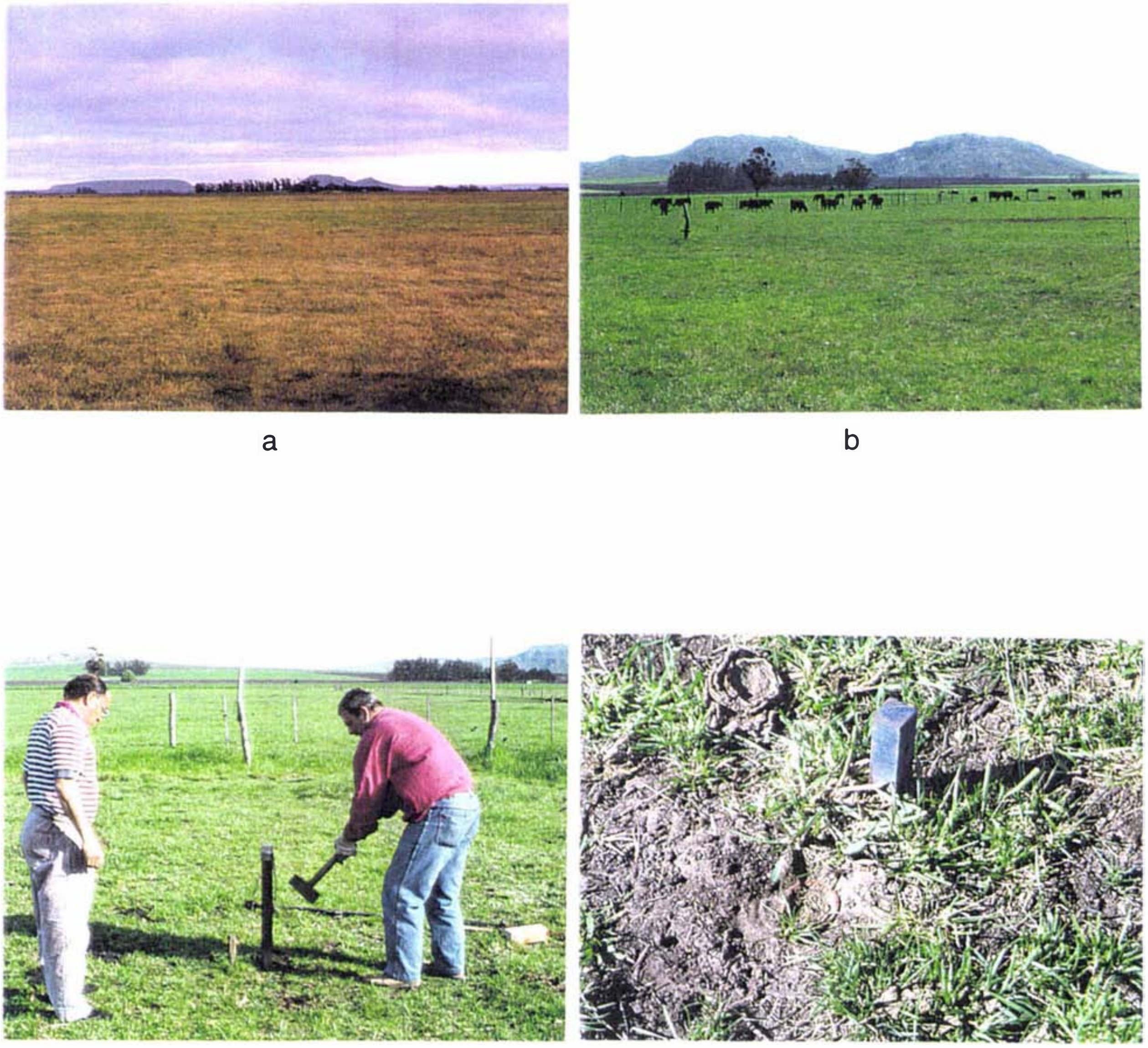


Figure 7.3. Monthly rainfall (mm) recorded at INTA Balcarce during 1996 and average monthly rainfall for 1986-1995.



a

b

c

d

Plate 7.1. Views of the grazing experiment on 4 July (a) and 8 October (b), respectively. In (a) the greener stripe visible is a paddock that had received N on 6 May. Plate (c) shows technicians taking root samples with a corer of 5 cm internal diameter and (d) a close-up view of a sand-filled core marked with a red ring.

- **Sward characteristics**

Herbage mass was measured by ten 300 x 150 mm quadrats per plot cut to ground level using hand-held electric shears on 6 May, 4 July, 14 August, 4 September and 1 October. The samples were separated in the laboratory into tall fescue, other grass species, lotus (*Lotus tenuis* L.), weeds and dead material. The number of tillers of tall fescue was counted and all components were oven dried and weighed (dry weight, DW). The green tall fescue fraction was ground and N analysed by a micro Kjeldahl N method, and *in vitro* digestibility (Tilley and Terry, 1963) determined.

- **Leaf area index measurement**

To determine LAI fifty sward height measurements per paddock were taken approximately every 10 days from 4 July to 4 October using a graduated stick on which a 2 x 1 cm clear window was lowered until it touched a green leaf (Bircham, 1981; Barthram, 1986). The relationship between sward height and LAI was established for four dates: 4 July, 14 August, 4 September and 1 October. Three height measurements were taken in each of the quadrats used for herbage mass and tiller number determinations. Other samples of around 300 g fresh weight (FW) per plot were collected, thoroughly mixed in the laboratory and a subsample was separated into sheath, folded green laminae and unfolded green laminae. Leaf area of the unfolded green laminae was measured using a LI-3100 area meter to estimate the specific leaf area (cm^2 g^{-1} leaf DM). Fractions were oven dried and the proportion of each fraction calculated. The values obtained were used to estimate quadrats LAI. A multiple regression between the average height of each quadrat and LAI was calculated taking into account cultivars, N treatments and month of measurement (Appendix 7.1). Plot LAI was calculated using the multiple regression equation above and mean plot heights.

- **Root mass**

Root mass (organic matter, OM) was measured twice, once on 30 April, before N fertiliser application, and again on 8 October. A 50 mm internal diameter corer and 1 m length was used (Plate 5.1c) to sample five soil cores per paddock to 400 mm depth (7.9×10^{-4} m^3). The soil cores were washed on a 1 mm mesh and the root organic matter determined as described in Chapter 3. No attempt was made to distinguish between live and dead roots.

Sand-filled cores were installed in the holes created by the extraction of the soil cores taken before fertiliser application (Plate 5.1d). These were to provide a comparative measure of the new root formation. On 8 October, sand cores were removed, washed and root mass determined as above.

- **Tissue turnover measurements**

During two experimental periods, P1 from 16 August to 3 September and P2 from 6 to 24 September, the following measurements were carried out.

Tissue turnover measurements were made on 100 tagged tillers per paddock at the beginning of the experimental periods. The method was adapted from those described by Davies (1993) and Mazzanti *et al.* (1994). Tillers were labelled with coloured fine plastic-coated wire rings. To keep the wire ring in position, one end of the wire was rolled onto an 80 mm length nail buried in the soil at approximately 5 cm from the tiller. Labelled tillers were grouped in series of ten equally spaced along a 2 m transect. Ten transects per paddock were randomly allocated.

Leaf elongation rate (LER) and leaf senescence rate (LSR) were estimated as described in Chapters 4 and 5. The persistence of necrotic tissue at the extremity of the leaf indicated that no defoliation had taken place. A small cut made with the ruler tip at the leaf tip of defoliated leaves allowed the identification of the occurrence of any subsequent defoliation. Leaf consumption rate (LCR) was estimated separately on mature leaves and on the youngest leaf (i.e. expanding leaf). The reduction in the length of mature leaves was directly attributed to animal consumption. The mean LER, estimated on the population of tillers for which the expanding leaf had not been defoliated during the measurement interval, was added to the change in length recorded on defoliated expanding leaves in order to achieve an indirect estimate of the contribution of this category of leaves to animal consumption. This estimate is considered a relatively unbiased one if it can be assumed that a single defoliation does not immediately or significantly modify the instantaneous LER (Arosteguy 1982) and that a small proportion of labelled tillers have their unexpanded leaves defoliated during the measurement interval. The LER, LSR and LCR expressed in mm tiller^{-1} day^{-1} were calculated for each measurement interval and were converted to gross growth rate (GGR), senescence rate (SR) and consumption rate (CR) expressed in mg DM tiller^{-1} day^{-1} using the average dry weight per unit of leaf length. The latter value was estimated for each cultivar-N combination from 150 tillers removed from each paddock at the beginning and end of each measurement period. Tissue fluxes were expressed in terms of kg DM ha^{-1} day^{-1} using the tiller density data. Defoliation Intensity of grazed leaves of each leaf age category was calculated as the ratio between leaf length removed by a single defoliation and leaf length before the defoliation, expressed as percentage. Tiller defoliation frequency was calculated as the ratio between the number of defoliation events and total number of tiller-days. Defoliation interval was estimated as the inverse of the defoliation frequency.

- **Site usage**

Number of developed tillers were recorded by nodal position (Matthew *et al.*, 1998) on fifteen mature tillers at approximately monthly intervals from 27 May to 17 September. A binocular dissecting microscope at 15x magnification was used. The reference point for numbering of nodes was the first fully expanded leaf, the point of attachment of this leaf being considered as node zero, with counting proceeding in a basipetal direction. Only nodes numbered one to six were examined. In this experiment, results are presented as an average for six sites on the tiller and were calculated using the formula:

$$P_t = \Sigma_1^6 n_t / 6T$$

where n_t is the total number of each nodal position that bear a tiller and T is the total number of tillers examined. Since nodal probability as defined by Matthew *et al.* (1998) refers to a single site it is probably more correct to term this data 'site usage'. The term site usage as defined by Skinner and Nelson (1992) involves the average of all sites on a tiller hierarchy over a period of time.

- **Animal measurements**

Animals were weighed unfasted on 16 July, 12 August, 11 September and 4 October. Only animals that stayed in the plots for the whole period were considered for daily live weight gain calculations. Beef production per ha was calculated from live weight gain (kg LW d^{-1}) and stocking rate (animal ha^{-1}) data.

7.4. Results and discussion

7.4.1. Sward characteristics

- **LAI**

One objective of the present experiment was to maintain the sward at uniform LAI by adjusting the stocking rate. Although the working LAI was expected to be approximately 2, this value of LAI was not reached, probably due to a low tiller number, the LAI target was lowered to 1-1.5.

MK had higher values of LAI than EP throughout the experiment with the difference being significant (P<0.05) up to 4 August (Fig. 7.4a). Similarly, N100 plots had higher LAI than N0 plots, the difference being significant (P<0.05) up to 4 August (Fig. 7.4). N-induced differences in LAI tended to be higher for MK than EP with the interaction being significant (P<0.05) on 4 July and 16 July (Fig. 7.5).

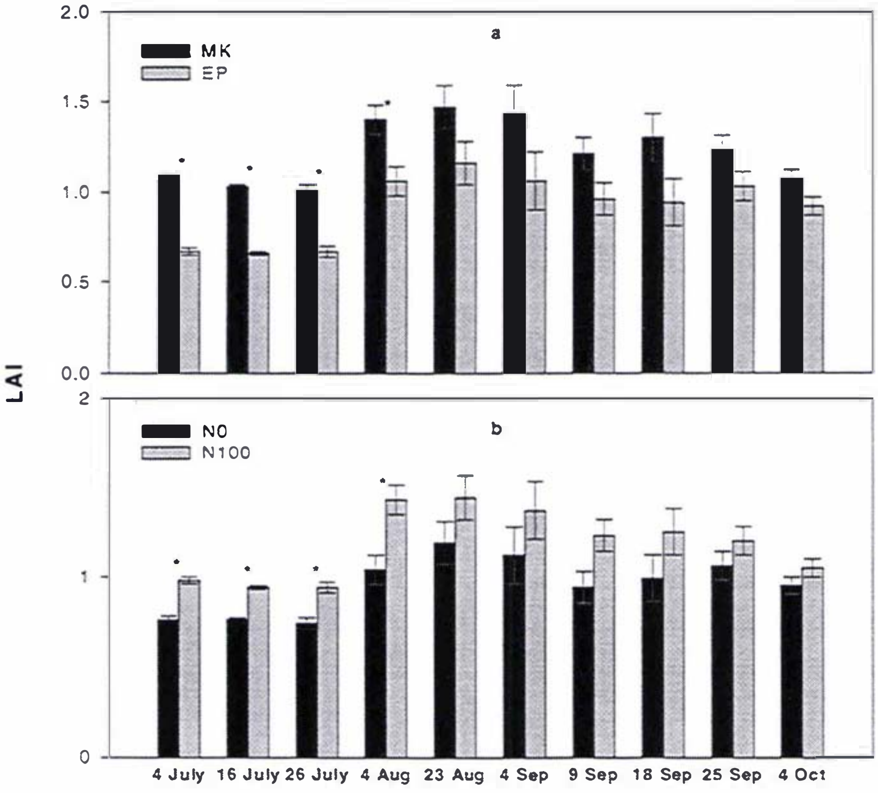


Figure 7.4. Leaf area index (LAI) for MK and EP (a) and N0 and N100 treatments (b). * indicates significant difference at P<0.05. Vertical lines represent standard errors.

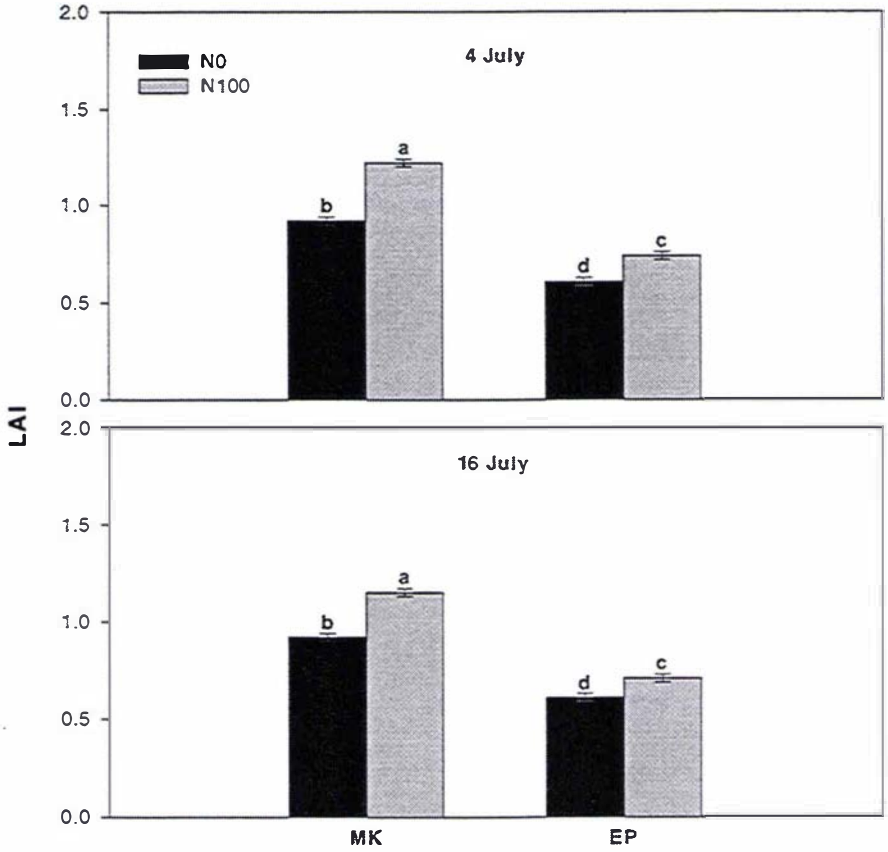


Figure 7.5. Leaf are index (LAI) for MK and EP cultivars at N0 and N100 treatments on 4 and 16 July. Letters indicate significant differences at $P<0.05$. Vertical lines represent standard errors.

When all measurement dates were analysed together, MK had higher LAI than EP ($P<0.05$). On the other hand, the contrasts between successive measurement dates showed that the overall LAI mean significantly increased ($P<0.05$) between 26 July and 4 August, and significantly decreased ($P<0.05$) between 25 September and 4 October.

It is worth noting that since the swards had been exposed to comparable previous grazing managements, and then cut to a similar height at the beginning of autumn, the differences in LAI observed between cultivars and N treatments in July confirmed the higher winter growth potential of the Mediterranean cultivar (Mazzanti and Arosteguy, 1985, Duhalde and Forjan, 1986) and the increased winter herbage production in response to N application in the SE of Buenos Aires Province (Femández Grecco *et al.*, 1995; Marino *et al.*, 1995)

- **Herbage mass**

Tall fescue herbage mass, of green and dead material, from May to October is shown in Tables 7.1 and 7.2, respectively. The herbage mass of other grasses, *Lotus* spp. and weeds is shown in Appendix 7.2. The combined contribution of the latter species to the sward dry matter was less than 10 % and diminished with time ($P<0.05$). In addition, no differences were found between cultivars, N treatment or interactions for herbage mass of any of these sward components in any of the measurement dates considered. The contrast tests between successive measurement dates indicated that the overall mean for weed herbage mass diminished significantly from July to August ($P<0.05$).

Tall fescue green dry mass was higher for MK during July and August ($P<0.05$, Table 7.1). No differences were found ($P>0.05$) between N treatments nor the cultivar x N interaction for the months in which measurements were made. On the other hand, when a repeated measures analysis was carried out, a significant N x month interaction was found ($P<0.05$). This was because the May sampling was done the same day as application of N fertiliser and no difference between N treatments was found, whereas after July, the N100 treatment tended to have a higher herbage mass than N0 (Table 7.1). There was also a significant month x cultivar interaction ($P<0.05$). No differences in green dry mass were found in May, while MK showed a higher green dry mass than EP in July ($P<0.05$, Table 7.1), again indicating the higher winter growth rate of MK than EP. In addition, overall green dry mass mean declined between September and October ($P<0.05$) as a consequence of a high actual efficiency of herbage use and a negative balance between net growth and consumption (Section 7.4.3.4).

The mass of dead tall fescue in the swards is shown in Table 7.2. The only significant difference was found in October, when N0 swards had a higher dead herbage mass than N100 swards. When data for the five months were analysed together in a repeated measures analysis, the month effect and the month x cultivar and month x cultivar x N interactions were significant ($P<0.05$). These results reflected a decrease of dead tall fescue material with time, this decrease being more pronounced in MK and in the N100 treatment.

Table 7.1. Tall fescue green dry mass (kg DM ha^{-1}) for MK and EP cultivars and two N treatments (N0 = 0 kg N ha^{-1} and N100 = 100 kg N ha^{-1}) from May to October.

	Green material (kg DM ha^{-1})					
Date	**MK**	**EP**	**N0**	**N100**	**s.e.**[1]	**Interaction (P=)**
6 May	1083	1155	1143	1094	134	0.773
4 July	1418 **a**[2]	891 **b**	1157	1151	23	0.843
14 August	1323 **a**	881 **b**	1070	1134	108	0.972
4 September	1515	1137	1189	1463	150	0.335
1 October	1149	1022	1052	1120	118	0.800

[1] Standard error.
[2] Means followed by different letters differ significantly at $P<0.05$.

Table 7.2. Dead herbage mass (kg DM ha^{-1}) for MK and EP cultivars and two N treatments (N0 = 0 kg N ha^{-1} and N100 = 100 kg N ha^{-1}) from May to October.

	Dead material (kg DM ha^{-1})					
Date	**MK**	**EP**	**N0**	**N100**	**s.e.**[1]	**Interaction (P=)**
6 May	2113	1293	1718	1688	315	0.466
4 July	1619	1398	1446	1572	70	0.942
14 August	1490	1446	1701	1235	122	0.577
4 September	1449	1103	1262	1290	117	0.950
1 October	1344	1347	1540 **a**[2]	1151 **b**	81	0.078

[1] Standard error.
[2] Means followed by different letters differ significantly at $P<0.05$.

- **Tiller density**

Tiller density was higher for MK than EP in July, August and October ($P<0.05$) but no N effect on tiller density was found ($P>0.05$) in any month (Table 7.3). In addition, the cultivar x N interaction was not significant ($P>0.05$) indicating that tiller density in both cultivars responded similarly to N application. When data for each month were considered together in a repeated measures analysis, the month effect and the month x cultivar and month x cultivar x N interactions were significant ($P<0.05$).

Table 7.3 shows that there was an increase in tiller density from May to September followed by a slight decrease from September to October. The month x cultivar interaction reflected a greater rise in tiller density between May and August for MK than EP ($P>0.05$, Table 7.3). Finally, the three-way interaction arose because tiller density for EP-N0 decreased from May to July, while afterwards, this treatment followed the general trend showed by the other cultivar-N combinations (Fig. 7.6). In addition, the increase in tiller density until August seemed higher in the N100 treatment than in N0 (Fig. 7.6). The interaction between month and N was significant at $P<0.10$, because in May, N100 had a lower tiller density than N0, while the opposite occurred after July, but as noted above, the differences between treatments were not significant for any month ($P>0.05$). However, there was a difference in overall tiller density between July and August ($P<0.05$).

Table 7.3. Tiller density for MK and EP cultivars and two N treatments (N0 = 0 kg N ha^{-1} and N100 = 100 kg N ha^{-1}) from May to October.

	Tiller density (tillers m^{-2})					
Date	**MK**	**EP**	**N0**	**N100**	**s.e.**[1]	**Interaction (P=)**
6 May	3452	2737	3381	2808	333	0.343
4 July	4260 **a**[2]	2687 **b**	3308	3639	148	0.934
14 August	5022 **a**	3098 **b**	3661	4459	350	0.807
4 September	5313	3899	4223	4988	400	0.976
1 October	4711 **a**	3612 **b**	3873	4450	285	0.515

[1] Standard error.

[2] Means followed by different letters differ significantly at $P<0.05$.

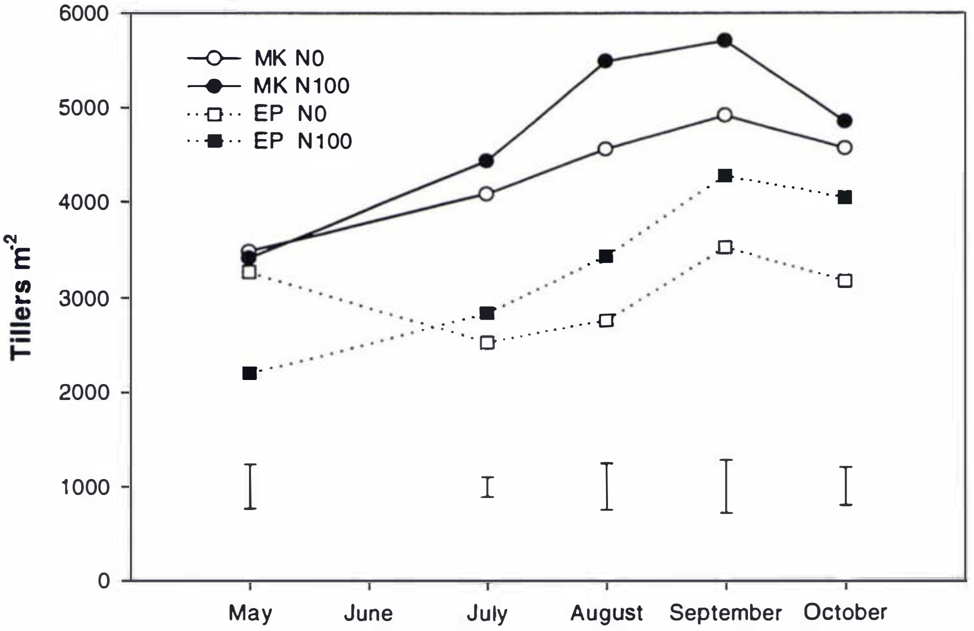


Figure 7.6. Tiller density from May to October for MK and EP at N0 and N100 treatments. Vertical lines represent standard errors.

The differences observed between cultivars support the results obtained in Balcarce by Mazzanti *et al.* (1985) who found higher tiller density for MK than EP from January to September. These authors also observed greater tiller density response to N in winter in MK than in EP evidenced by a 30 % increase in winter tiller density in MK swards that had received two applications of 50 kg N ha^{-1} in the previous spring and autumn while no response was observed in EP. In the current experiment, however, only a trend for a higher increase in MK-N100 tiller density from May to August compared with EP-N100 (Fig. 7.6) could be observed. Tiller density in the N100 treatment tended to be higher than in N0, and this was also expected due to the relatively low LAI of the swards (Simon and Lemaire, 1987; Mazzanti and Lemaire, 1994).

7.4.2. Root mass

The root mass before N application was significantly higher for MK ($P<0.05$) with no differences between N treatments ($P>0.05$, Table 7.4). The same results were observed in October for the soil core samples and, interestingly, no significant effect of N fertiliser was observed (Table 7.4). When both sampling dates were analysed together, a significant effect of sampling dates was found ($P<0.05$) as a consequence of the increased root mass harvested at the October sampling.

Root mass recovered from sand cores, which provided an estimate of the amount of root growth during the period considered (i.e. from 30 April to 8 October), also indicated a higher root production for MK, and again no N effects were found (Table 7.4).

October root mass data and root mass from sand cores were first analysed using April root mass as a covariate. However, as the covariate was not statistically significant ($P>0.05$) it was omitted in the final analysis.

Table 7.4. Root mass (g OM m^{-2}) to 400 mm depth for MK and EP cultivars and two N treatments (N0 = 0 kg N ha^{-1} and N100 = 100 kg N ha^{-1}) for 30 April and 8 October soil cores and for 8 October sand cores.

	Root mass (g OM m^{-2})					
	MK	**EP**	**N0**	**N100**	**s.e.**[1]	**Interaction (P=)**
30 April Soil	497 **a**[2]	259 **b**	363	393	36	0.75
8 October Soil	823 **a**	470 **b**	598	695	69	0.95
8 October Sand	273 **a**	160 **b**	210	223	24	0.29

[1] Standard error.

[2] Means followed by different letters differ significantly at $P<0.05$.

Since root mass and tiller density are expected to be correlated (Deinum, 1985), the higher root mass of MK could be partially explained by its higher tiller number density (Table 7.3).

Conflicting results have been published on the effect of N on root weight (Schuurman and Knot, 1974). For instance, while Oswalt *et al.* (1959) found that root weight of bromegrass decreased in response to increased N availability, Schuurman and Knot, (1974) found the opposite in Italian ryegrass. In the present experiment no effects of N on root mass were found ($P>0.05$, Table 7.4).

On the other hand, there is general agreement that N decreases root growth in relation to shoot growth, often as a consequence of a greater increase in shoot growth with respect to root growth (Davidson, 1969; Troughton, 1977; Hilbert, 1990 ; Bélanger *et al.*, 1992b). This agrees with Brouwer (1983), who affirmed that root growth is favoured when overall growth is limited by supply of a substance to be absorbed by the roots. Similarly, in the present experiment a higher shoot growth in response to N was found. This result was observed both directly, from the tissue turnover data, and indirectly through the higher stocking rates necessary to maintain similar LAI between N treatments (see Sections 7.4.3.2 and 7.4.5). This higher shoot growth and the lack of significant differences in root mass between N treatments, suggests that shoot growth was proportionally higher than root growth in N100 swards.

7.4.3. Tissue turnover

7.4.3.1. Plant characteristics

- **Leaves per tiller**

The mean number of leaves per tiller ranged between 2.8 and 3.5 (Table 7.5). These figures are slightly higher than 2.5, a value commonly quoted in the literature as characteristic for tall fescue (Lemaire and Chapman, 1996). In the first period, the average number of leaves per plant was similar for all cultivars and N treatments (Table 7.5). In the second period, however, N0 plants had a significantly higher number of leaves per plant ($P<0.05$, Table 7.5). When both periods were analysed together the plants had a lower number of leaves during the second period ($P<0.05$).

- **Leaf length**

The lengths of individual leaves, classified by position on the tiller, and the average mean leaf lengths are shown in Table 7.5. Leaf age increases from leaf 1 to leaf 6. No significant effects were found for the first three leaves within a measurement period ($P>0.05$). However, when periods were analysed together the length of leaves 2 and 3 decreased significantly with time($P<0.05$, Table 7.5). The period x N interaction was significant ($P<0.05$) for all the first three leaf categories because, during the first period, leaves in N100 swards were longer than leaves in N0, while the opposite was observed in the second period (Table 7.5). This result is consistent with the higher increase in stocking rate in N100 swards from the first to the second period than in N0 (Table 7.11).

In both periods MK had a longer leaf 4 than EP (Table 7.5). For leaf 5, the cultivar x N interaction treatment was significant ($P<0.05$, Table 7.6) in the first period. That was because MK plants had longer leaves under N100 swards than in N0 swards, while the opposite was true for EP plants. A significant effect of period for leaves 4 and 5 was found ($P<0.05$) because, as with leaves 2 and 3, leaves 4 and 5 were shorter in the second period.

Tillers with 6 leaves were found only in EP (both periods) and only in the N100 treatment in the second period. This variable was therefore not analysed statistically but the corresponding treatment means are shown in Table 7.5.

The average leaf length was similar between cultivars and N treatments in both periods. However, as this variable reflects the general trend of the individual leaves, the average leaf length was found to be shorter ($P<0.05$) during the second period. This result can be explained by the higher rates of consumption per hectare estimated during the second period (Table 7.10), with a consequent negative balance between growth and consumption (Table 7.11).

- **Leaf appearance interval**

The leaf appearance interval (LA) was longer for MK in both periods of measurement ($P<0.05$, Table 7.5) and was longer ($P<0.05$) for both cultivars during the second period. A close relationship between leaf appearance rate, which is the reciprocal of leaf appearance interval, and temperature has been reported for grasses (Langer, 1954; Davies and Calder, 1969; Peacock 1975b). While, in this experiment the differences in mean temperature do not seem to be large enough to explain the different LA between periods (Period 1 = 10.9 °C and Period 2 = 10.6°C, Fig. 7.2), it is possible that the higher rainfall recorded in August than in September (Fig. 7.3) could be responsible for this result.

Table 7.5. Mean number of leaves per plant, length of individual leaves (1 = youngest, 6 = oldest) and leaf appearance interval (LA) for MK and EP cultivars and two N treatments (N0 = 0 kg N ha^{-1} and N100 = 100 kg N ha^{-1}) in the two periods studied.

Variable	MK	EP	N0	N100	s.e.[1]	Interaction (P=)
	Period 1 (16 August to 3 September)					
Number of leaves	3.3	3.5	3.5	3.3	0.1	0.472
Leaf 1 length (mm)	46.4	41.6	41.9	46.1	2.3	0.451
Leaf 2 length (mm)	73.1	65.5	66.8	71.8	4.2	0.573
Leaf 3 length (mm)	61.9	55.2	57.8	59.3	2.2	0.907
Leaf 4 length (mm)	55.2 **a**[2]	43.6 **b**	47.4	51.4	2.5	0.132
Leaf 5 length (mm)	37.9	36.4	32.1	42.2	3.8	**0.047**
Leaf 6 length (mm)	-	19.6	14.6	5	-	-
Average length (mm)	57.5	49.4	51.5	55.4	2.8	0.769
LA (d leaf^{-1})	23.8	19.9	23	20.8	0.9	0.828
	Period 2 (6 to 24 September)					
	MK	**EP**	**N0**	**N100**	**s.e.**	**Interaction (P=)**
Number of leaves	2.8	2.9	2.9 **a**	2.8 **b**	<0.1	0.097
Leaf 1 length (mm)	48.4	44.1	48	44.6	1.8	0.228
Leaf 2 length (mm)	55.9	51.8	57.3	50.3	2.6	0.278
Leaf 3 length (mm)	43.1	37.5	42.2	38.3	2.1	0.518
Leaf 4 length (mm)	36.3 **a**	28.6 **b**	31.3	33.6	1.4	0.647
Leaf 5 length (mm)	16.3	17.7	19.3	14.7	9.5	0.795
Leaf 6 length (mm)	-	10	-	10	-	-
Average length (mm)	45.4	40.8	45.5	40.7	2.5	0.275
LA (d leaf^{-1})	34.5	26.5	30.3	30.7	1.8	0.599

[1] Standard error.
[2] Means followed by different letters differ significantly at P<0.05.
– Not estimated.

Table 7.6. Length of leaf 5 for MK and EP cultivars and two N treatments (N0 = 0 kg N ha^{-1} and N100 =100 kg N ha^{-1}) in the first period studied (16 August to 3 September).

	MK	EP
N0	25.2 **b**[1]	39.0 **ab**
N100	50.6 **a**	33.9 **ab**
s.e.[2]	5.40	

[1] Means followed by different letters differ significantly at P<0.05.
[2] Standard error.

- **Site usage**

The change in site usage for cultivars and N treatments is shown in Fig. 7.7. MK had a significantly higher site usage ($P<0.05$) on 22 July and 17 September. The N100 treatment showed higher values of site usage from May to September, but the difference was significant only on 22 July. When the months were analysed together there was a significant effect of cultivar and N indicating different growth strategies of the cultivars and the effect of N in promoting tillering. There was also significant effect of month due to an increase in mean site usage from 0.38 in May to 0.71 in August and a decrease to 0.58 in September, the difference between month being significant only between May and June. The month x cultivar interaction was also significant ($P<0.05$) reflecting the greater difference between the two cultivars especially in September ($P<0.05$).

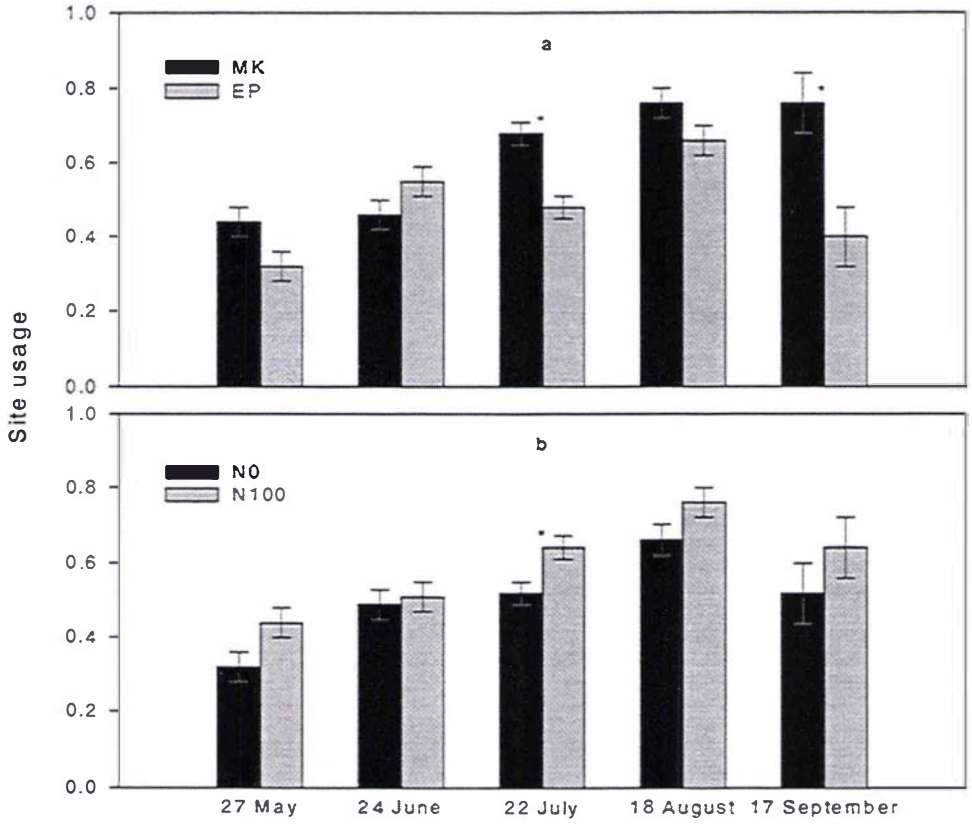


Figure 7.7. Site usage for MK and EP (a) and N0 and N100 treatments (b). * indicates significant differences at P<0.05. Vertical lines represent standard errors.

7.4.3.2. GGR, SR and NGR

The GGR, SR and NGR for the two periods studied are shown in Fig. 7.8. Neither cultivar nor N effects were significant for GGR or SR during the first period ($P>0.05$, Fig. 7.8a), however, the application of N significantly increased NGR ($P<0.05$, Fig. 7.8a). The same results were found for the second period, but in addition, EP showed a higher NGR than MK ($P<0.05$, Fig. 7.8b). Mean GGR was significantly higher ($P<0.05$) in Period 1 (23.6 kg ha^{-1} d^{-1}) than in Period 2 (17.4 kg ha^{-1} d^{-1}). No significant differences between periods were found for SR ($P<0.05$). Consequently, NGR decreased significantly ($P<0.05$) from the first to the second period (Period 1 = 11.8 kg ha^{-1} d^{-1} and Period 2 = 7.0 kg ha^{-1} d^{-1}).

In agreement with the results of the individual analyses, the combined analysis also found a significant effect of N on NGR. Application of N increased the NGR by 78 % and 58 % during Periods 1 and 2, respectively. This higher production in N100 was the result of an increased GGR, though the difference between N treatments was not statistically significant, and a similar SR. The increased GGR was directly associated with a higher leaf extension rate (LER). Increases in LER of up to four-fold in response to N have been observed in field conditions (Gastal *et al.*, 1992). These results confirm previous experimental evidence in that winter forage growth in the SE of Buenos Aires Province is limited due to N unavailability (Femández Grecco *et al.*, 1995; Marino *et al.*, 1995).

The higher winter growth potential of MK than EP in the SE of Buenos Aires Province has been confirmed by several cutting experiments (Mazzanti and Arosteguy, 1985; Mazzanti *et al.*, 1985; Duhalde and Forjan, 1986). In this experiment, for instance, it was necessary to graze MK paddocks earlier and with a higher stocking rate (Table 7.12) than EP, because MK had higher winter growth. However, by mid August, when tissue tumover measurements began, temperatures had already begun to increase and EP was beginning to show spring growth. Consequently, in August MK and EP cultivars had a similar NGR. Moreover, by the second period, despite the lower absolute values of GGR and NGR estimated for both cultivars, EP showed a higher NGR than MK. Similar results were found by Lattanzi[1] in a cutting trial carried out in enclosures located inside the paddocks used in the present grazing experiment (Fig. 7.9). In Lattanzi's experiment 30 % of the fertiliser was applied on 30 May and 70 % on 17 July. Cumulative herbage production was measured by 7 cuts carried out between 22 May and 15 October. In Fig. 7.9 is shown that from late May to mid

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[1] Personal communication, Escuela de Postgrado en Producción Animal, Unidad Integrada Balcarce **INTA-FCA**.

August MK-N100 herbage accumulation was higher than for the other treatments, indicating that N application was required to achieve the growth potential of MK. By the beginning of September MK-N0 showed a similar herbage accumulation to EP-N100, which shows that winter growth potential of MK was similar to EP that had received 100 kg N ha^{-1}. However, during September, coinciding with Period 2 of the grazing experiment, for both EP-N0 and EP-N100 the herbage accumulation rate was higher than for their respective MK counterparts (Fig. 7.9).

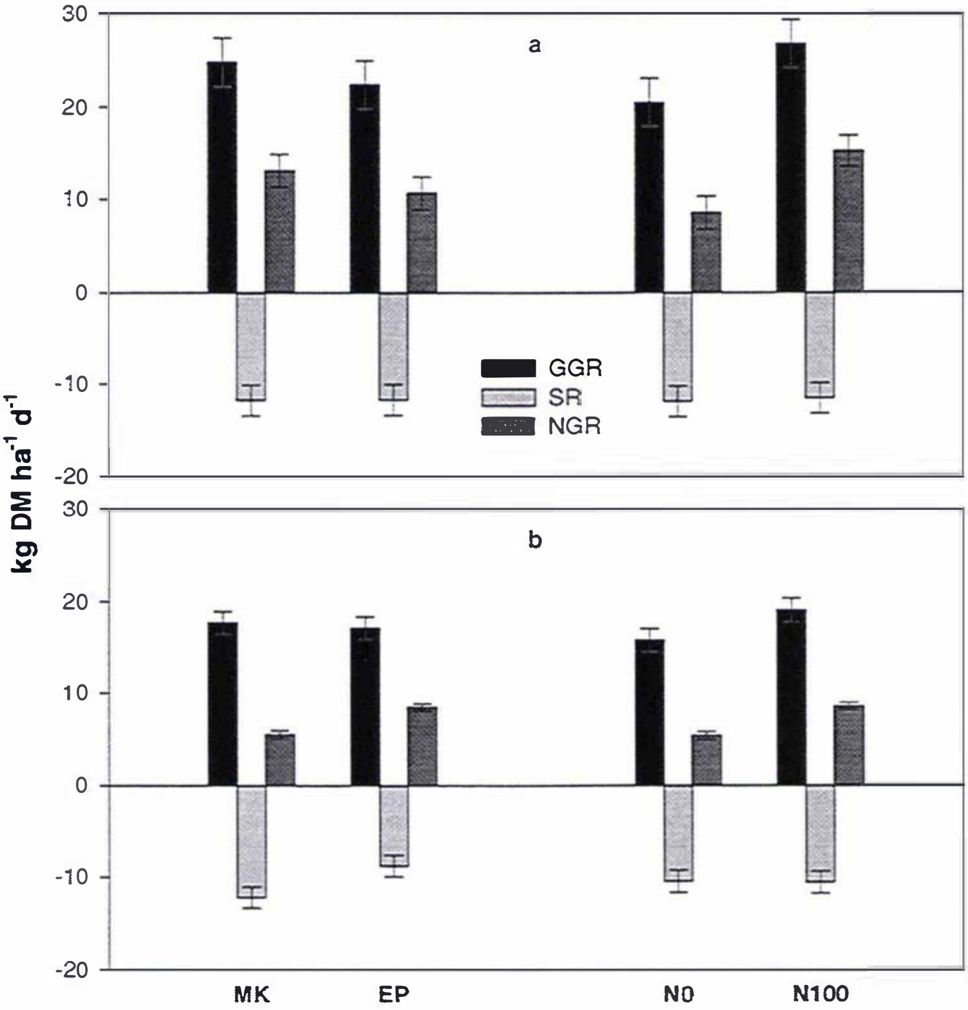

Figure 7.8. Gross growth rate (GGR), senescence rate (SR) and net growth rate (NGR) for MK and EP and N0 and N100 treatments. a: 16 August to 3 September 1996. b: 6 to 24 September 1996. Vertical lines represent standard errors.

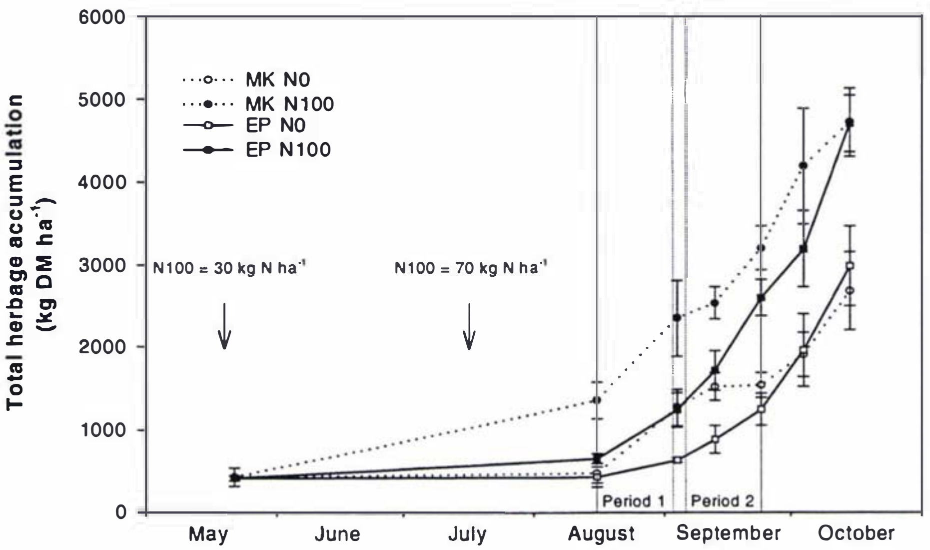

Figure 7.9. Total herbage accumulation for MK and EP at N0 and N100 treatments. Each point is the average of 4 replicates. Vertical lines represent standard errors. Reference lines indicate periods of tissue turnover measurement in the grazing experiment. From Lattanzi (unpublished).

7.4.3.3. Consumption

- **Intensity of defoliation**

The intensity of defoliation expressed as percentage of lamina removed and the interval between consumption events on each tiller are shown in Table 7.7. For leaves 1, 2 and 3 the percentage of lamina removed was higher for EP than MK in the first period ($P<0.05$) while no differences were found between cultivars for these leaves in the second period ($P>0.05$).

For leaf 1 when both periods were analysed together, a significant effect of cultivar was found ($P<0.05$), and no differences between periods were observed ($P>0.05$).

For leaf 2 the cultivar x N interaction in the second period ($P<0.05$) was significant because the proportion removed was higher for EP-N0 and lower for MK-N0, while both cultivars showed an intermediate and similar value under N100 (Table 7.8). The effect of period was significant, the percentage of lamina removed being higher during the second period ($P<0.05$, Period 1 = 56.4 % and Period 2 = 59.5 %).

No effects of period were found for leaf 3. None of the effects were significant ($P>0.05$) for leaves 4 and 5 in the individual periods, nor when both periods were analysed together. Values for leaf 6 shown in Table 7.7 are for completeness and were not statistically analysed because the majority of tillers had less than 6 leaves.

Although the consumption percentages for leaves 2 and 3 were higher for EP than for MK in the first period ($P<0.05$, Table 7.7), no differences between cultivars were found in the actual leaf length consumed ($P>0.05$). Conversely, for leaf 1, the effects of cultivar and N treatment on length of leaf consumed during the first period were significant. This result was mainly due to a significant cultivar x N treatment interaction (MK-N0 = 22.6 mm, MK-N100 = 22.5 mm, EP-N0 = 22.4 mm and EP-N100 = 29.5 mm, $P<0.05$). The reason of the higher length removed in leaf 1of EP-N100 swards is not clear since on the 23 August this treatment did not have a different LAI than the other treatments (MK-N0 = 1.36, MK-N100 = 1.58, EP-N0 = 1.02 and EP-N100 = 1.30, s.e. = 0.17, $P>0.05$) and the stocking rate for EP-N100 was similar to MK-N100 (MK-N100 = 5.6 an. ha^{-1} and EP-N100 = 5.8 an. ha^{-1}, s.e. = 0.08 an. ha^{-1}, $P>0.05$).

The variation between the proportion of leaf removed in each defoliation between cultivars observed here, for the first 3 leaves during the first period, and the increase in the proportion removed between periods for leaf 2 differ from results reported by Mazzanti and Lemaire (1994). These researchers observed a constant percentage of leaf removed by sheep at each defoliation irrespective of the stocking rate, tall fescue genotype or N treatment. While in their experiment the proportion removed was about 50 %, in the present experiment it ranged between approximately 50 and 70 % for the first four leaves. The differences between experiments could be partially due to the different LAI, the higher actual efficiency of herbage use estimated for the second period in the present experiment (Table 7.11), and/or to differences in grazing behaviour between sheep and steers.

- **Tiller defoliation interval**

None of the effects considered were significant ($P>0.05$) for the tiller defoliation interval during the first period (Table 7.7). However, in the second period the cultivar, and N treatment effect and the interaction between them were all significant ($P<0.05$). The defoliation interval was higher for MK than for EP, and higher for N0 than for N100. The interaction is shown in Table 7.9, where it can be observed that EP-N100 had the lowest interval ($P<0.05$), while all the other cultivar x N treatment combinations had a similar value ($P>0.05$). This result was directly associated with the stocking rates necessary to maintain a similar LAI among treatments during the second period (MK-N0 = 4.3 an. ha^{-1}, MK-N100 = 6.4 an. ha^{-1}, EP-N0 = 5.0 an. ha^{-1} and EP-N100 = **9.6** an. ha^{-1}).

There was a significant period effect (P<0.05) and period x cultivar interaction (P<0.05). The effect of period was due to the longer tiller defoliation interval estimated for the first period. The interaction occurred because, while the interval was shorter for MK in the first period, it was longer in the second period.

Table 7.7. Percentage of individual leaf lamina removed for the different leaf categories (1 = youngest, 6 = oldest) and tiller defoliation interval (days) for MK and EP cultivars and two N treatments (N0 = 0 kg N ha^{-1} and N100 = 100 kg N ha^{-1}) in the two periods studied.

	Period 1 (16 August to 3 September)					
Variable	**MK**	**EP**	**N0**	**N1**	**s.e.**[1]	**Interaction (P=)**
Leaf 1 percentage (%)	49.1 **b**[2]	62.2 **a**	54.3	56.9	2.9	0.397
Leaf 2 percentage (%)	52.4 **b**	60.5 **a**	58.4	54.5	1.9	0.152
Leaf 3 percentage (%)	51.9 **b**	64.0 **a**	59.8	56.2	2	0.788
Leaf 4 percentage (%)	58.5	59.3	53.3	64.6	4.4	0.998
Leaf 5 percentage (%)	18	68.9	43.6	43.3	14.4	0.15
Leaf 6 percentage (%)	-	22.9	22.9	0	-	-
TDI (d $tiller^{-1}$)	24.7	37.7	38.3	24	4.3	0.727
	Period 2 (6 to 24 September)					
	MK	**EP**	**N0**	**N1**	**s.e.**	**Interaction (P=)**
Leaf 1 percentage (%)	58.2	61.2	59.2	60.2	1.4	0.113
Leaf 2 percentage (%)	58.1	60.8	59.5	59.5	1.3	**0.047**
Leaf 3 percentage (%)	56.7	59.8	53	63.5	3.8	0.851
Leaf 4 percentage (%)	69.1	55.7	54.2	70.7	16.7	0.625
Leaf 5 percentage (%)	0	25.5	17.2	8.3	13.5	0.667
Leaf 6 percentage (%)	-	0	-	0	-	-
TDI (d $tiller^{-1}$)	19.8 **a**	16.2 **b**	19.1 **a**	16.9 **b**	0.4	**0.007**

[1] Standard error.

[2] Means followed by different letters differ significantly at P<0.05.

- Not estimated.

Table 7.8. Percentage of lamina removed from leaf 2 for MK and EP cultivars with two N treatments (N0 = 0 kg N ha^{-1} and N100 = 100 kg N ha^{-1}) in the second period studied (6-24 September).

	MK	EP
N0	55.7 **b**[1]	63.4 **a**
N100	60.6 **ab**	58.3 **ab**
s.e.[2]	1.8	

[1] Means followed by different letters differ significantly at P<0.05
[2] Standard error.

Table 7.9. Interval between defoliation events on each tiller for MK and EP cultivars and two N treatments (N0 = 0 kg N ha^{-1} and N100 = 100 kg N ha^{-1}) in the second period studied (6-24 September).

	MK	EP
N0	19.4 **a**[1]	18.7 **a**
N100	20.1 **a**	13.7 **b**
s.e.[2]	0.5	

[1] Means followed by different letters differ significantly at P<0.05
[2] Standard error.

- **Herbage consumption rate**

The herbage consumption rate (kg DM ha^{-1}d^{-1}), during the first period, was significantly higher for MK and for the N100 treatment (P<0.05, Table 7.10). No significant differences were found for this rate in the second period (P>0.05, Table 7.10). The effect of period was significant (P<0.05) because the overall mean of the first period was lower than for the second period. The period x cultivar interaction was also significant (P<0.05).

The consumption (Table 7.10) and stocking rates (Table 7. 11) allowed calculation of the DM intake per animal (Table 7.10.). No significant effects were found during the first period (P>0.05). During the second period, consumption rate per animal was higher for MK than EP (P<0.05) and higher for N0 than N100 (P<0.05) this is a consequence of the lower stocking rates in MK compared with EP and in N0 in relation to N100 (P<0.05, Table 7.11). The cultivar x N interaction was not significant (P>0.05). When both periods were analysed together, a significant effect of cultivar was found (P<0.05), but no differences were found between periods.

The values of consumption per animal estimated in this experiment were moderately lower than the tabulated value of 5.5 kg DM $an^{-1}d^{-1}$ (Agricultural and Food Research Council, 1993) for steers of 200 kg LW (average animal weight for the grazing period) gaining 1 kg LW d^{-1} (Table 7.12). The measurements involved in the calculation of consumption by the tissue turnover method are potentially subject to systematic error and it is unclear which of these measurements might have resulted in the low estimate in this study.

Table 7.10. Herbage consumption rate per ha (kg DM $ha^{-1}d^{-1}$) and per animal (kg DM $an.^{-1}d^{-1}$) for MK and EP cultivars and two N treatments (N0 = 0 kg N ha^{-1} and N100 = 100 kg N ha^{-1}) in the two periods studied.

	Period 1 (16 August to 3 September)					
Consumption	**MK**	**EP**	**N0**	**N100**	**s.e.**[1]	**Interaction (P=)**
Per ha	20.9 **a**[2]	12.9 **b**	12.2 **b**	21.7 **a**	1.9	0.192
Per animal	4.4	3	3.5	3.8	0.5	0.274
	Period 2 (6 to 24 September)					
	MK	**EP**	**N0**	**N100**	**s.e.**	**Interaction (P=)**
Per ha	21.9	21.9	19.7	24.1	1.4	0.355
Per animal	4.2 **a**	3.2 **b**	4.2 **a**	3.1 **b**	0.2	0.907

[1] Standard error.

[2] *Means followed by different letters differ significantly at P<0.05.*

In a previous grazing experiment conducted in Balcarce (Escuder *et al.*, 1992), a higher intake per animal on MK than in EP swards was also found during winter and spring. In that experiment, both cultivar swards had similar initial dry mass and stocking rate, but DM intake [g DM $(kg\ LW\ d)^{-1}$] was higher in MK than in EP during spring 1982 (33 %), and winter (17 %) and spring (23 %) 1983. As no differences in bite size of steers grazing either type of sward were found (Cangiano *et al.*, 1986), the higher intake in MK was attributed to a lower ratio of pseudostem:leaf (P : L) in the sward, and also to a higher proportion of leaf in the diet of animals grazing MK, which in turn, resulted in a lower rumen retention time (Escuder *et al.*, 1992). The latter finding was probably associated with the smaller size of leaf particles than pseudostem particles in the rumen (Minson, 1982). Coincidentally, in the present experiment the P : L ratio was significantly lower in MK swards in July (MK = 0.31 and EP = 0.50, s.e.=0.02, P<0.05), in August, September and October the difference was not significant, but the ratio was still lower for MK than EP.

7.4.3.4. Actual efficiency of herbage use and balance between herbage net growth and consumption

The actual efficiency of herbage use (AEHU), defined as the ratio between herbage consumption and herbage growth (Mazzanti and Lemaire, 1994), is shown in Table 7.11. No differences were found for cultivars or N treatments for any period ($P>0.05$). However, the cultivar x N interaction was almost significant ($P= 0.054$) in the second period due to a higher AEHU in MK in N0 than in N100, while the opposite took place in EP (MK-N0 = 1.34, MK-N100 = 1.17, EP-N0 = 1.17, EP-N100 = 1.36, s.e. = 0.07). There was a higher AEHU in Period 2 than Period 1 ($P<0.05$). In Period 1 MK tended to have a higher AEHU than EP ($P>0.05$), while both cultivars had the same AEHU during the second period, resulting in a significant period x cultivar interaction ($P<0.05$).

According to Bircham and Hodgson (1983), when swards under continuous stocking management are in a steady state, the net herbage accumulation would be zero. To achieve this it is necessary that the net accumulation of green material (i.e. growth minus senescence) is the same as the consumption of green material. Under this situation, values of the AEHU between 50 % and 80 %, for laxly grazed swards and for hard grazed swards, respectively, have been found (Hodgson, 1990). The results of the first period fell approximately between these values (Table 7.11). However, during the second period the AEHU values were higher than 1.0. Under this situation, a decrease in the herbage mass is occurring. The decreases in both the GGR and NGR observed during the second period could be explained in this way. Moreover, when the balance between net herbage production and consumption was analysed, it was found that during the first period the balance was not sufficiently different from zero to be statistically detected ($P>0.05$) for EP and N0 treatments. This result indicated that for the latter treatments, in which the AEHU was also lower, the swards were maintained close to equilibrium. Conversely, the balance for both cultivars and N treatments during the second period was more negative and significantly different from zero ($P<0.05$) indicating that animals were removing herbage faster than it was growing. Consequently, when both periods were analysed together, the period effect was significant ($P<0.05$). The latter analysis also found a significant effect of cultivar ($P<0.05$) because MK tended to have a lower mean than EP in both periods for the balance between herbage net growth and consumption.

Table 7.11. Actual efficiency of herbage use (AEHU=herbage consumption/ herbage growth), balance between net herbage production (kg DM ha^{-1} d^{-1}) and stocking rate (an. ha^{-1}) for MK and EP cultivars and two N treatments (N0 = 0 kg N ha^{-1} and N100 = 100 kg N ha^{-1}) in the two periods studied.

	Period 1 (16 August to 3 September)					
Variable	**MK**	**EP**	**N0**	**N100**	**s.e.[1]**	**Interaction (P=)**
AEHU	0.84	0.58	0.6	0.82	0.1	0.722
Balance	-7.89*	-2.27	-3.64	-6.52*	1.83	0.910
Stocking rate	4.66	4.47	3.42 **b**	5.72 **a**	0.06	**0.007**
	Period 2 (6 to 24 September)					
	MK	**EP**	**N0**	**N100**	**s.e.**	**Interaction (P=)**
AEHU	1.25	1.27	1.25	1.26	0.1	0.054
Balance	-16.36*	-13.45*	-14.28*	-15.53*	1.41	0.363
Stocking rate	5.39 **b**	7.32 **a**	4.67 **b**	8.04 **a**	0.45	0.120

[1] Standard error.

[2] Means followed by different letters differ significantly at $P<0.05$.

* Different from zero at $P<0.05$.

7.4.4. Sward digestibility and N content

The changes in sward digestibility from N fertilisation to the end of the experiment, for cultivars and N treatments, is shown in Fig. 7.10. Digestibility was significantly higher ($P<0.05$, Fig. 7.10a) for EP than MK from May to September, and was significantly higher ($P<0.05$, Fig. 7.10b) under N100 treatment in July and August. Although in the present experiment MK had lower digestibility values during winter than EP, results from a previous experiment showed that the *in vitro* digestibility of the diet of fistulated steers grazing MK and EP showed no differences in digestibility between these cultivars in winter or spring (Assuero, 1984). However, lower digestibility values were observed in MK during summer.

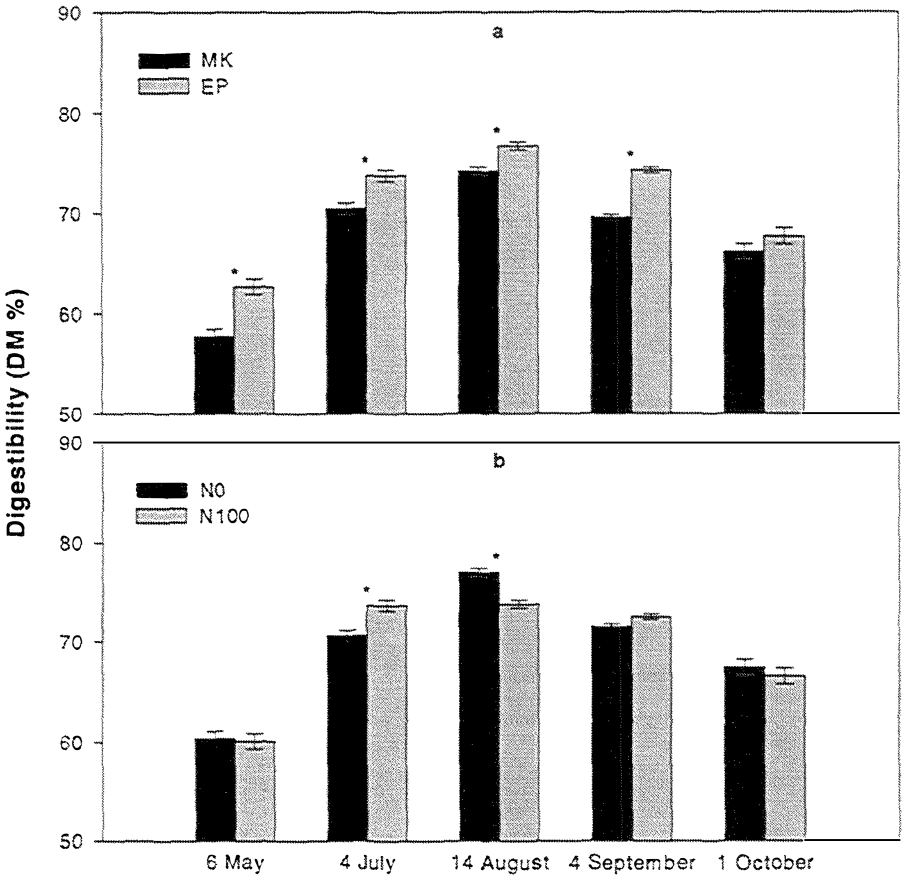

Figure 7.10. Dry matter digestibility (%) for MK and EP (a) and N0 and N100 treatments (b). * indicate significant difference at $P<0.05$. Vertical lines represent standard errors.

Changes with time in sward N content for cultivars and N treatments are shown in Fig. 7.11. No differences were found between cultivars in any month. The N100 treatment significantly increased ($P<0.05$) N content in August, September and October. Considering all the months together, a significant N treatment effect was found. The month and month x N effects were also significant ($P<0.05$). A significant difference in N treatment between May and July was observed (Fig. 7.11b) reflecting the effects of N application in May and July. A decrease in the N content of the sward was observed between September and October ($P<0.05$, Fig. 7.11).

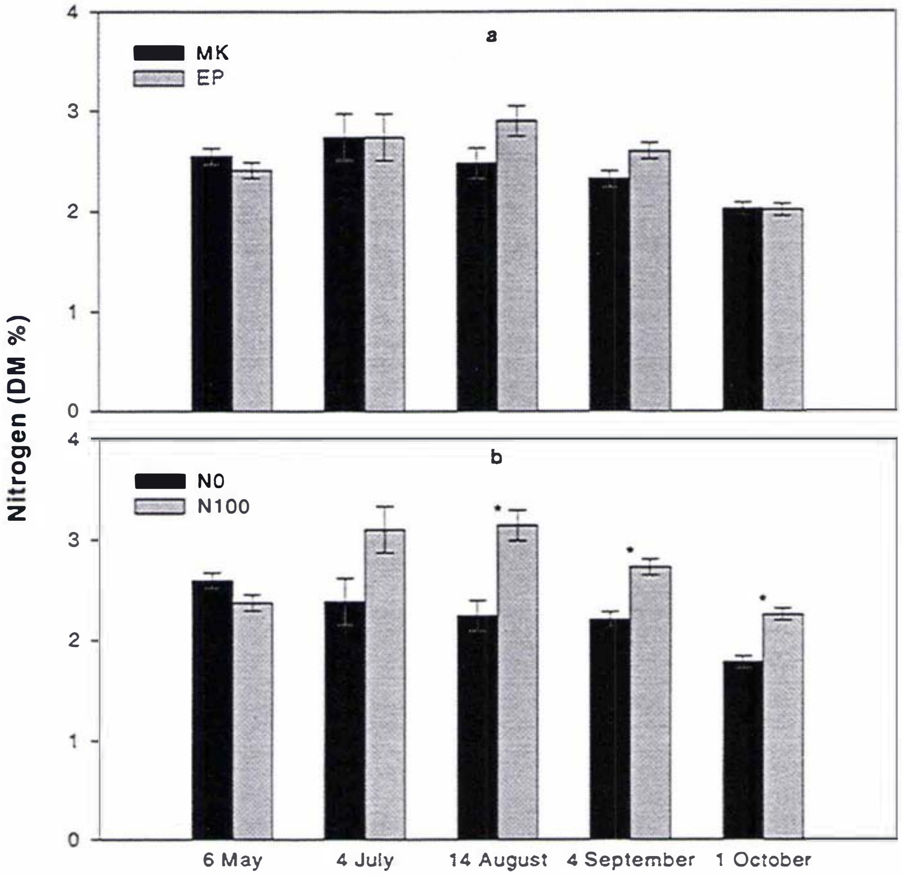

Figure 7.11. Nitrogen content (DM%) for MK and EP (a) and N0 and N100 treatments (b). * indicates significant difference at P<0.05. Vertical lines represent standard errors.

7.4.5. Stocking rate, daily live weight gain and beef production

The mean stocking rate per ha from 16 July to 4 October is shown in Table 7.12. To maintain a similar LAI in the swards it was necessary to increase the stocking rate by 18 % in MK swards with respect to EP swards and, at the same time, to increase the stocking rate 67 % in the N100 treatments in comparison with N0. The differences in stocking rate, both between cultivars and between N treatments, were significant (P<0.05). The daily live weight gain, however, was similar for cultivars and N treatments (Table 7.12). Consequently, the beef production per ha was significantly higher in MK (26 % higher than EP cultivar) and in N100 treatment (66 % higher with respect to N0, Table 7.12). The cultivar x N interaction was not significant (P>0.05) for any of these variables.

Table 7.12. Mean stocking rate, beef production and daily live weight gain (DLWG) for MK and EP and two N treatments (N0 = 0 kg N ha^{-1} and N100 = 100 kg N ha^{-1}).

Cultivar	**Stocking rate (an. ha^{-1})**	**DLWG (kg LW d^{-1})**	**Beef Production (kg LW ha^{-1})**
MK	3.2 **a**[1]	1.02	260 **a**
EP	2.7 **b**	0.97	206 **b**
Nitrogen			
N0	2.2 **b**	1.00	175 **b**
N100	3.7 **a**	0.99	291 **a**
s.e.[2]	0.1	0.04	6
Interaction (P=)	0.148	0.273	0.959

[1] Means followed by different letters within cultivars or N treatments differ significantly at $P<0.05$.
[2] Standard error.

The average prices 1991-96[2] were US\$ 0.288 per kg urea, US\$ 5 fertiliser application per ha, and the farmer received US\$ 0.835 per kg beef (US\$ 0.885 per kg beef at Mercado de Liniers less 5.6 % for taxes). In the case of the split application of 100 kg N as in this study, it is necessary to increase the beef production by more than 87.5 kg ha^{-1} for the fertilisation to be profitable. The figures in Table 7.12 indicate that the N100 treatment increased beef production 116 kg ha^{-1}, resulting in net profit of approximately US\$ 24 ha^{-1}.

N application in winter and the complementary use of Mediterranean and temperate cultivars can be recommended for animal production systems in the SE of Buenos Aires Province. The promotional effect of N on pasture growth and the increased pasture production of Mediterranean material in winter, help bridge the feed deficit which occurs at this time of the year.

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[2] Personal communication Ing. Agr. Néstor Scheggia, Administracion Rural, FCA, Balcarce, Argentina.

7.5. Summary

7.5.1. Nitrogen effects

- A higher LAI in response to N application during July. By August it was possible to maintain a comparable LAI between treatments.

- The expected increase in tiller density in response to N fertilisation was not of sufficient magnitude to be statistically significant. However, site usage was slightly higher for the N100 treatment. NGR was significantly enhanced by N fertilisation. The amount of N applied here increased the stocking rate by 67 % and the beef production per hectare by 66 % during the period considered.

7.5.2. Cultivar effects

- MK had a higher LAI during July. As above, by August it was possible to maintain a homogeneous LAI between treatments.

- MK showed a higher tiller density and a greater root mass and root production during winter than EP.

- In late winter-early spring, when NGR was estimated, EP showed a higher growth rate. However, because of the higher winter growth of MK prior to commencing measurements it was necessary to graze MK paddocks earlier.

- Despite an increase in EP stocking rate later in the season, the mean stocking rate for the whole period was higher for MK.

- Although higher consumption rates were measured for MK, no differences in LWG were observed between cultivars. However, a higher beef production for the whole grazing period was found for MK, due to a higher mean stocking rate.

CHAPTER 8. CONCLUSIONS

In comparison with the temperate cultivars EP and GA, the Mediterranean cultivar MK is characterised by smaller plant size, higher tiller number and a higher root : shoot ratio. Although, MK had a lower stomatal resistance, which was confirmed by its higher Δ compared with EP and GA, its small shoot size and high root : shoot ratio seem to confer on MK the capacity of delaying the effects of water deficit when growing in monoculture in pots. In addition, MK had a lower content of reducing and fructosyl sugars and a lower growth rate under high temperatures than the temperate cultivar EP.

All tall fescue cultivars exhibited decreased growth rates, diminished evaporative surface area, increased root : shoot ratio and osmotic adjustment in response to water deficit. The proportion of root mass and length increased with depth in the profile and the mean root diameter was higher as soil moisture decreased. The Δ of leaf tissue did not reflect the changes that took place in response to the short term application of the different water regimes.

Plant water status was generally similar for the contrasting cultivars under comparable soil water availability. However, EP and GA showed a greater stomatal resistance at lower soil moisture and a greater ability for osmotic adjustment than MK under the most stressful water deficit conditions studied.

Further studies of these cultivars would be necessary to evaluate their capacity for recovery after drought, their ability for osmotic adjustment in the meristematic tissues, and also their behaviour under field conditions.

The unique response observed in this study for each endophyte strain-grass genotype combination demonstrates, as several other experiments have done, that endophyte presence in a mixed population increases the phenotypic variability. It would therefore be recommended that any endophyte strain was evaluated in combination with the plant genotypes with which it is likely to be associated. The experimental evidence suggests that MK-KY31 and EP-AR501 combinations would tolerate water deficit conditions more effectively than other combinations studied.

To maintain MK swards at the same LAI as EP swards it was necessary to graze them earlier, which resulted in a higher stocking rate and a 26% higher beef production on MK swards during mid winter-early spring. The impact of N application was more pronounced with beef production increased by 66% during the same period. However, when tissue turnover measurements were taken, by the end of August no differences in NGR were found between cultivars, while in September EP showed a higher NGR than MK. For both periods N fertiliser application increased the NGR of the swards. These results support earlier reports that proposed the complementary use of temperate and Mediterranean cultivars in animal production systems of the SE Buenos Aires Province to improve feed supply during winter. In addition, winter herbage growth and animal production of both cultivar types can be considerably improved with the application of N fertiliser.

Appendix 2.1. Plant water relations

Appendix 2.1.1. Cell structure

The plant cell consists of a cytoplasm with many compartments bounded in the outside by a membrane and cell wall (Kramer and Boyer, 1995). The plant cell membrane, the plasmalemma, makes osmosis possible because it is highly permeable to water molecules but slowly and selectively permeable to solutes (Salisbury and Ross, 1992; Kramer and Boyer, 1995). The primary cell wall is commonly highly permeable to both water and solutes, and provides the rigidity to allow an increase in pressure (Salisbury and Ross, 1992). As the cell ages, more layers of wall are often deposited on the inside of the primary wall. These secondary wall layers may include lignins, suberins and other compounds that confer properties such as cell wall rigidity and imperviousness to water. Two kinds of pores exist in the wall, a few large pores filled with protoplasm (plasmodesmata) and many much smaller pores (4.0 to 6.5 nm in diameter) filled with the external cell solution. The compartments or organelles (vacuole, mitochondria, nucleus, and plastids) are each bounded by a membrane similar to the plasmalemma that exchanges water and solutes with the cytosol (Kramer and Boyer, 1995).

Inside the cells there is always a concentrated solution that includes metabolites, inorganic salts and macromolecules. In contrast, on the outside of the cells there is a dilute solution (except in seawater or around embryonic cells). In response to the difference in solute concentration water enters the cell and the cell swells. As the plasmalemma becomes restrained by the cell wall, expansion is resisted and turgor pressure develops inside the cell. Cell wall rigidity contributes to the form and strength of tissues, but in herbaceous plants, much of the form of leaves and stems depends on the turgor pressure in their cells (Kramer and Boyer, 1995).

When the solute concentration outside the cell is high or when transpiration occurs, the cell loses water and shrinks and, if dehydration becomes severe, the organelles can become deformed. The cell wall frequently develops folds as the cell shrinks, however in some cases, when the wall has layers of rigid material, folding does not occur and the cell content may come under tension (Kramer and Boyer, 1995). It is evident that osmosis is the process that moves water into and through plants and that the plasmalemma is the key to the process. Any disruption in the plasmalemma caused by external factors (e.g. freezing and thawing or chemical agents) will bring about an abrupt decrease in the water transport and the plant will desiccate to the air-dry state (Kramer and Boyer, 1995).

Appendix 2.1.2. Water potential

The most useful way to characterise the water status is in terms of chemical potential. This avoids some of the biological system's intrinsic variation, permits the water status to be reproduced and indicates the force that moves water from place to place. When the potential is expressed in pressure units it is termed water potential (Kramer and Boyer, 1995).

The water potential (Ψ_w) comprises several components attributable to solute, pressure, porous matrices and gravity. The components are expressed as:

$$\Psi_w = \Psi_o + \Psi_p + \Psi_m + \Psi_g \quad (A2.1)$$

where:

Ψ_o = the solute or osmotic potential
Ψ_p = the pressure potential or turgor
Ψ_m = the matric potential
Ψ_g = the gravitational potential.

Each component refers to the same point in the solution and is algebraically additive according to whether it increases (positive) or decreases (negative) Ψ_w at that point compared to the reference potential. The reference potential is pure, free water at atmospheric pressure and at a defined gravitational position, at the same temperature as the system of interest (Kramer and Boyer, 1995).The Ψ_m is not strictly additive with $\Psi_{o,}$ Ψ_p and Ψ_g, but no alternative equation has yet been developed (Tyree, 1976).

Solutes lower Ψ_w by decreasing the number of water molecules able to move compared to pure water. The following relationship, discovered empirically by van't Hoff in 1887 (Salisbury and Ross, 1992), allows the calculation of approximate Ψ_o from the molal concentration of a solution:

$$\Psi_o = -CiRT \quad (A2.2)$$

where:

C = concentration of the solution expressed as molality (moles of solute per kg H_2O)
i = a constant that accounts for ionization of the solute and/or other deviation for perfect solutions (for nonionized molecules, such as glucose in dilute solutions i = 1)

R = the gas constant (0.00831 kg MPa mol^{-1} K^{-1})
T = absolute temperature (K) = degrees C + 273

Wetable matrices also decrease the number of molecules able to move because of surface attractions. When the external pressure is higher than the atmospheric pressure the ability of water to move increases while the opposite occurs when external pressure is below atmospheric. Similarly, gravity increases or decreases the ability of water to move depending on whether local pressure is increased or decreased by the weight of water (Kramer and Boyer, 1995).

Appendix 2.1.2.1. Plant water potential

Gravitational potential becomes significant at heights greater than one metre in vertical water columns, as in trees, but can often be ignored. Then, according to Kramer and Boyer (1995) Eq. (*A*2.1) becomes:

$$\Psi_w = \Psi_o + \Psi_p + \Psi_m \quad (A2.3)$$

In plant tissues it is necessary to consider structure because of the presence of the symplast or protoplast inside and the apoplast or external solution outside. The protoplast contains a solution under pressure (turgor) applied by the walls and its water potential is:

$$\Psi_{w(p)} = \Psi_{o(p)} + \Psi_{p(p)} \quad (A2.4)$$

where the subscript *p* denotes the protoplast. Matric potential ($\Psi_{m(p)}$) can be ignored because generally the water content is high and there are no air-water interfaces.

In comparison, the apoplast contains a solution in the porous cell wall subjected to pressures generated by surface effects. Then its water potential is:

$$\Psi_{w(a)} = \Psi_{o(a)} + \Psi_{m(a)} \quad (A2.5)$$

where the subscript *a* denotes the apoplast. Pressure potential ($\Psi_{p(a)}$) can be ignored because external pressure is atmospheric.

Water moves readily into and out of cells depending of the water potential differences between the protoplast and the apoplast. The differences in water potential do not need to be high to originate large flows. In fact, water potentials across membranes are so small that the protoplast and its cell wall are almost at equilibrium. For this reason it is assumed that:

$$\Psi_{w(p)} = \Psi_{w(a)} \qquad (A2.6)$$

and substituting Eqs. (*A*2.4) and (*A*2.5) in Eq. (*A*2.6)

$$\Psi_{o(p)} + \Psi_{p(p)} = \Psi_{o(a)} + \Psi_{m(a)} \qquad (A2.7)$$

There is a substantial difference in the solute concentration across the membrane, the inside being more concentrated. In addition, the turgor in the cells is positive ($\Psi_{p(p)}$) but the water in the apoplast in a multicellular plant is under tension ($\Psi_{m(a)}$). Because of this, an important pressure difference accross the plasmalemma originates, which would burst the cell if the restraining effect of the cell wall was not present (Kramer and Boyer, 1995).

Appendix 2.2. Water movement in the soil-plant-air system

Usually, Ψ_w is highest in the soil and lowest in the atmosphere, with intermediate values in the plant. But, the components of the water potential are not the same for all elements of the soil-plant-air system (Salisbury and Ross, 1992).

The roots must develop a water potential lower than the combined matric potential and any osmotic potential of the soil solution (Kramer and Boyer, 1995). It has been proposed that the major hydraulic resistance at the interface between root and soil is caused by poor contact. Apparently, if soil Ψ_w diminishes rapidly, the roots may shrink because they may not be able to maintain turgor. This probably does not happen if soil Ψ_w changes slowly and the root is able to maintain turgor by osmoregulation. Within the roots the main radial resistance was considered to be the endodermis, however there is now evidence suggesting that it is the epidermis instead (Passioura, 1982). In the xylem, where membranes are not present at maturity, water moves because of pressure differences produced by the surrounding cells (Kramer and Boyer, 1995). The force that moves water into a cell containing viable membranes is the difference in Ψ_w across the plasmalemma. Leaf cells contain a more concentrated solution (Ψ_o is quite negative), water moves in and raises Ψ_p. As atmospheric Ψ_w is even more negative, water evaporates and moves out of the leaves towards the atmosphere (Salisbury and Ross, 1992).

Appendix 2.3. Measuring the water content, water potential components and carbon isotope discrimination

- **Water content**

The water content of the plants is easily calculated by weighing the material immediatly after sampling and reweighing it after drying it for 24 h at 60-105 °C. The water content (*WC*) can be expresed either in a dry weight (*DW*) or fresh weight (*FW*) basis by the following equations (Turner, 1981):

$$WC_{(DW\ basis)} = \frac{FW - DW}{DW} \times 100 \qquad (A2.8)$$

$$WC_{(FW\ basis)} = \frac{FW - DW}{FW} \times 100 \qquad (A2.9)$$

As dry weight can change diurnally and/or seasonally comparisons of water content on a dry weight basis are inadequate. However, comparisons on a fresh weight basis are worse because they still have the problem of changing dry weight, but in addition, water content on a fresh weight basis tends to minimise changes in water content as expressed on other bases (Turner, 1981).

To overcome the above inconveniences the water content should be expressed on the basis of the water content at full turgor (e.g. fully turgid weight, *TW*). It is termed then relative water content (*RWC*) or water saturation deficit (*WSD*):

$$RWC = \frac{FW - DW}{TW - DW} \times 100 \qquad (A2.10)$$

and

$$WSD = \frac{TW - FW}{TW - DW} \times 100 \qquad (A2.11)$$

$$WSD = 100 - RWC \qquad (A2.12)$$

Therefore the determination of *RWC* needs an additional step, the measurement of turgid weight. This is done by placing tissue in contact with, or on, water in a humid chamber and permitting it to take water until it is fully turgid (Turner, 1981).

- **Water potential and its components**

The determination of the total water potential of a tissue has become routine since the development of two basic instruments: the thermocouple psychrometer and the pressure chamber (Turner, 1981).

The thermocouple psychrometer is based on the principle that the relative vapour pressure (e/e_o) of a solution or piece of plant material is related to its water potential according to the following equation:

$$\Psi = \frac{RT}{\bar{V}} \ln \frac{e}{e_o} \qquad (A2.13)$$

where:
R = the gas constant
T = absolute temperature (K)
V = partial molal volume of water

When a sample of plant material is placed in a hermetically sealed chamber and it is allowed to equilibrate it will produce a relative vapour pressure or relative humidity in the chamber equivalent to the total water potential of the tissue at the time of sealing into the chamber. In the thermocouple psychrometer, the humidity in the chamber is measured by determining the wet bulb depression (Turner, 1981).

There are two basic types of thermocouple psychrometer, the Peltier and the wet-loop psychrometer (Turner, 1981). In the Peltier type, the thermocouple is situated inside the chamber and it is cooled by a weak current (Peltier's effect, Salisbury and Ross, 1992) until condensation forms a fine film of liquid over the thermocouple, the cooling is then stopped and the evaporative cooling of the thermocouple, that corresponds to the wet bulb depression, is measured (Turner, 1981).

In the wet-loop psychrometer, the thermocouple junction is maintained permanently wet by a silver ring or wire loop containing a droplet of water. Water continuously moves from the droplet to the tissue and the difference of temperature of this thermocouple and that of the sample chamber, usually measured by a dry thermocouple in the chamber, is the wet bulb depression (Turner, 1981).

The measure obtained by the thermocouple psychrometer is the water potential of the cell walls $\Psi_{w(a)}$ (which is the same as the protoplast, $\Psi_{w(p)}$ as was shown in Eq. (*A*2.6) above) because the water surface of the sample is located there and the vapour pressure developed there is in equilibrium with it. It is also possible to measure the osmotic potential $\Psi_{o(a)}$ in the apoplast by placing in the psychrometer chamber exudate obtained from the tissue by applying pressure to the cells using a pressure chamber (Scholander *et al.*, 1965).

In the pressure chamber a leaf or a branch is placed with the cut end of the petiole or stem just protruding from the chamber through a rubber gland used to seal the chamber. The pressure in the chamber is gradually raised by compressing air (nitrogen or another inert gas from a cylinder, but not oxygen) (Turner, 1981). In this way the water is forced from the protoplast into the apoplast, displacing the original wall solution into the xylem from which it exudes onto the cut surface of the xylem. The pressure (P_{gas}) needed to displace the water until the sap just returns to the severed ends of the xylem vessels gives $\Psi_{m(a)}$ because it counteracts the tensions pulling water into the wall pores ($-P_{gas} = \Psi_{m(a)}$). The wall water potential ($\Psi_{w(a)}$) measurement determined by the psychrometer can be checked by these two potentials ($\Psi_{o(a)} + \Psi_{m(a)}$) according to Eq. (*A*2.5) (Kramer and Boyer, 1995). As the osmotic potential of the apoplastic water is near zero (usually greater than -0.1 MPa), the $\Psi_{m(a)}$ can be considered similar to the water potential of the leaf cells ($\Psi_{w(p)}$) (Turner, 1981).

To measure the osmotic potential of the protoplast with the psychrometer it is possible to use expressed sap, usually placed in the chamber on filter paper, or killed tissue (e.g. by freezing and thawing or by heating). Only measurements of osmotic potential on expressed sap are recommended, because the disruption of the cell membranes by freezing may create matric potentials not present in unfrozen tissue (Turner, 1981). Vapour pressure osmometers can also be used to measure total water potential of soils and plants, as well as osmotic potentials of solutions (Turner, 1981).

The symplastic sap obtained from killed tissue is a mixture of that from the apoplast and that from the symplast. As the osmotic potential of the apoplastic liquid is almost near zero, as it was mentioned previously, the symplastic water is diluted by apoplastic water. Because the volume of apoplastic water is unlikely to change as leaf dehydrates, the dilution of symplastic water by apoplastic water in dehydrated tissue is much greater (Tumer, 1981).

The osmotic potential can be calculated using the pressure chamber. The turgor is reduced to zero, not by killing the tissue but by applying pressure, and the osmotic potential is obtained from the pressure-volume relationship (Salisbury and Ross, 1992; Turner, 1981)

Indirect methods of measuring water status have been proposed. For example visible wilting, colour change, and leaf rolling. Other characters can be correlated with leaf water potential like: leaf temperature, leaf thickness, stem diameter and stomatal conductance. Nevertheless, all these methods suffer from disadvantages compared with direct measurement of water potential. Some of them are not linearly related to leaf water potential and others can change in sensitivity or response is subject to a time-lag behind changes in water potential. However, these are nondestructive techniques that can be used when the plant material is scarce or when screening large populations or large numbers of treatments for differences in plant water status (Turner, 1981).

- **Carbon isotope discrimination**

Two carbon dioxide (CO_2) isotopes are found in the atmosphere ^{12}C and ^{13}C, their respective proportions being 98.9% and 1.1%. The ratio of ^{13}C:^{12}C in plant tissue is less than that in atmospheric CO_2, indicating a discrimination against the heavier isotope being incorporated into plant biomass (Farquhar *et al.,* 1989). This happens for two reasons, in first place because $^{12}CO_2$ diffuses through stomata faster, and in second place because ribulose 1,6 biphosphate carboxylase (Rubisco) has greater affinity for $^{12}CO_2$ (Craufurd *et al.*, 1991). As isotopes are stable, their ratio of abundance in the plant is invariant as long as carbon is not lost. According to Farquhar *et al.* (1989) considerable theoretical and experimental progress has been made over the last decade in understanding how biochemical, metabolic and environmental factors affect the isotopic plant compositions. In relation to water stress, when plants are subjected to drought the stomata aperture eventually decreases so that water loss is restricted while photosynthesis continues, proportionately less restricted. Because Rubisco discriminates against $^{13}CO_2$, the ratio of $^{13}CO_2$: $^{12}CO_2$ increases within the leaf. As stress becomes more pronounced, Rubisco has a lower opportunity for discriminating $^{13}CO_2$ and the proportion of this isotope fixed increases, giving a higher ratio of ^{13}C:^{12}C in plant biomass.

The discrimination (Δ) is calculated according to the following formula (Farquhar *et al.*, 1989):

$$\Delta = \frac{R_a}{R_p} - 1 \qquad (A2.14)$$

where:
R_a = isotopic abundance (^{13}C:^{12}C molar ratio) in air
R_p = isotopic abundance in the plant

As Δ is not simple to measure, the deviation of the isotopic composition of the sample from a standard (δ) is measured by the mass spectrometer instead. The reference material traditionally has been carbon in CO_2 generated from a fossil belemnite from the Pee Dee Formation (PDB) which R is 0.01124. The Δ is calculated then as (Farquhar *et al.*, 1989):

$$\Delta = \frac{\delta_a - \delta_p}{1 + \delta_p} \qquad (A2.15)$$

where *a* and *p* refer to air and plant, respectively. Conversely to δ, the Δ is independent of the isotopic composition of the standard and of the isotopic abundance in the air, and it is dimensionless. Free atmospheric CO_2 has currently a deviation, δ_a, of -0.008 and a typical plant material a deviation, δ_p, of -0.0276 which results in a typical plant material Δ = (-0.008 + 0.0276/(1 - 0.0276)) = 20.1‰ (Farquhar *et al.*, 1989).

Appendix 3.1. Derivation of mean root diameter formula

Since volume of a cylinder is = $\frac{1}{4} \pi D^2 L$
where:
D = mean diameter
L = length

Then assuming:

- root fresh weight (FW) density = 1 g cm^{-3} (Barker *et al.*, 1988)
- root dry weight (DW) = 8% root FW (Schuurman and Knot, 1974)

$FW (g) = \frac{1}{4}\pi D (cm)^2 \cdot L (m) \cdot 100 (cm \cdot m^{-1})$
$DW (g) \cdot 0.08^{-1} (g^{-1}/cm^3) = \frac{1}{4}\pi D (cm)^2 \cdot L (m) \cdot 100 (cm \cdot m^{-1})$
$D (cm)^2 = (\frac{1}{4}\pi)^{-1} \cdot L (m)^{-1} \cdot 0.01 (mc \cdot m^{-1}) \cdot 0.08^{-1} DW (cm^3)$
$D (cm)^2 = 0.15915 DW (cm^3) \cdot L (cm)^{-1}$
$D (cm) = 0.39894 \surd (DW \cdot L^{-1})$
$D (mm) = 3.9894 \surd (DW \cdot L^{-1})$

and root surface area (m^2):

$A (m^2) = \pi D (mm) \cdot L (m) \cdot 0.001 (m \cdot mm^{-1})$

Appendix 3.2. Percentage of total root mass, total root length and coarse root length in each of the four soil strata before the application of water treatments (Harvest 1).

	Root mass percentage (%)			
Cultivar	**0-75 mm**	**75-150 mm**	**150-300 mm**	**below 300mm**
MK	24.9 (29.9) **b**[1]	15.9 (23.5)	20.9 (27.2)	38.3 (38.3)
GA	35.0 (36.2) **a**	16.9 (24.3)	18.0 (25.1)	30.1 (33.2)
EP	30.1 (23.3) **ab**	15.4 (23.1)	17.3 (24.5)	37.1 (37.5)
s.e.[2]	(1.1)	(0.4)	(1.2)	(1.7)

	Root length percentage (%)			
Cultivar	**0-75 mm**	**75-150 mm**	**150-300 mm**	**below 300mm**
MK	16.6 (23.8)	20.0 (26.5)	23.1 (28.6)	40.3 (39.4) **a**
GA	29.1 (32.4)	21.1 (27.3)	20.5 (26.9)	29.3 (32.7) **b**
EP	22.6 (28.4)	16.2 (23.7)	19.2 (25.9)	42.0 (40.4) **a**
s.e.	(1.8)	(0.9)	(1.7)	(1.3)

	Coarse root length percentage (%)			
Cultivar	**0-75 mm**	**75-150 mm**	**150-300 mm**	**below 300mm**
MK	13.0 (15.9)	6.9 (15.5) **a**	6.2 (15.0) **a**	6.7 (16.1) **a**
GA	9.3 (15.2)	4.4 (13.2) **b**	5.2 (11.7) **b**	4.4 (10.3) **b**
EP	8.7 (15.1)	6.5 (13.0) **b**	4.0 (11.9) **b**	2.9 (9.1) **b**
s.e.	(0.7)	(0.6)	(0.6)	(0.8)

Data within brackets are arcsin square root transformed percentages.

[1] Letters indicate groupings of means that are significantly different at P<0.05.

[2] Standard error.

Appendix 3.3. Percentages of total root mass recovered from each of the four soil strata after the application of water treatments.

	Root mass percentage (%)			
Harvest	**0-75 mm**	**75-150 mm**	**150-300 mm**	**below 300mm**
2	29.9 (33.1) **a**[1]	15.2 (22.9)	19.6 (26.2)	35.3 (36.4)
3	30.0 (33.1) **a**	15.9 (23.4)	20.0 (26.5)	34.0 (35.6)
4	27.5 (31.6) **b**	15.1 (22.8)	20.3 (26.7)	37.0 (37.4)
s.e.[2]	(0.6)§	(0.4)	(0.5)	(0.6)
Cultivar				
MK	26.2 (30.9) **b**	16.4 (24.0) **a**	22.1 (28.1) **a**	35.3 (36.1) **b**
GA	34.2 (35.7) **a**	15.3 (22.7) **b**	19.3 (26.1) **b**	31.3 (34.1) **b**
EP	27.4 (31.2) **b**	14.8 (22.5) **b**	18.1 (25.2) **b**	39.7 (39.2) **a**
s.e.	(0.6)	(0.4)	(0.5)	(0.6)
Water				
Control	29.4 (32.7)	15.9 (23.5)	19.7 (26.5)	34.9 (36.0) **b**
Stressed	29.0 (32.5)	14.9 (22.7)	19.9 (26.4)	36.2 (37.0) **a**
s.e.	(0.5)§	(0.3)	(0.4)	(0.5)
Interactions (P=)				
Cult. * Water	0.377	0.865	0.117	**0.010**
Harvest * Cultivar	0.725	0.484	0.846	0.621
Harvest * Water	0.593	0.790	0.723	0.340
Harvest*Cult.*Wat.	0.716	0.076	0.721	0.176

Data within brackets are arcsin square root transformed percentages.

[1] Letters indicate groupings of means that are significantly different at P<0.05 within harvests, cultivars or water treatment.

[2] Standard error.

§ Mean standard error.

Appendix 3.4. Percentage of total root mass recovered below 300 mm after the application of water treatments.

	Percentage of total root mass below 300 mm stratum	
Cultivar	**Control**	**Stressed**
MK	34.4 (35.0) **cd**[1]	36.6 (37.2) **bc**
GA	29.6 (32.8) **d**	33.7 (35.4) **bcd**
EP	40.6 (40.3) **a**	38.4 (38.2) **ab**
s.e.[2]	(1.06)	

Data within brackets are arcsin square root transformed percentages.

[1] Letters indicate groupings of means that are significantly different at $P<0.05$.

[2] Standard error.

Appendix 3.5. Percentage of total root length recovered from each of the four soil strata after the application of water treatments.

	Root length percentage (%)			
Harvest	**0-75 mm**	**75-150 mm**	**150-300 mm**	**below 300mm**
2	23.6 (28.9)	17.1 (24.3)	22.8 (28.3)	36.5 (36.9)
3	25.4 (30.1)	16.5 (23.8)	21.1 (27.2)	37.0 (37.4)
4	20.8 (26.8)	15.6 (22.9)	22.6 (28.0)	41.0 (39.9)
s.e.[1]	(1.2)[§]	(0.9)[§]	(1.0)	(2.8)[§]
Cultivar				
MK	23.9 (29.9) **a**[2]	18.7 (25.4) **a**	24.6 (29.8) **a**	32.7 (33.9) **b**
GA	26.3 (30.4) **a**	16.3 (23.1) **ab**	21.4 (27.6) **ab**	35.9 (37.4) **b**
EP	19.4 (25.5) **b**	15.3 (22.6) **b**	19.7 (26.1) **b**	45.4 (43.0) **a**
s.e.	(1.2)	(0.9)	(1.0)	(3.8)
Water				
Control	24.6 (29.9) **a**	17.4 (24.1)	21.5 (27.6)	36.5 (36.8)
Stressed	21.4 (27.2) **b**	16.0 (23.2)	22.5 (28.1)	40.1 (39.3)
s.e.	(1.0)[§]	(0.7)[§]	(0.8)	(1.1)[§]
Interactions (P=)				
Cult. * Water	**0.003**	0.711	0.976	**0.011**
Harvest * Cult.	0.915	**0.035**	0.330	**0.002**
Harvest * Water	0.380	0.886	0.550	0.423
Harvest*Cult.*Wat.	0.371	0.426	0.828	0.205

Data within brackets are arcsin square root transformed percentages.

[1] Standard error.

[§] Mean standard error.

[2] Letters indicate groupings of means that are significantly different at $P<0.05$ within harvests, cultivars or water treatment.

Appendix 3.6. Percentage of total root length recovered from the 75-150 mm and below 300 mm strata after the application of water treatments averaged for cultivars and harvests.

	Percentage of total root length in the 75-150 mm stratum		
Harvest	**MK**	**GA**	**EP**
2	20.7 (26.9) **a**[1]	15.7 (23.3) **ab**	15.0 (22.7) **b**
3	16.4 (23.8) **ab**	15.3 (22.9) **b**	17.8 (24.8) **ab**
4	18.5 (25.4) **ab**	15.5 (23.1) **ab**	12.9 (20.2) **b**
s.e.[2]		(1.5)	

	Percentage of total root length in the below 300 mm stratum		
Harvest	**MK**	**GA**	**EP**
2	25.6 (30.1) **fg**	38.5 (38.3) **bc**	45.3 (42.3) **ade**
3	34.6 (35.9) **b**	35.4 (36.4) **b**	41.1 (39.9) **ab**
4	34.0 (35.6) **bdg**	37.3 (37.5) **bcef**	51.6 (46.7) **acd**
s.e.		(1.9)	

Data within brackets are arcsin square root transformed percentages.

[1] Letters indicate groupings of means that are significantly different at P<0.05.

[2] Mean standard error.

Appendix 3.7. Percentage of total root length recovered from the 0-75 mm and below 300 mm strata averaged for cultivars and water treatments.

	Percentage of total root length in the 0-75 mm stratum	
Cultivar	**Control**	**Stressed**
MK	24.8 (31.4) **a**[1]	22.7 (28.3) **ab**
GA	28.0 (31.6) **a**	24.0 (29.2) **a**
EP	20.9 (26.7) **ab**	17.5 (24.2) **b**
s.e.[2]	(1.7)	

	Percentage of total root length in the below 300 mm stratum	
Cultivar	**Control**	**Stressed**
MK	32.0 (32.4) **c**	33.6 (35.3) **bc**
GA	33.1 (35.8) **b**	39.8 (39.0) **ab**
EP	44.4 (42.2) **a**	46.8 (43.7) **a**
s.e.	(2.2)	

Data within brackets are arcsin square root transformed percentages.

[1] Letters indicate groupings of means that are significantly different at $P<0.05$.

[2] Mean standard error.

Appendix 3.8. Coarse root length as percentage of total root length recovered from each of the four soil strata after the application of water treatments.

	Coarse root length as percentage of total root length			
Harvest	**0-75 mm**	**75-150 mm**	**150-300 mm**	**below 300mm**
2	6.90 (14.98)**ab**[1]	5.36 (13.18)	4.59 (12.16) **b**	4.15 (11.31) **b**
3	6.40 (14.46) **b**	5.79 (13.64)	4.55 (11.98) **b**	3.29 (9.91) **b**
4	8.56 (16.79) **a**	6.69 (14.86)	6.46 (14.44) **a**	6.78 (14.18) **a**
s.e.[2]	(0.65)	(0.51)	(0.58)	(0.64)
Cultivar				
MK	7.78 (15.87)	7.27 (15.45) **a**	6.97 (14.97) **a**	8.23 (16.06) **a**
GA	7.03 (15.21)	5.35 (13.20) **b**	4.21 (11.74) **b**	3.34 (10.28) **b**
EP	7.07 (15.14)	5.22 (13.03) **b**	4.43 (11.87) **b**	2.65 (9.05) **b**
s.e.	(0.65)	(0.51)	(0.58)	(0.64)
Water				
Control	6.90 (15.00)	5.99 (13.97)	5.06 (12.81)	4.29 (11.43)
Stressed	7.69 (15.82)	5.90 (13.82)	5.34 (12.91)	5.19 (12.17)
s.e.	(0.53)	(0.42)	(0.47)	(0.52)
Interactions (P=)				
Cult. * Water	0.590	0.894	**0.012**	0.066
Harvest * Cult.	0.291	0.556	0.867	0.086
Harvest * Wat.	0.167	0.115	0.365	0.451
Harv.*Cult.*Wat.	0.171	0.056	0.863	0.170

Data within brackets are arcsin square root transformed percentages.

[1] Letters indicate groupings of means that are significantly different at P<0.05 within harvests, cultivars or water treatment.

[2] Standard error.

Appendix 3.9. Coarse root length as percentage of the total root length recovered from the 150-300 mm stratum averaged for cultivars and water treatments.

	Percentage of coarse root length in the 150-300 mm stratum	
Cultivar	**Control**	**Stressed**
MK	5.54 (13.45) **b**[1]	8.40 (16.49) **a**
GA	4.49 (12.14) **bc**	3.93 (11.33) **bc**
EP	5.15 (12.84) **bc**	3.70 (10.90) **c**
s.e.[2]	(0.82)	

Data within brackets are arcsin square root transformed percentages.

[1] Letters indicate groupings of means that are significantly different at $P<0.05$.

[2] Standard error.

Appendix 3.10. Carbon isotope discrimination averaged for harvests, cultivars and water treatments.

Harvest 2 Cultivar	**Carbon isotope discrimination**	
	Control	**Stressed**
MK	20.24 **abc**[1]	20.86 **a**
GA	17.98 **efg**	17.89 **efg**
EP	19.58 **bcd**	17.55 **g**
Harvest 3 Cultivar		
MK	20.63 **ab**	20.73 **ab**
GA	18.95 **def**	17.79 **fg**
EP	17.64 **g**	18.39 **defg**
Harvest 4 Cultivar		
MK	19.10 **cde**	20.36 **ab**
GA	17.52 **g**	17.91 **efg**
EP	17.63 **g**	18.36 **defg**
s.e.[2]	0.45	

[1] Letters indicate groupings of means that are significantly different at $P<0.05$.
[2] Standard error.

Appendix 4.1. Methods used to measure reducing and fructosyl sugars

a) Somogyi-Nelson's Method (Somogyi, 1952)

Reagents:

- **Somogyi A:**

5 g $NaHCO_3$ + 3.75 g tartrate + 45 g Na_2SO_4 + distilled water to complete 250 ml

- **Somogyi B:**

2 g $CuSO_4$ + 18 g Na_2SO_4 + distilled water to complete 100 ml

Both solutions should be kept above 20°C and should be mixed in the ratio 4A:1B before used.

- **Nelson**

25 g $(NH_4)Mo_7O_{24}$ are added to 450 ml distilled water and mixed, then 20 ml of concentrate H_2SO_4 are added and the solution is shaken. Three grams of $Na_2HAsO_4.7H_2O$ are dissolved in 25 ml distilled water and are added to the solution above. The solution should be kept for 24 hs at 37°C in a dark flask.

- **Standard solution:** glucose 180 $\mu g ml^{-1}$

Procedure

Five hundred µl of sample (could be part of sample - or standard - and part of distilled water depending of the reducing sugar concentration of the sample) are put in a test tube, 500 µl of Somogyi solution A+B are added, tubes are shaken and boiled for 15 minutes, then tubes are cool down in a water bath. Five hundred µl of Nelson's solution and 3,500 µl of distilled water are added. Blank tubes containing water and reagent are prepared for correction. Absorbance at 540 nm is measured.

b) Kulka's method (Kulka, 1956)

Reagents

- **Solution A:**

Resorcinol 0.05% w/v in absolute ethanol

- **Solution B:**

0.216 g l^{-1} $FeNH_4(SO_4)_2.12H_2O$ in HCl (sp.gr. 1.18)

- **Standard solution:** fructose 180 μgml^{-1}

Procedure

One ml of sample (could be part of sample - or standard - and part of distilled water, depending of the reducing sugar concentration of the sample) is put in a test tube, 0.5 ml of solution A is added, the tube is shaked and 0.5 ml of solution B under fume hood is added. Tubes are covered with glass marbles and boiled at 100°C for 1 h. At the end of the heating period tubes are cooled in ice water. Blank tubes containing water and reagent are also prepared for correction. Absorbance at 480 nm is measured. Colour is stable for more than 5 hr.

Appendix 5.1. Surface sterilization of seeds

The following methods of seed surface sterilization were used.

Method 1

1. Soak seeds for 20-30 min in 50% sulphuric acid.
2. Rinse in water.
3. Soak seeds for 20-30 min in sodium hypochlorite solution (3.15 g l^{-1}).
4. Rinse 3 times in sterile water.
5. Place seeds with sterile forceps onto blotting for a few minutes before placing the seeds onto agar plates to germinate.

Method 2

1. Place seeds in a gauze bag and soak in water at approximately 24-27°C for 3 hrs.
2. Place seeds (in the gauze bag still) in a water bath for 3-4 min at 57°C.
3. Place seeds (in the gauze bag still) in sterile water to cool promptly.
4. Take out of water, remove seeds from the bag, place them into small jar containing sodium hypochlorite solution (6.30 g l^{-1}) and soak for 20 min.
5. Rinse 3 times with sterile water.
6. Place seeds with sterile forceps onto blotting for a few minutes before placing the seeds onto agar plates to germinate.

Appendix 5.2. Osmotic potential (Ψ_o) adjusted by RWC for both periods (1 and 2) and water treatments.

Water	**Ψ_o 1 (MPa)**	**Ψ_o 2 (MPa)**
Control	-1.79 **a**[1]	-1.89 **a**
S1	-2.08 **b**	-2.11 **b**
S2	-2.21 **bc**	-2.18 **b**
S3	-2.55 **c**	-2.29 **b**
s.e.[2]	1.01	0.87

[1] Letters indicate groupings of means that are significantly different at P<0.05 within cultivars, water treatment or endophyte.

[2] Mean standard error.

Appendix 5.3. Photosynthesis (µm ol CO_2 $m^{-2}s^{-1}$) averaged for cultivars and endophytes .

Endophyte	**MK**	**EP**
Nil	7.99 **ab**[1]	6.18 **bc**
AR501	6.90 **bc**	7.37 **bc**
KY31	9.60 **a**	5.69 **c**
s.e.[2]	0.77	

[1] Letters indicate groupings of means that are significantly different at P<0.05.

[2] Standard error.

Appendix 5.4. Stomatal resistance averaged for cultivars, water treatments and endophytes.

	Stomatal resistance (s cm^{-1})			
MK	**Control**	**S1**	**S2**	**S3**
Nil	0.108 **ij**[1]	0.142 **ghij**	0.159 **fghij**	0.178 **efghij**
AR501	0.153 **fghij**	0.114 **hij**	0.155 **fghij**	0.216 **cdefgh**
KY31	0.160 **fghjj**	0.089 **j**	0.225 **cdefg**	0.137 **ghij**
EP				
Nil	0.316 **abc**	0.280 **abcde**	0.332 **ab**	0.254 **bcdef**
AR501	0.221 **cdefg**	0.204 **defghi**	0.306 **abcd**	0.270 **abcde**
KY31	0.314 **abc**	0.361 **a**	0.266 **abcde**	0.338 **ab**
s.e.[2]	0.037			

[1] Letters indicate groupings of means that are significantly different at $P<0.05$.
[2] Standard error.

Appendix 5.5. Average relative water content (%) between measurement periods and green tissue percentage averaged for cultivars and endophytes.

MK	**RWC (%)**	**Green tissue (%)**
Nil	84.4	68.2
AR501	84.9	72.9
KY31	83.2	65.3
EP		
Nil	75.8	72.2
AR501	78.5	75.7
KY31	80.8	72.5

Appendix 7.1. Calculation of LAI

A multiple regression was calculated using the average quadrat height as independent variable and the quadrat LAI as dependent variable as has been already described in Chapter 6.

The dummy variables used were:

D1: intercept for cultivar, Maris Kasba =1 and El Palenque =0
DD1: intercept for nitrogen fertiliser, N0=1 and N1=0
D7: intercept for month, July=1 August=0 September=0 October=0
D8: intercept for month, July=0 August=1 September=0 October=0
D9: intercept for month, July=0 August=0 September=1 October=0
DA1: slope for cultivar, Maris Kasba =1 and El Palenque =0
DDA: slope for nitrogen fertiliser, N0=1 and N1=0
DA7: slope for month, July=1 August=0 September=0 October=0
DA8: slope for month, July=0 August=1 September=0 October=0
DA9: slope for month, July=0 August=0 September=1 October=0

Sas output was as follows:

```
Dependent Variable: LAI
                             Analysis of Variance
                                Sum of          Mean
       Source          DF      Squares        Square       F Value        Prob>F
       Model           11     51.56777       4.68798        53.843        0.0001
       Error          305     26.55560       0.08707
       C Total        316     78.12337
           Root MSE        0.29507     R-square       0.6601
           Dep Mean        0.99315     Adj R-sq       0.6478
           C.V.           29.71069

                             Parameter Estimates
                    Parameter      Standard    T for H0:
         Var. DF     Estimate         Error    Parameter=0     Prob > |T|
INTERCEP    1        0.333551    0.13207367          2.525         0.0121
HEIGHT      1        0.101812    0.02433285          4.184         0.0001
D1          1       -0.127844    0.09550335         -1.339         0.1817
DD1         1        0.077086    0.08958963          0.860         0.3902
D7          1        0.085390    0.13491381          0.633         0.5273
D8          1       -0.250234    0.14663912         -1.706         0.0889
D9          1       -0.405028    0.15548479         -2.605         0.0096
DA1         1        0.037006    0.01554495          2.381         0.0179
DDA1        1       -0.028616    0.01354041         -2.113         0.0354
DA7         1       -0.048582    0.02380557         -2.041         0.0421
DA8         1        0.045023    0.02585033          1.742         0.0826
DA9         1        0.104144    0.02685465          3.878         0.0001
```

Another multiple regression was calculated taking into account only the dummy variables which parameters had resulted significant at 10% significance level. The following analysis de variance and parameter estimates were obtained. The resulting regression was used to estimate LAI from the height measurements.

```
Dependent Variable: LAI

                               Analysis of Variance

                                  Sum of         Mean
       Source            DF      Squares        Square        F Value        Prob>F
       Model              8     51.30031       6.41254         73.633        0.0001
       Error            308     26.82306       0.08709
       C Total          316     78.12337
           Root MSE         0.29511      R-square       0.6567
           Dep Mean         0.99315      Adj R-sq       0.6477
           C.V.            29.71416

                               Parameter Estimates
                    Parameter      Standard     T for H0:
         Var. DF    Estimate         Error      Parameter=0    Prob > |T|
INTERCEP    1       0.379230     0.06068647         6.249        0.0001
HEIGHT      1       0.093402     0.01424707         6.556        0.0001
D8          1      -0.310425     0.11031502        -2.814        0.0052
D9          1      -0.484925     0.12231273        -3.965        0.0001
DA1         1       0.017435     0.00581556         2.998        0.0029
DDA1        1      -0.017128     0.00509985        -3.359        0.0009
DA7         1      -0.031853     0.00876812        -3.633        0.0003
DA8         1       0.057447     0.01884962         3.048        0.0025
DA9         1       0.119726     0.02023546         5.917        0.0001
```

Appendix 7.2. Other grasses, *Lotus* spp. and weeds dry matter accumulation in MK and EP paddocks and the two nitrogen treatments (N0= 0 kg N.ha^{-1} and N100=100 kg N.ha^{-1}) from June to October.

	Other grasses (kg DM ha^{-1})				
Date	**MK**	**EP**	**N0**	**N1**	**s.e.**[1]
6 May	43	49	59	33	15
4 July	48	39	30	57	26
14 August	11	26	22	15	8
4 September	9	8	7	9	6
1 October	0	0	0	0	0

	Lotus **spp. (kg DM ha^{-1})**				
Date	**MK**	**EP**	**N0**	**N1**	**s.e.**
6 May	62	71	57	75	34
4 July	18	28	34	11	15
14 August	3	2	3	2	3
4 September	4	3	7	1	3
1 October	2	7	8	1	2

	Weeds (kg DM ha^{-1})				
Date	**MK**	**EP**	**N0**	**N1**	**s.e.**
6 May	119	77	114	81	35
4 July	23	49	44	28	13
14 August	2	3	3	2	2
4 September	0	1	0	1	1
1 October	2	0	2	0	2

[1] Standard error.

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