# NUTRIENT UPTAKE STUDIES OF THREE GRASS SPECIES IN RELATION TO WATER STRESS

by

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## ABSTRACT

Nutrient uptake was studied in common millet (*Panicum milieaceum*), wild oat (*Avena fatua*) and an arid zone wild grass (*Aristida funiculata)*, three grass species of different origins and habitats, in relation to water stress. The main objective of this study was to look for evidence of differences and similarities between the three species in their ability to take up and use nutrients under conditions of water stress and with varying levels of nutrients.

Two methods were used to induce water stress in the present investigation; withholding water from the soil and using an osmotic agent (polyethylene glycol). Using gradually increasing concentrations of PEG either in conjunction with a semi-permeable membrane, or in a circulating nutrient solution, showed the advantage of inducing precise levels of osmotic stress whilst maintaining adequate root aeration. This system resembled the stresses created by withholding water, but did so in a manner allowing more precise comparison between species. Similar inter-species differences were obtained by both methods.

The response of the three species to a range of N and/or K supply, and soil moisture regimes showed that water stress caused the largest effects on the total dry weights of *A. fatua*, followed by *P. milieaceum*, and with no obvious changes in weights of *A. funiculata*. *A. funiculata* showed the ability to produce maximum growth at very low levels of nutrients compared with *P. milieaceum* and *A. fatua*. The ecological implications were discussed, and these differences were suggested to be related with their natural background.

Although water stress had varied effects on nutrient concentrations and contents in the three species, some trends were consistent. Dry matter yields generally decreased faster than nutrient uptake in *P. milieaceum* and *A. funiculata*, and in contrast, total content in *A.fatua* was reduced more than its growth. The accumulation of divalent cations in both *P. miliaceum* and *A. funiculata* was reduced under water stress, whilst that of *A. fatua* was unaffected. The uptake of monovalent ions was consistently proportional to growth, except for an enhanced uptake of $K^+$ in *A. funiculata*, which might well be an adaptive feature of this species.

The nutrient uptake of the three species was highly correlated with water use under soil moisture stress conditions. The uptake of well-watered plants was not closely related to water use. However, the uptake of N in osmotically stressed plants of *A. funiculata* was not affected by reduced water uptake, so that any change in growth was due to water stress *per se* and not to interacting effects with N nutrition. The effect of water stress on transport of nutrients to the shoots was independent of its effect on uptake by the roots in all the species. Transport of nutrients from the roots to the shoots occurred even at reduced transpiration rates.

Split-root experiments which involved growing plants in nutrient film channels, were found to be very useful by permitting half of each root system to be supplied with nutrients whilst the other half was kept in a deficient solution. Water stress which was imposed by incorporating PEG into the flowing solutions, allowed nutrient uptake to be distinguished from overall water uptake, and gave a better indication of species differences in their responses to water stress. *A. fatua* showed a large reduction in shoot growth due to water stress, which was associated with an increase in the total proportion of N that was cycled. In contrast, *A. funiculata* which grew more normally at the low N level used (50 ppm) exhibited smaller changes in the patterns of N distribution between the shoots and roots. The experiments revealed the amounts of N which were cycled as a consequence of differences in the balance between the species capacities to absorb and incorporate N. The implications of these findings are discussed.

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# CHAPTER ONE

## General Introduction and Review of Literature

# INTRODUCTION

## 1.1 General Introduction

Water availability is one of the principal environmental limitations of crop productivity throughout the world. The water deficits, which are a consequence of either continuous or transitory periods of drought, cause significant yield reductions on presently cultivated land, and greatly restrict the cultivation of crops on over one-third of the earth's land surface considered to be arid or semiarid (Kramer 1983). These restrictions on yield potential are of great concern in the face of food demands for an ever increasing world population. The problem becomes complicated further by the fact that supplies of suitable irrigation water are dwindling rapidly, and the costs of irrigation are becoming prohibitive. As a result, studies on the effects of water stress on plant survival and yield are attracting extra interest in plant science research.

Plants which are adapted to arid or semi-arid conditions, however, cover large areas of the world, and part of their ability to grow in these conditions is due to an adaptation to water stress. Kramer (1983) considered the mechanisms of adaptation of plants to water deficits into two categories: drought avoidance and drought tolerance. Drought avoidance plants exhibit growth habits which allow them to avoid water stress conditions. Drought tolerant plants consists of those that postpone dehydration and those that tolerate dehydration. Dehydration postponement is accomplished by mechanisms which either reduce transpiration or increase water absorption so as to prevent cells of the plant from reaching detrimentally low cell water potentials. Dehydration tolerance involves processes which allow the plant to survive and/or grow after cells actually have been exposed to water deficits and low cell water potentials. If the water deficits are severe enough, plant cells either lose turgor or turgor is reduced to a point restricting cell expansion (growth). However, only those cells that are young enough to expand must adjust their internal osmotic potential and sufficiently increase turgor to resume cell expansion and growth, and this process of osmotic adjustment is important as a drought stress tolerance mechanism.

Though there have been extensive studies of the water relations of the plants there have been fewer investigations of their nutrient uptake. The effects of water stress on nutrient uptake has been studied in various ways but there is still a serious lack of research to establish the nature and magnitude of any interaction between water and nutrient uptake (Krisknakumari *et al.*,

1988; Al-Mustafa and Al-Omran, 1989). There is much recent information on the effects of water stress on photosynthetic $CO_2$ fixation and related process (Schulze, 1986a, 1986b; Patterson, 1986; Otoo, *et al.*, 1989; Zhang and Davies, 1989). However, nitrogen nutrition for example, also contributes substantially to plant growth and yet the effects of water stress on nitrogen uptake and utilization have been little investigated (Bradford and Hsiao, 1982; Khondaker *et al.*, 1983; Rego *et al.*,1986; Talouizite and Champigny, 1988).

Plant species differ extensively in the uptake, translocation, accumulation, and use of mineral elements. There have been many studies on the subject, such as Gerloff (1963), Epstein (1972), Lafever (1981), Saric (1981), Clark (1983), Barker (1989), Glass (1989), and Sussman and Gabelman (1989). All came to the conclusion that the observed differential response among species was due to a difference in either nutrient uptake and use in adaptation to their natural habitats or inherent differences in genetic constitution. Clark (1983) however suggested that large differences are under genetic control, but their expression may be altered dramatically when plants are grown under different environments. These genotype differences helped to explain plant adaptations to mineral stress or to the arid and semi-arid conditions which cover large areas of the world. These adaptations in which some plant species are able to grow and reproduce under nutrient or water stress while others do not, or in which one species is able to accumulate higher amounts of an element compared to another, differ with the mineral element as well as the plant species.

Polyethylene glycols (PEG) have been widely used in culture solution experiments to create specific water stress levels that can not only be repeated but also more easily and accurately maintained than water stresses in soil culture (Gergely *et al.*, 1980a, 1980b; Krizek, 1985). Further, in dry soil, nutrients become unavailable, leading to reduced ion uptake as the soil moisture decreases. However, there is still limited information relating plant water stress to ion uptake without regard to soil factors. Plant water stress induced by decreasing the solution water potential might cause reductions in nutrient uptake by its effect on the rate of water flow through plants (Greenway and Klepper 1969) or possibly by its effect on active ion uptake mechanisms and/or the passive efflux of ions (Erlandsson 1975, 1979).

In general, water stress impairs numerous metabolic and physiological processes in plants (Begg and Turner, 1976; Levitt, 1980; Sullivan and Eastin, 1974; Turner and Kramer, 1980; Simpson, 1981). Increasing resistance to transpiration, osmotic adjustment, and other adaptive mechanisms leading to xerophytism enables the plants to tolerate water stress. Nutrient uptake by plants is decreased under water stress conditions due to reduced transpiration (Greenway and Klepper, 1969; O'Toole and Baldia, 1982; Yambao and O'Toole, 1984) and impaired active

transport and membrane permeability (Hsiao, 1973) resulting in reduced root absorbing power (Tanguilig *et al.*, 1987). On the other hand, nutrient uptake from the soil solution is also closely linked to soil water status. Thus, in order to have a better understanding of the adverse effects of water stress on nutrient uptake, study should focus more on nutrient movement in the soil and the plant response to applied nutrient and water stress. Therefore in the following discussion the main attention is directed toward the effects of water stress on plant growth and function, and on the interaction of ion uptake with plant and water stress.

## 1.2 Plant Water Relations

Water is the most common and most important substances on the earth's surface (Kramer, 1983). It plays an important role in all physiological processes in plant life and metabolism (Craft, 1968; Hsiao, 1973). Many excellent books and review articles have been written on the subject of plant water relations and plant responses to water stress. Plant water relations and their interaction with other environmental and physiological factors therefore occupy a central position in the study of plant physiology. Meidner (1983) has presented an historical account of the development of plant water relations and Slatyer (1960) and Kramer (1983) have both written comprehensive reviews of the subject.

The availability of water to the plant depends on a combination of three main factors; they are the soil, the plant itself and the weather conditions. Reviews of these subjects have been given by, for example, Arnon (1972) and Kramer (1983). The complex plant responses to water deficit also have been comprehensively discussed by Hsiao (1973), Morgan (1984 and 1986) and Levitt (1980). Such responses will only be referred to in passing during the course of this review. The main attention is directed towards to the effects of variation in water availability on the plant's growth and function. The way in which such changes affect the metabolism of the plant as a whole will also be considered.

## Movement of water from soil to atmosphere

The movement of water through the soil-plant-atmosphere system can be considered in terms of a series of interrelated but independent processes (Kramer, 1983). There are two general concepts recognised in study on plant water relations (Kramer, 1988a, 1988b; Passioura, 1988). One is the plant water balance concept, which treats the plant water status as dependent on relative rates of deposits (water absorption) and withdrawal (transpiration) with the water status of the plant, ranging from full turgor, through various degrees of wilting to death by desiccation. The other is the soil-plant-atmosphere continuum (SPAC) concept and the related Ohm's law analogy which provide a system for studying water movement through plants. The consideration of this pathway of movement as a continuum, has certainly led to considerable improvement in understanding of the physical processes involved in transpiration, water flows and its control (Passioura, 1982).

The SPAC concept have been developed by van den Honert (1948), , presented by Slatyer and Taylor (1960), Cowan (1965), and others. This 'catenary hypothesis' for the movement of water through plants has been widely discussed and has formed the basis of much work in plant water relations. Essentially, it states that at each stage in the transport pathway, water will encounter resistances to its flow, analogous to Ohm's law concerning electrical resistance. However, there seem to be considerable differences of opinion regarding the relative importance of resistance to water movement in the soil, at the soil-root interface, and in the plant. Some workers, such as Newman (1969), Taylor and Klepper (1975) and Blizzard and Boyer (1980) concluded that soil resistance is small except in very dry soil. Herkelrath *et al.* (1977a, 1977b) suggested that the larger part of soil-plant resistance is located at the root-soil interface since in the dry soil it decreased the contact between them. It is also well established that roots can shrink in diameter when they become dehydrated, further reducing contact with the soil (e.g. Huck *et al.*, 1970). However, Passioura (1982) suggested that roots could still maintain partial contact with the soil and water films even when both soil and roots were shrunken, because of the mucilaginous coating (mucigel) and roots hairs found on many roots. This subject has been reviewed in detailed, for example by Weatherly (1982), and Kramer (1983).

## Importance of turgor pressure in plant growth

The water potential ($\psi_w$) of the cell is generally expressed in the form:

$$\psi_w = \psi_s + \psi_p$$

where $\psi_p$ is a pressure potential identified with turgor pressure due to the presence of the rigid cell wall around the plant cell; and $\psi_s$ is a solute or osmotic potential due to the presence of dissolve solutes in the cytoplasm. For certain applications a matric potential, $\psi_m$ due to the retention of water by surface forces in the cell matrix is also included in the above equation. In practice, $\psi_m$ in the cytoplasm breaks down into either $\psi_p$ or $\psi_s$ and therefore $\psi_w$ can be considered to arise essentially from osmotic and pressure components (Passioura 1980).

A lowering of the turgor pressure due to a restriction in the water supply to the plant has a major effect on the growth of plants cells. Turgor pressure is the driving force of a cell extension (Cleland, 1971), and is therefore important in elongating roots, leaves and stems. It also regulates the movement of stomatal guard cells. Enzymatic processes are also reduced by lowered turgor pressure (Jones, 1973; Jones and Mansfield, 1972). It therefore follows that if any cell growth occurs then there must be an adequate supply of water to the tissues involved.

## Development of Water Deficits

Water deficits develop inevitably as a consequence of water loss from the leaf as the stomata open to allow the uptake of carbon dioxide from the atmosphere for photosynthesis. The water lost by transpiration from the leaf mesophyll cells is replaced by water drawn from the soil through the root, stem, and leaf via the xylem (Turner 1986a, 19686b). The pathways of movement through the plant, as mentioned earlier, have been reviewed by Passioura (1982), Weatherley (1982). The loss of water from plants (transpiration) is partly controlled by the stomata. As a soil dries and the soil water potential decreases, uptake of water by the root is decreased. If this situation continues, the water potential throughout the whole soil-root-shoot system will gradually be reduced until the leaf water potential falls sufficiently to cause stomatal closure, leading to a reduction in the transpiration rate. The control of this is not fully understood although recently the discovery that root signals can influence shoot responses to soil drying has generated considerable discussion in the literature about whether root signals or shoot water status is the true regulator of shoot responses. The subject has been discussed in detail, for example by Saab and Sharp (1989), Kramer (1988a) and Passioura (1988).

While the rate of transpiration and processes of water flow may be coupled to the total water potential, the physiological and morphological growth processes are more closely coupled to turgor maintenance, where osmotic adjustment is the major mechanism. Osmotic adjustment is the accumulation of solutes under conditions of water deficits thereby decreasing the osmotic potential and hence increasing the turgor pressure of the cells (Turner, 1986b). Osmotic adjustment of shoots has been demonstrated in a range of species (Turner and Jones 1980) and has been observed to maintain stomatal opening (Ludlow *et al.* 1985) and photosynthesis (Ackerson *et al.* 1980), at lower leaf water potentials, and to defer leaf rolling and leaf death (Hsiao *et al.* 1984) until lower leaf water potentials are reached. The recognition of these abilities of the plants to accumulate solutes in response to water deficits (Begg and Turner, 1976; Turner *et al.*, 1978) prompted the hypothesis that turgor pressure is the transducer of water deficits in the cell. Nevertheless, not all data have provided such confirmation. Turner (1975) observed that stomata in adequately watered maize plants were open and those in stressed plants were closed at the same turgor pressure. Jones and Rawson (1979) and Gollan *et al.* (1985) working with several species observed a range of leaf conductances and rates of net photosynthesis at zero turgor, depending on the size of container and rate of drying utilized. Increasingly the evidence is pointing to the root as a sensor of plant water deficits. Turner *et al.* (1985), Gollan *et al.* (1986) with sunflower, and Gollan *et al.* (1985) with oleander, all concluded that photosynthetic rate and/or stomatal conductance was more closely correlated with soil water content or soil water potential than with leaf water potential or leaf turgor.

Although a number of hormones have been observed to change when a plant is subjected to water deficits (Hsiao, 1973; Bradford and Hsiao, 1982; Davies *et al.*, 1986; Hartung *et al.*, 1988), the discovery that endogenous levels of abscisic acid (ABA) increased severalfold when plants were subjected to water stress (Wright and Hiron, 1969) and that ABA closed stomata and reduced transpiration (Jones and Mansfield, 1972; Raschke, 1975; Steuer, *et al.*, 1988) generated a flurry of activity on ABA as the stress hormone. Whether ABA is the messenger transmitting root signals to the stomata, or whether other plant growth regulators are involved is still an open question. Snaith and Mansfield (1982) and Blackman and Davies (1984) showed, respectively, that both indole-3-acetic acid and cytokinins (kinetin and zeatin) reversed ABA-induced closure of stomata. Moreover, the interaction between ABA and kinetin was affected by the level of nitrogen and phosphorus in the leaf (Radin *et al.*, 1982; Radin, 1984). While it is known that ABA can be synthesized in the mesophyll cells of the leaf, it was also produced in the roots (Walton *et al.*, 1976; Hubick *et al.*, 1986). If it then moves in the transpiration stream, it could act as the communicator between the root and shoot in its own right.

## 1.3 Methods of inducing water stress

Numerous studies have been conducted on the relationship between soil water content and plant growth (e.g. Crafts, 1968; Kramer, 1983; Levitt, 1980; Paleg and Aspinall, 1981). For many years, there were two opposing points of view; Firstly, soil moisture is equally available to plants over the entire range from field capacity to permanent wilting percentage and secondly, availability of water declines as soil water potential decreases. Data in support of the latter viewpoint have been obtained by many investigators including Kaufmann (1981) and Ritchie (1981). As discussed earlier, the relationship between growth and soil water status depends not only the physiology of the plant but also on environmental conditions affecting the rate of water uptake and moisture loss from the plant. Krizek (1985), stated that the methods for regulating water deficits in plant tissues are perhaps some of the most difficult of environmental variables to control experimentally because of the dynamic nature of water in the plant and its surrounding substrate.

There are four primary methods of inducing water stress in plants (Krizek 1985). The easiest and most frequently used method is the withholding of water from the soil or other substrate. It is the most obvious and natural method of inducing water stress in plants but also one of the most difficult to control. The plant may experience gradual or rapid dehydration depending upon the choice of media, size and type of container, and species used but the greatest difficulties are to reach a quantifiable end point and to maintain a uniform level of water deficit (Krizek *et al.*, 1985). The second involves the use of various osmotica such as NaCl, polyethylene glycol (PEG), mannitol, sorbitol, dextran, or mixtures of these compounds (see later). The third involves the use of semipermeable membranes or other techniques to separate the osmotic agent from direct contact with the root system (see later). The fourth involves the regulation of hydraulic conductivity by adjusting the height of a water column. Snow and Tingey (1985) developed this method for imposing long-term water stress without the disavantages of PEG toxicity, by suspending plant roots above a water column of known height. They were able to impose different levels of water stress over a period of several weeks by changing the height of the water column and/or the hydraulic conductivity of the medium in the column and thereby controlling the matric potential at the root surface.

However, the appropriate experimental approach to use in inducing water stress and in making measurements of the plant water relations will depend on the objectives and duration of the study, the experimental location, the type of media, the number of species or cultivars being examined, and the amount of time and support available. If PEG treatments are to be utilized,

it is useful to have comparable plants that are stressed by withholding water to ensure that toxic effects of the osmoticum are not involved in the stress response. If long term water stress treatments are planned, then controlling the hydraulic conductivity would be preferable. This advantage would be seen even if a membrane system were to be employed since membranes degrade after 14 to 20 days (Tingey and Stockwell, 1977; Snow and Tingey, 1985).

### 1.4 Use of polyethylene glycol in inducing water stress

Polyethylene glycols (PEG) are nonionic, long-chain polymers [$HOCH_2$-($CH_2$-O-$CH_2$)$_x$ $CH_2OH$]. They are available in a wide range of molecular weights and are highly soluble in water. Numerous investigators such as Greenway and Leahy (1970), Henzell *et al.* (1975), Jane (1974), Lawlor (1970), Mougou *et al.* (1984), Hasegawa 1984), and Oertli, (1984, 1986) have used PEG as osmotic agent to simulate water stress effect either for whole plants or for tissues, cells, and organelles. As early as 1952, PEG was used as an osmoticum to replace sugar or salts for the preparation of red alga plastids (McClendon and Blinks, 1952). It became a popular technique for applying stress by decreasing the water potential of the growing media.

PEG has been widely used in experimental solution cultures to create specific water stress levels that can be repeated and also more easily and accurately maintained than water stresses in soil culture (Gergely *et al.*, 1980a). The two water stresses are different. That imposed by PEG solution cultures is created by an osmotic potential, whereas in soil the stress is primarily a matric potential. It seems that they both produce similar effects on plant growth. Kaufmann and Eckard (1971) concluded that PEG 6000 produced changes in plant water relations similar to those caused by drying soil to the same water potential. Gergely, *et al.* (1980a), compared transpiration rates of apple seedling from the two methods. When plotted, they showed that PEG-induced osmotic stresses in soil of -0.05 MPa and -0.4 MPa were equivalent to greenhouse potting soil at 75% of field capacity and approaching the wilting point, respectively.

Despite its frequent use there are strong differences of opinion concerning the efficacy of PEG as an osmoticum for inducing water stress in plants (Tingey and Stockwell, 1977). Toxic effects of unpurified PEG on plants and P contamination in PEG have been reported by several investigators (Jackson 1962; Kaufmann and Eckard 1971; Leshem 1966; Reid 1978). Lagerwerff *et al.* (1961) attributed the toxicity of PEG 6000 to associated heavy metals (especially Al and

Mn) and recommended dialysis or passage through an ion exchange column to remove these contaminants. However, Lawlor (1970) was unable to detect differences in these elements before and after running PEG samples through ion exchange resins. PEG has been considered as a nonpenetrating osmoticum by various workers (Applegate 1960; Mexal *et al.* 1975; Michel 1971) although PEG 400 had toxic side effects (Kaufmann and Eckard 1971). Several undesirable side effects of PEG-treatment have been reported, such as: significant amounts of PEG are absorbed and transported unmetabolized through plants (Chapman and Fisher, 1988; Jane, 1974; Lawlor, 1970; Jacomini, 1988; Yaniv and Werker, 1983), reduction in P uptake and translocation (Emmert, 1974; Resnik, 1970; Plaut and Federman, 1985), reduction in oxygen content of the nutrient solution (Mexal, *et al* 1975) and visible leaf damage (West *et al.* 1980).

One way of overcoming possible PEG toxicity is to use a membrane system for inducing water stress. Several workers (Zur, 1966, 1967; Tingey and Stockwell 1977; Yambao and O'Toole 1984) have used a semipermeable membrane to enclose the root system and then growing the plants in nutrient solutions containing PEG. Tingey and Stockwell (1977) showed that dialysis tubing or seamless cellulose tubing (Spectrapor 1) with minimum molecular weight exclusion limits of 12,000 to 14,000 and 6,000 to 8,000 respectively, excluded PEG 20,000 (average molecular weight 14,000 t0 16,000) from roots but allowed water and nutrients to diffuse readily. Plants equilibrated with the nutrient solution within one to four days. Although including PEG directly in nutrient solutions inhibited P uptake and translocation (Emmert 1974; Resnik 1970), by using a semipermeable membrane system to separate the PEG from the root system, Tingey and Stockwell (1977) found an increase in P concentration in the leaves and stems with time suggesting that P uptake and translocation were not inhibited.

Several factors need to be considered before deciding upon a particular molecular weight of PEG, such as its possible uptake and metabolism by the plant. The most commonly used molecular weight compounds are PEG 4000 and PEG 6000, but molecular weights as large as PEG 20,000 have been used (Tingey and Stockwell 1977). Lower molecular weight PEG compounds e.g. PEG 600, also have been used successfully with little evidence of uptake in sorghum, cowpea, and other plants (Schaefer *et al.* 1977). Uptake of PEG appears to be greatly dependent on the plant material and the condition of the roots used in the experiment (Lawlor 1970). The importance of using undamaged roots cannot be overemphasized. Since any handling causes some damage, care should be taken to minimize this effect. It is generally believed that PEG molecules with a molecular weight of 3000 to 4000 or greater do not pass through cell membranes (Carpita *et al* 1979; Michel 1971). Yaniv and Werker (1983) however, found that PEG-containing drops were secreted by leaves when tomato and other solanaceous plants were put in solutions of different molecular weight PEGs (PEG 1500, PEG 4000 and

PEG 6000). They indentified the secreted material using several methods, and showed that PEG molecules as high as 10,000 were absorbed and secreted through ordinary epidermal cells.

Handa *et al.* (1982, 1983), working on tomato cell suspensions, showed that PEG 6000 and $^{3}H$-labelled PEG 4000 did not penetrate tomato cells but remained in a single compartment (apoplastic spaces), which would exclude direct interaction between the osmoticum and cell metabolism. They also suggested that tomato cells which have been exposed to PEG stress, respond in a similar manner as in the natural dessication conditions. In addition, Hasegawa *et al.* (1984) concluded that the use of PEG, resulted in the cells being exposed to water stress, but not to the deleterious effects caused by absorption of toxic levels of the osmotic agent. The capacity of solutes to cross cell walls and to plasmolyze the cells was used to determine wall pore size by Carpita *et al.* (1979). Working on several plant species they produced evidence that PEG 6000 did not plasmolyze but cytorrhyzed cells (due to water moving out of the cells). However, Lawlor (1970) suggested that PEG adhered to the matrix cell wall and caused interruption of the transpiratory route but that small amounts of PEG do not interfere with water movement. A recent study by Jacomini *et al.* (1988) on young tomato plants grown in 10% and 25% PEG 6000 found that PEG was accumulated in extracellular spaces of the tissues and this could lower the osmotic potential of those spaces and draw the water out. This water would remain in the tissue for some time but be inaccessible for cell metabolism. These authors also showed a rise in the levels of soluble-sugars, amino acids and organic-inorganic ions, and suggested that it was a typical adaptive response of plants in water stress to reduce cellular osmotic potential, as reported by other workers (Hanson and Hitz 1982; Hasegawa *et al.* 1984).

### 1.5 **The effects of water stress on ion uptake and transport**

The pathways of nutrient and water movement from the soil to the shoot system are essentially the same: through the cortex of the roots to the stele and upwards in the xylem. However, as early as in 1923, Hoagland showed that barley plants absorbed major nutrients such as nitrogen, phosphate, and potassium more readily than water from an aqueous solution. Whilst, in contrast other ions such as calcium, magnesium and sulphate were taken in more slowly than water. This shows that the plant exerts a degree of selectivity over which ions are absorbed. The mechanisms of uptake of minerals have been review in detail by Scott Russell (1977), Nye and Tinker (1977).

The effect of water stress on nutrient uptake has been studied in various ways. The direct approach of varying the availability of water in the soil has been widely used to study plant responses to nutrient and water stress together, and has shown the need to consider movement of ions in the soil as well as the properties of plants in assessing the effects of water flow on nutrient uptake (Pitman 1981). It also has been convenient to use osmotic agents (e.g. PEG), avoiding the problems of nutrient transfer in dry soil, even though often inducing other difficulties.

## Movement of ions in the soil and to the roots

It is well documented that low soil water potentials reduce the diffusion rates of ions (e.g. Dunham and Nye, 1976; Mackay and Barber, 1985a, 1985b) and it would seem likely that reduced levels of ion uptake by plants growing in dry soils is due to this reduced availability of the ion as well as to the actual impairment of uptake ability of the root itself. A discussion of the movement of ions is presented by Brewster and Tinker (1972).

Surprisingly, little work has been done in analysing the contribution of various factors to the reduction of uptake of ions at low water potentials, so that the effect of low water content of the soil may be separated from the effects of low water potentials on the uptake mechanism of the root. Danielson and Russell (1957) compared soils with solutions of varied osmotic pressures. Although uptake was inhibited when soil moisture was low, an equivalent osmotic stress (to -1.2 MPa) in solution had no effect even though the relative water content of the roots was reduced by both treatments. A more detailed analysis was made by Dunham and Nye (1974, 1976) for the uptake of $Cl^-$, $K^+$, $Ca^{2+}$, $Mg^{2+}$ and phosphate (discussed in Nye and Tinker, 1977). In general, it appeared that influx to root cells was much less sensitive to stress than transport across the root to the xylem. Greenway *et al.* (1969) measured the effect of culture solutions of varied osmotic pressure (produced by adding mannitol) on phosphate ($^{32}P$) uptake by intact tomato plants and showed that transport to the shoot was much more sensitive to external osmotic pressure than the uptake by root cells. Pitman *et al.* (1974) also reported that transport of $^{86}Rb$ and $^{14}C$-leucine to the shoot was inhibited when seedlings were wilted, and did not recover after two hours even though transpiration had recovered within 30 minutes. Uptake of $^{86}Rb$ by the roots was increased during this period showing again that uptake into the cells of the root was less affected than transport to the shoot.

## Effects of water stress on root growth

Plant growth is generally decreased when soil water is limited but root growth is often less inhibited than shoot growth (Blum *et al.* 1983; Sharp and Davies 1979; Sepaskhah and Boersma, 1979). However, studies of the effect of water stress on the growth of roots have produced various results, such as decreases (Newman, 1966 ; Mackay and Barber, 1985a; Stevenson and Laidlaw, 1985), increases in the production of lateral roots (Kmoch *et al.*, 1957; Huck *et al.*, 1983) increases in root length (Sharma and Ghildyal, 1977; Osonubi and Davies, 1978) and increases in total root dry weight (Malik *et al.*, 1979). Jupp and Newman (1987 ) using *Lolium perenne* demonstrated that roots responded to severe drought by producing new lateral roots originating from the pericycle and often growing through a dead cortex. Furthermore, recent studies of maize have shown that root growth is intrinsically less sensitive than growth of the aerial plant parts to low water potentials in the growing region, indicating some form of internal regulation (Schildwacht, 1988 and Sharp *et al.* 1988).

Many studies have shown that as water deficits develop, there is a substantial capacity for osmotic adjustment in the growing zone of roots. Molyneux and Davies (1983) compared the rooting patterns of several pasture grasses growing in drying soil and found considerable differences in their responses. They found that *Phleum pratense* showed a reduction in root growth which corresponded with a reduction in root turgor while *Dactylis glomerata* (which is reputed to be a drought resistant species) showed an increase in root growth, and maintained the root turgor throughout the drying cycle. This maintenance of root turgor was suggested to be the main mechanism by which roots of some species continued to grow in drying soil. Hsiao and Acevedo (1974) and, Sharp and Davies (1979) further suggested that continued root growth at low soil water potentials could be the result of a high capacity for solute accumulation in the roots, and thus maintenance of root turgor. Shone and Flood (1983) using PEG to induce water stress in barley, found that tips of nodal roots treated with PEG accumulated sugars, and this was accompanied by a significant decrease (by comparison with untreated plants) in the fresh weight of the untreated parts of the root system. This suggested that sugars were being moved into the root tips from unstressed parts of the root system and thus maintained turgor pressure.

## The interactions between the nutrient uptake, plant growth and water stress

Water stress can affect plant growth through its influence on nutrient uptake. Early reviews of the subject have been given by Wadleigh and Richards (1951) and Russell and Barber (1960). The effect of water stress on ion uptake and transport within the plant has been reviewed by Hsiao (1973), and Nye and Tinker (1977). Pitman (1981) reported that it is difficult to separate effects of water stress on ion uptake on the one hand and dry matter production on the other. For example, in many plants the rate of accumulation of N, P and K is proportional to the rate of growth, so that relative contents tend to be constant. The proportionality in uptake and growth can be attributed variously to increasing root size, to feedback processes in the plant, or to rate of supply of sugars to the root. In drying soil, nutrients become unavailable because their transport to the root-soil interface is impeded by the diminished rate of diffusion and mass flow (Viets 1972). These reasons are often associated with reduced ion uptake as the soil moisture decreases. There is still limited information relating plant water stress to ion uptake without regard to soil factors. Plant water stress by decreasing solution water potential can cause reduction in nutrient uptake by its effect on the rate of water flow through plants (Greenway and Klepper, 1969) or possibly by its effect on active ion uptake mechanisms and the passive efflux of ions (Erlandsson 1975, 1979).

Yambao and O'Toole (1984), found that rice plants with PEG induced water stress showed reduced total N uptake in shoots of both low and high nitrogen treatments. Shoot growth which was decreased by water stress, low N supply or both, led to a decrease in total water transpired. They demonstrated that transpiration and N uptake were correlated. Furthermore, the same experiment showed that the nitrogen content was reduced in water-stresssed plants by the integrated effects of plant water stress *per se* on the accumulation of dry matter and transpiring leaf area as well as the changes in soil physical properties of a drying root medium. Frota and Tucker (1978) observed a high positive correlation between N and water uptake under both water and salt stress. Greenway and Klepper (1969) concluded that water flow accelerated anion transport to the shoot after the ions had been taken up by the roots. They found that total P uptake decreased with nutrient solution potential as a result of reduced P transport to the shoot. Reduced N uptake by plants could possibly also arise from the effect of plant water on the active ion uptake mechanism and the release of ions as suggested by Erlandson (1975, 1979).

Tanguilig *et al.* (1987) reported that maize and soybean could maintain high leaf water potentials for longer periods of water stress than rice, whilst rice transpired more than soybean and maize at the same leaf water potential. They also found that nutrient uptake under water stress conditions was influenced more by the capacity of the roots to absorb nutrients than by transpiration. Transport of nutrients to the shoots may occur even at reduced transpiration rates. They suggested that the ability to maintain turgor was a result of the slow decline in leaf water potential brought about by the low transpiration rate and the continued uptake of nutrient, especially K, which must have allowed osmotic adjustment to occur. Similarly, Sinha (1978) observed that drought-tolerant wheat varieties accumulated more K than susceptible varieties and that plants well supplied with K had higher stomatal resistance which resulted in low transpiration rates.

It is well-established that under water stress conditions the plant's nutrient uptake decreases because of reduced transpiration (Greenway and Klepper, 1969; Verasan and Philips, 1978; O'Toole and Baldia, 1982), impaired active transport and increased membrane permeability (Hsiao, 1973), all of which reduce the root's absorbing power. Although the results of such experiments are varied there seems to be general agreement that the uptake of ions into the root cells, and subsequent transport across the roots to the shoots are affected differently. It would therefore be important that these two processes, although related should be considered separately when investigating the effect of water stress on the mineral nutrition of plants. As early as 1938, Crafts and Boyer suggested that ions were taken up actively at the plasmalemma of epidermal and cortical cells of the roots and then transported passively down their activity gradients through the symplasm into xylem vessels in the stele. There is now much evidence (reviewed by Lauchli, 1972) that there is a second active step in the stele whereby the xylem parenchyma cells secrete ions into the xylem vessels. One could postulate therefore that water stress might have two kinds of effect. First, an influence on ion transport due to reduced transpiration and hence reduced water flow (the passive step) and secondly, effects on the active transport mechanism and on membrane permeability.

Although water stress conditions affect several aspects of plant metabolism it is generally accepted that the first effect is on the cell membrane structure, with consequent modifications in their viscosity and permeability. The nature of this damage is not well understood yet (Izzo *et al.*, 1988), but it is known that it can influence the regular exchange capacity of the biological membrane (Tanguilig *et al.* 1987) and increasing cytoplasmic solute leakage. There are reports (e.g. Maas *et al.*, 1979; Migliaccio, 1980; Rubinstein, 1982a, 1982b) that roots subjected to an osmotic shock release protein to the surrounding solution. It has been suggested that these

proteins may have come from the cell membranes and might have contained the components of the active transport systems for ion uptake. This evidence is very circumstantial, however, and is based on the observations that first, a close relationship exists between loss of ion transport activity and the release of proteins, and secondly that recovery of transport activity seems to require protein synthesis. The question arises, therefore, as to the location of these released proteins in the roots. An answer to this would give a greater understanding of the pathway of ion uptake and transport across the root and how this is affected by water stress. It is known that little of the endogenous vacuolar solutes in root cells are lost during osmotic shock (Maas *et al.*, 1979) so it would seem unlikely that the tonoplast is damaged. The plasmodesmata, however, are strongly affected (e.g. Rubenstein, 1982a, 1982b). This question was investigated by Migliaccio *et al.* (1984) working with maize. They osmotically shocked 4-day old excised roots by 30 minutes treatment with saline solution and found that uptake of orthophosphate and its transport to the xylem was inhibited, due to decreased uptake rather than any efflux of phosphate from within the root cells. They also found evidence that a gradual recovery of uptake occurred concomitantly with the synthesis of membrane proteins. Phosphate transport to the xylem failed to recover completely, however, suggesting that long-term damage to the plasmodesmata had occurred.

The work discussed above shows fairly conclusively that in young excised roots osmotically shocked for a short period of time, mineral nutrient uptake into the root and transport across the root to the xylem are affected differently. It would be wrong, however, to assume that identical responses would be found in mature plants growing in soil and subjected to longer periods of low soil water potential. In addition, much of the work discussed so far has imposed the osmotic shock using solutions of osmotica such as NaCl. It is possible that such solutions would cause plasmolysis of cells, separating the plasmalemma from the cell wall and tearing plasmodesmata in the process, and so may have their own unique effects on ion transport. Little work has been done on the effect of water stress on the metabolic ability of roots to take up nutrients grown in soil, probably due to a large extent to the difficulty in separating effects on the plant's physiology from effects on the availability of minerals in the soil.

## 1.6 The use of split-root technique for study plant water relation and nutrient uptake

The split-root growth system has been used to study the effects of various stresses on plant growth and root development: salinity (Singleton, 1983; Jeschke and Wolf, O., 1988), water uptake (Lawlor, 1973), plant water relations (Herkelrath et al., 1977a; Michel and Elsharkawi, 1970), root growth (Coutts, 1982), nutrient availability (Hackett, 1972; Gergely, *et al.*, 1980b). Hinson (1975) inoculated half the root system of soybeans grown in vermiculite to study the localized effect of mineral N on nodule development. However, most work using split-root systems has been directed toward investigations of root growth, water and nutrient uptake and translocation from dry soil or osmotic stress solutions. Lawlor (1973) subjected wheat roots to a water stress by adding PEG 4000, and found that the growth of roots decreased with the decrease in osmotic potential and stopped at -1.0 MPa. Solutions of high osmotic potential, used to treat one part of the root system, did not influence the growth of the other part where the osmotic potential was low. However, it did allow greater growth of leaves and faster transpiration rates, because plant water potential was not decreased. He also showed that the rate of water transport slowed with decreasing osmotic potential but with part of the root system under stress, absorption from solutions of high potential increased.

Blackman and Davies (1985) used a split root system to investigate the effect of drying part of the root system of maize plants. They found that compared to plants grown with their roots divided between two pots of moist soil, drying of part of the root system resulted in partial closure of the stomata, but leaf water potential, leaf turgor and abscisic acid content remained unaffected. These workers present evidence which suggests that the soil drying interrupted the supply of cytokinin from the roots to the leaves which is necessary to maintain stomatal opening.

Similarly, Saab and Sharp (1989) grew maize with the root system divided between two containers one of which was allowed to dry while the other was kept well-watered. They found that soil drying had no effect on stomatal conductance and suggested that the non-hydraulic inhibition of leaf elongation may act to conserve water as the soil dries before the occurrence of shoot water deficits.

Lambers *et al.* (1982) used the split-root system to obtain further evidence for the idea that shoots of wheat seedlings are able to redirect significant quantities of nitrogen back to the roots. They used the wheat seedlings whose roots were pruned leaving only two seminal roots. The plants were replanted into split-pots so that each root was grown in an independent free-draining pot. One of the seminal roots was supplied with different levels of $NO_3^-$ whilst the

other received a N-free nutrient solution. They found that at optimum N supply, 11% of all N transported to the shoots was retrans located to the roots, and both a supra-optimal and a limiting N supply increased the translocation of N back to the roots to 18% of that translocated to the shoot. They also showed that at optimal N supply, 40% more N was translocated to the roots from the shoot than was incorporated direct or in total whilst, at lower supply of N, 80% more N was imported from the shoots than was incorporated by these roots.

### 1.7 Reasons for selecting the species and general aims of the study

Plant responses to water stress vary with species and are modified by environmental and physiological factors. This explains why one species survives or yields better than another under a limited supply of water and nutrients. Brown (1985) studied four *Panicum* species; *P.prionitis* ($C_4$), *P.laxum* ($C_3$), *P.grumosum* ($C_3$) and *P.spathellosum* ($C_3/C_4$), and bermuda grass, *Cynodon dactylon* ($C_4$) at low N levels, and found that the growth rates were twice as high, whilst the N concentrations in live shoot and root tissues were lower for the $C_4$ than the $C_3$ or $C_3/C_4$ species. The root weights and the dry matter produced per unit of N absorbed were twice as high in the $C_4$ as in the other species. Tanguilig *et al.* (1987) suggested that the ability of maize ($C_4$) and soybean ($C_3$) to grow better than rice under water stress conditions may have been due to their ability to maintain turgor as a result of slower decline in leaf water potentials brought about by low transpiration rates and continued uptake of nutrients, especially K, which must have allowed osmotic adjustment to occur.

Therefore, this study sought to determine and compare the differences between three grass species of different origins and habitats, in the way in which they respond to water stress, specifically for nutrient uptake and utilization. *Panicum miliaceum* (a non-arid zone native pasture, $C_4$ plant), *Aristida funiculata* (an arid zone wild, $C_4$ plant) and *Avena fatua* (a non-arid zone weed, $C_3$ plant) were chosen in this study because of their differences in origins and contrasting in natural habitats. Their botanical features and agronomic importance are described in appendices I - III.

Since little information is available on the effects of water stress on the functioning of roots, this study was also designed to consider these effects on the efficiency of roots of the above species in the absorption and/ or utilization of mineral nutrients. Our general objective therefore was to look for evidence in favour of the hypothesis that *Aristida funiculata* would be different, if not better, than *Panicum miliaceum* and *Avena fatua* in its ability to take up and use nutrients under conditions of water stress.

# CHAPTER TWO

## General Materials and Methods

# CHAPTER TWO

# GENERAL MATERIALS AND METHODS

## 2.1 Soil

In all the experiments in Chapters Three and Four except for experiment 3.2.3, the plants were grown in a clay-loam soil of the 'Winchester series' collected from an open hilltop on the Wye College Farm. The site was permanent grassland, regularly grazed by sheep and received very little added fertilizer. Soil collected for all experiments came from the top 25 cm of the soil profile. The soil was spread on the glasshouse floor and left to dry with the help of a hot air fan. The dry soil was crushed and sieved to pass through a 7.0 mm mesh to remove stones and large roots. Samples of the sieved soil were sent to the Agricultural Development and Advisory Service for analysis of some soil properties, particularly pH ($CaCl_2$ extract) and nutrient status. The analysis showed that the soil had a pH of 5.5, phosphorus at 4 ppm (0.129 mM), potassium 51 ppm (1.308 mM) and magnesium 174 ppm (7.250 mM) on a soil dry weight basis. The lime requirement was 3 tonnes/ha of ground chalk. The soil was mixed 3:1 with river sand and the final pH of the mixture was 5.37. The soil used in experiment 3.2.3 was collected from the College experimental plots near the glasshouse, which had not been cropped in the previous year. The soil moisture-matric potential curve for this soil is shown in the Appendix IV.

The relationship between PEG concentrations (g/1000g distilled water) and water potential (MPa) for PEGs used in all the experiments was shown in Fig 5.5e (page 119).

## 2.2 Plants and Growth Conditions

The species used in the experiments were *Aristida funiculata, Avena fatua* (wild oats) and *Panicum miliaceum*. Wild oat seeds were obtained from B & S Weed Seed Supplies of Whatton-in-the-Vale, Nottinghamshire, whilst, seeds of *A.funiculata* and *P.miliaceum* were obtained from departmental colleagues. Unless otherwise stated, seeds were germinated on nutrient-free damp vermiculite in seed trays. The time to reach the transplanting stage (2 leaves) varied: in particular, *A.funiculata* seedlings grew at a slower rate than the other two species during the first few weeks and were therefore sown 8 to 10 days earlier. Seedlings at the 2 leaf stage were transplanted (2 per pot) into 12 cm diameter plastic pots of soil. The soil was thoroughly mixed before use, and the dry weights of random soil samples were determined so that the dry weight of soil in each pot could be estimated. The two plants in each pot were pooled for experimental analysis.

All plants were grown in cubicles of the Wye College glasshouse with supplementary heating and watered with distilled water to maintain approximate field capacity i.e. 50% soil moisture

content. When nec essary additional light was provided by suspending 400 watts high pressure mercury fluorescent lamps approximately 150 cm above the level of the pots. A total of approximately 16 hours light was maintained and the average daily maximum, minimum and mean air temperatures of the cubicle were recorded. The relative air humidity was not controlled. In these experiments, the treatments were imposed 2 weeks after transplanting.

## 2.3 Harvesting

Root and shoot systems were separated by cutting the shoot just above the soil surface. Both were washed carefully with tap water and rinsed with distilled water. Excess water was removed by blotting between two layers of paper towel before weighing. Unless otherwise mentioned, plant attributes such as root and shoot fresh and dry weights were measured at each harvest. Roots and shoots were oven-dried at 70 °C for up to 3 days before dry weights were recorded.

## 2.4 Measurement of root length and root volume

Estimates of root length were obtained using the line interception method described by Tennant (1975). Root volume was determined with the apparatus shown in (Plate 1). This apparatus consisted mainly of a large glass tube (30 mm diameter) connected to a smaller U-tube (5 mm diameter), and fitted against a scale. The glass tubes of the apparatus contained water and the change in height of the water column in the smaller tube when roots were lowered gently into the system, was measured with a screw-mounted cursor. The volume of roots was then read from a graph of calibrated values.

## 2.5. Analysis of Plant Material

The dried shoots and roots were analysed separately. They were ground in a ball mill grinder into fine powder to pass through a 0.5 mm sieve. The ground materials were digested and analysed for total nitrogen, phosphate, potassium, magnesium and calcium.

The method of Thomas *et al.* (1967) was used to digest samples for analysis. To 0.1g sample of dried ground plant material was added 3 $cm^3$ of digestion mixture, consisting of 20g lithium sulphate and 20g salicylic acid dissolved in 500 $cm^3$ of concentrated sulphuric acid. The sample was soaked overnight. Before digestion, 0.3g of sodium thiosulphate was added. The digestion was carried out in an aluminium digestion block, for one hour at 200 °C followed by 2 hours at 350 °C. The digests were then cooled, 1 $cm^3$ of hydrogen peroxide added, and reheated.

If necessary, this was repeated until the digest was clear. Care was taken to add the same amount of hydrogen peroxide to all samples and to the blanks. After digestion the solutions were made up to 50 $cm^3$ in volumetric flasks.

The nitrogen in the sample was determined using an Auto Analyser. A continuous flow system, built in the department laboratory and incorporating a Pye Unicam SP6-500 UV-visible spectrophotometer was used. Signals from the spectrophotometer were passed via an interface to a computer which converted the readings to mg/l (ppm) and printed them. The analysis was based on reactions between three reagents (Varley, 1966). 'Reagent A' (Buffer solution) consisted of 50g potassium sodium tartrate, 14.2g anhydrous disodium hydrogen phosphate and 54g sodium hydroxide dissolved in 2 litres deionised water and 2 $cm^3$ Brij 35 (polyoxyethylene lauryl ether). 'Reagent B' (Salicylate/nitroprusside) consisted of 129.4g salicylic acid, 40g sodium hydroxide and 0.6g sodium nitroprusside were dissolved in 2 litres deionised water and 2 $cm^3$ Brij 35 added. 'Reagent C' (Sodium hypochlorite) was prepared by diluting 15 $cm^3$ sodium hypochloride (10-14% available chlorine) to 500 $cm^3$ with deionised water. Approximately 2 $cm^3$ of sample was pipetted into a sample cup and placed on a Carlo Erba turnable capable of holding 40 cups. Samples were then picked up by a Teflon tube and drawn into the system by a Carlo Erba peristaltic pump. Reagents entered the system separately through flow-measured pump tubing, and mixed with the sample in a glass coil. Delivery rates ($cm^3$/min) of the sample, reagent buffer, salicylate and sodium hypochlorite were 0.05, 2.50, 1.20 and 1.20 respectively, and the time that the pick-up tube remained in the sample cup was 0.05 min. Ammonium salts in the sample react with sodium salicylate, sodium nitroprusside and sodium hypochlorite in an alkaline buffer to form a bluish-green indo-phenol colour which was read in the spectrophotometer at 660 nm.

The method of Murphy and Riley (1962) was used for determination of phosphate. 'Reagent A' consisted of 12g ammonium molybdate, 0.2908g of antimony tartrate, 149 $cm^3$ sulphuric acid made up to 2 litres with deionised-distilled water. To this was added ascorbic acid at the rate of 1.32g ascorbic to 250 $cm^3$ 'reagent A' to make 'reagent B'. Then, 4 $cm^3$ of 'reagent B' were added to the aliquot to be analysed (1 to 5 $cm^3$) in a 25 $cm^3$ volumetric flask and made up to the mark with deionised-distilled water. The samples were left for about 30 minutes to allow full development of a blue complex, which was measured at a wavelength of 712nm on a Pye-Unicam SP8-100 uv/vis spectrophotometer. John (1970) showed that this method is very sensitive to pH. In order to achieve the correct pH with the digests, it was necessary to neutralize them with 0.5M NaOH using p-nitro-phenol indicator and to reacidify using 0.5M $H_2SO_4$ until the colours just slightly changed from yellow to colourless (pH 5.5).

Calcium and magnesium were measured by an atomic absorption spectrophotometer, and potassium by a flame photometer. In order to remove any depressive effects due to interference from other elements, lanthanum chloride (final concentration 400 ppm La) was added as a releasing agent to both samples and standards. All standards were made up using blank digests.

### 2.8. Experimental Design and Statistical Analysis of Data

Unless otherwise stated, all the experiments are factorial combinations of treatments in randomized complete block designs with varying numbers of replicates. All experimental data were statistically analysed with ANOVA (analysis of variance) followed by comparisons of treatment means using LSD (least significant differences) where applicable.

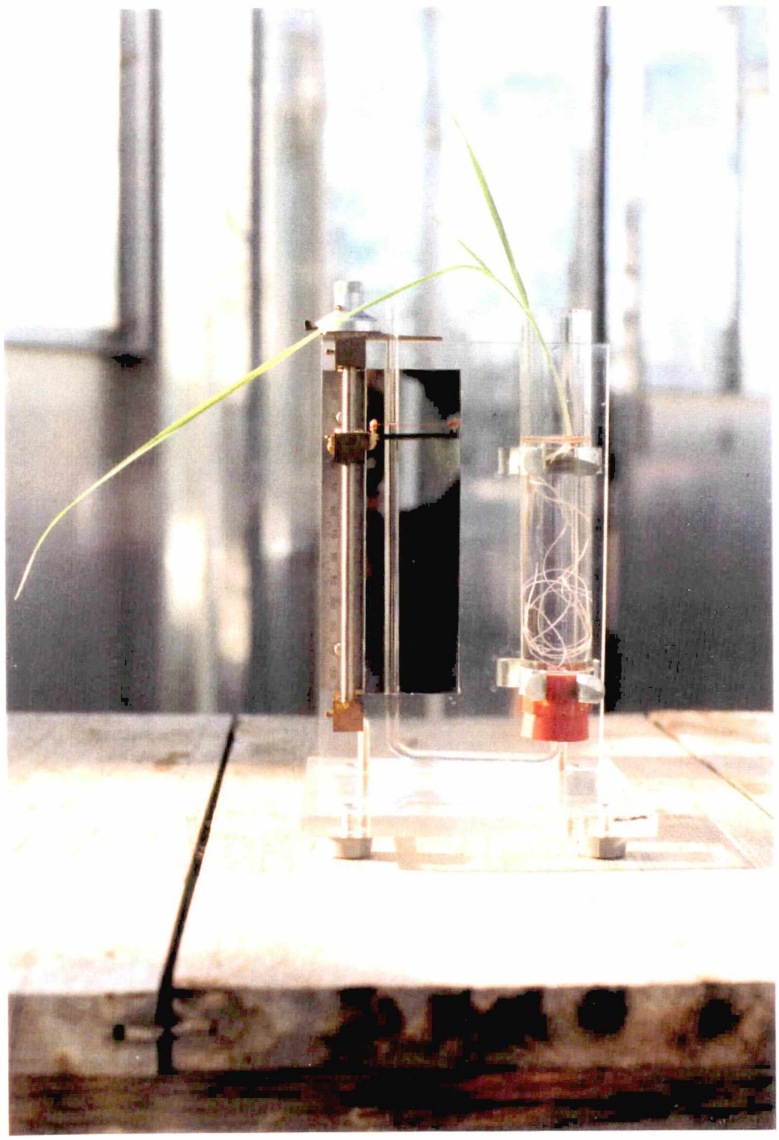


Plate 1. Root volume measuring apparatus. The apparatus consisted mainly of a large glass tube (30 mm diameter) connected to smaller U-tube (5 mm diameter) and fitted against a scale. The operation are given in the text.

# CHAPTER THREE

## Response of *Aristida funiculata*, *Avena fatua* and *Panicum miliaceum* to Differential Nutrition for Nitrogen and Potassium Under Conditions of Soil Water Stress.

# CHAPTER THREE

## RESPONSE OF *Aristida funiculata* (Aristida), *Avena fatua* (Avena) AND *Panicum miliaceum* (Panicum) TO DIFFERENTIAL NUTRITION FOR NITROGEN AND POTASSIUM UNDER CONDITIONS OF SOIL WATER STRESS.

### 3.1 Introduction

The main aim of this study was to look for evidence of differential responses of *A.funiculata, A.fatua* and *P.miliaceum* to the levels of N and K under soil water stress conditions and relate where possible any observed differences to the nutritional backgrounds and adaptations of these species. The experiments in this chapter were carried out to achieve the following specific objectives:

1) To study the responses of *A.funiculata, A.fatua* and *P.miliaceum* in dry matter production under soil water stress conditions (in the glasshouse) and to compare the differences with the control (unstressed).

2) To determine species differences in the uptake and accumulation of N, K, Mg and Ca.

3) To determine the efficiencies of nutrient uptake and utilization:

   i. In terms of uptake per unit weight of root (Root Efficiency)

   ii. In terms of dry matter produced per unit nutrient taken up (Utilization Efficiency)

## 3.2 Experiments

### Experiment 3.2.1 The response of *Panicum miliaceum* (Panicum) and *Avena fatua* (Avena) to varied levels of nitrogen and soil water stress.

*Materials and Methods*

Plants were grown as described in Chapter Two. The seeds were sown on 4/7/87 and ten days later the seedlings were transplanted into the pots. The Long Ashton nutrient recipe, based on nitrate nitrogen was used (Hewitt and Smith, 1975). The seedlings were treated with solutions of four N concentrations and subjected to three levels of soil moisture during the growing period as follows:

(N0) 0 mM (0 ppm N)
(N1) 1.35 mM (18.9 ppm N)
(N2) 4.04 mM (56.7 ppm N)
(N3) 12.13 mM (170 ppm N or full strength Long Ashton solution)
(W0) FC (Field Capacity or unstressed i.e. 50% soil moisture)
(W1) 3/4FC
(W2) 1/2FC

The different N concentrations were obtained by replacing some of the $KNO_3$ and $Ca(NO_3)_2$ with equivalent concentrations of KCl and $CaCl_2$ respectively, and were applied weekly. The soil moisture content and the amount of distilled water needed to maintain the required moisture levels were estimated by weighing the pots daily.

A randomised complete block design with four replication was used. The experiment was terminated after six weeks (i.e. four weeks after treatments applied). Fresh and dry weights of shoot and root were determined, and shoot nutrients analysed. The efficiency of nutrient uptake of roots (mMol/g root dry weight) was measured as the total amount of nutrient in shoot per unit of root weight. Whilst, the utilization efficiency (g dry weight/mMol) was measured as total dry matter produced per unit nutrient taken up.

## *Results*

Root and shoot growth

The effects of the four N concentrations and three soil moisture levels on the final size of Avena and Panicum are shown in Table 3.1.

The total dry weight of Avena plants were consistently and significantly larger than Panicum across all treatments (analysis of variance Table 3.2). There were differences in the responses of the two species to the treatments imposed particularly water stress. The total dry weight of both stressed and unstressed Panicum responded to increases in N concentration from N0 to N2 but then levelled off or declined at the highest N concentration (Fig 3.1a)*. The unstressed (W0) plants of Panicum showed significantly the lowest total dry matter accumulation at N3. Water stress showed very little effect on the total dry weight of Panicum except for the difference at the N3 level. On the other hand, Avena showed a continuing increase in total dry matter up to the highest N level except for the W2 stressed plants which levelled off at N2. The response of Avena was significantly different between the unstressed (W0), W1 and W2 stressed plants, particularly at the higher N concentration (N2 and N3). The unstressed and W2 stressed plants accumulated the most and least dry matter respectively at the highest N level.

Dry matter content (ratio of total dry weight to total fresh weight) showed highly significant effects of species and water stress and their interaction, but not effects due to N. Avena had generally higher levels than Panicum and showed no consistent changes with water stress, whilst Panicum showed consistently increasing ratios as water stress increased (Fig 3.1b).

The partitioning of dry matter between roots and shoots, as revealed by the root/shoot ratios showed that the main effects were all significant but none of the interactions (Table 3.1). The ratio was consistently lower for Panicum than Avena, declined quite strongly as N supply increased and declined to a smaller extent as water supply decreased (Fig 3.1c).

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* The low value for the FC treatment at 12mM N was unexpected, but all four replicates showed the same effect.

Table 3.1 Effects of nitrogen levels on some growth variates of Panicum (PM) and Avena (WO) under varying soil moisture conditions

| N Conc (N) Soil moisture levels (WS) | | Whole Plant dry wt (g/plt) | | Root/Shoot ratio (dry wt) | | Dry Matter Ratio (Total DW/Total FW) | |
|---|---|---|---|---|---|---|---|
| | | PM | WO | PM | WO | PM | WO |
| N(mM) | WS | | | | | | |
| 0 | FC | 0.995 | 1.862 | 0.4124 | 0.5682 | 0.0965 | 0.1166 |
| | 3/4FC | 1.020 | 1.950 | 0.3732 | 0.4809 | 0.1084 | 0.1318 |
| | 1/2FC | 1.165 | 1.900 | 0.2756 | 0.4157 | 0.1285 | 0.1380 |
| 1.32 | FC | 1.565 | 2.725 | 0.3231 | 0.4576 | 0.0978 | 0.1303 |
| | 3/4FC | 1.550 | 2.415 | 0.2937 | 0.5785 | 0.1127 | 0.1258 |
| | 1/2 | 1.752 | 2.107 | 0.2286 | 0.4013 | 0.1342 | 0.1380 |
| 4.04 | FC | 2.360 | 3.525 | 0.2745 | 0.3992 | 0.1074 | 0.1330 |
| | 3/4FC | 2.395 | 2.875 | 0.2499 | 0.4356 | 0.1233 | 0.1292 |
| | 1/2FC | 2.097 | 2.397 | 0.2303 | 0.3678 | 0.1399 | 0.1290 |
| 12.13 | FC | 1.595 | 3.732 | 0.1444 | 0.3251 | 0.0802 | 0.1228 |
| | 3/4FC | 2.190 | 2.947 | 0.1961 | 0.2649 | 0.1043 | 0.1308 |
| | 1/2FC | 2.180 | 2.292 | 0.2001 | 0.2885 | 0.1410 | 0.1208 |
| Significance level | | | | | | | |
| N | | *** | | ** | | ns | |
| WS | | ** | | ** | | *** | |
| SP | | *** | | ** | | *** | |
| N x WS | | * | | ns | | ns | |
| N x SP | | ns | | ns | | ns | |
| WS x SP | | *** | | ns | | *** | |
| N x WS x SP | | ** | | ns | | ns | |
| LSD (P< 0.05) | | | | | | | |
| N | | 0.1718 | | 0.0444 | | - | |
| WS | | 0.1488 | | 0.0385 | | 0.00690 | |
| SP | | 0.1216 | | 0.0314 | | 0.00562 | |
| N x WS | | 0.2976 | | - | | - | |
| N x SP | | - | | - | | - | |
| WS x SP | | 0.2104 | | - | | 0.00974 | |
| N x WS x SP | | 0.4210 | | - | | - | |

Table 3.2 Analysis of variance of the effects of nitrogen levels on total dry weight of Panicum and Avena under varying soil moisture conditions experiment

| Source of variation | df | SS | MS | VR |
|---|---|---|---|---|
| Blocks | 3 | 0.988 | 0.329 | |
| Nitrogen (N) | 3 | 18.927 | 6.309 | 71.208*** |
| Moisture (M) | 2 | 1.538 | 0.769 | 8.679** |
| Species (S) | 1 | 16.220 | 16.220 | 183.070*** |
| N x M | 6 | 1.453 | 0.242 | 2.731* |
| N x S | 3 | 0.384 | 0.128 | 1.445ns |
| M x S | 2 | 3.712 | 1.856 | 20.948*** |
| N x M x S | 6 | 2.105 | 0.351 | 3.962** |
| Residual | 69 | 6.113 | 0.0886 | |
| Total | 92 | 50.451 | 0.548 | |

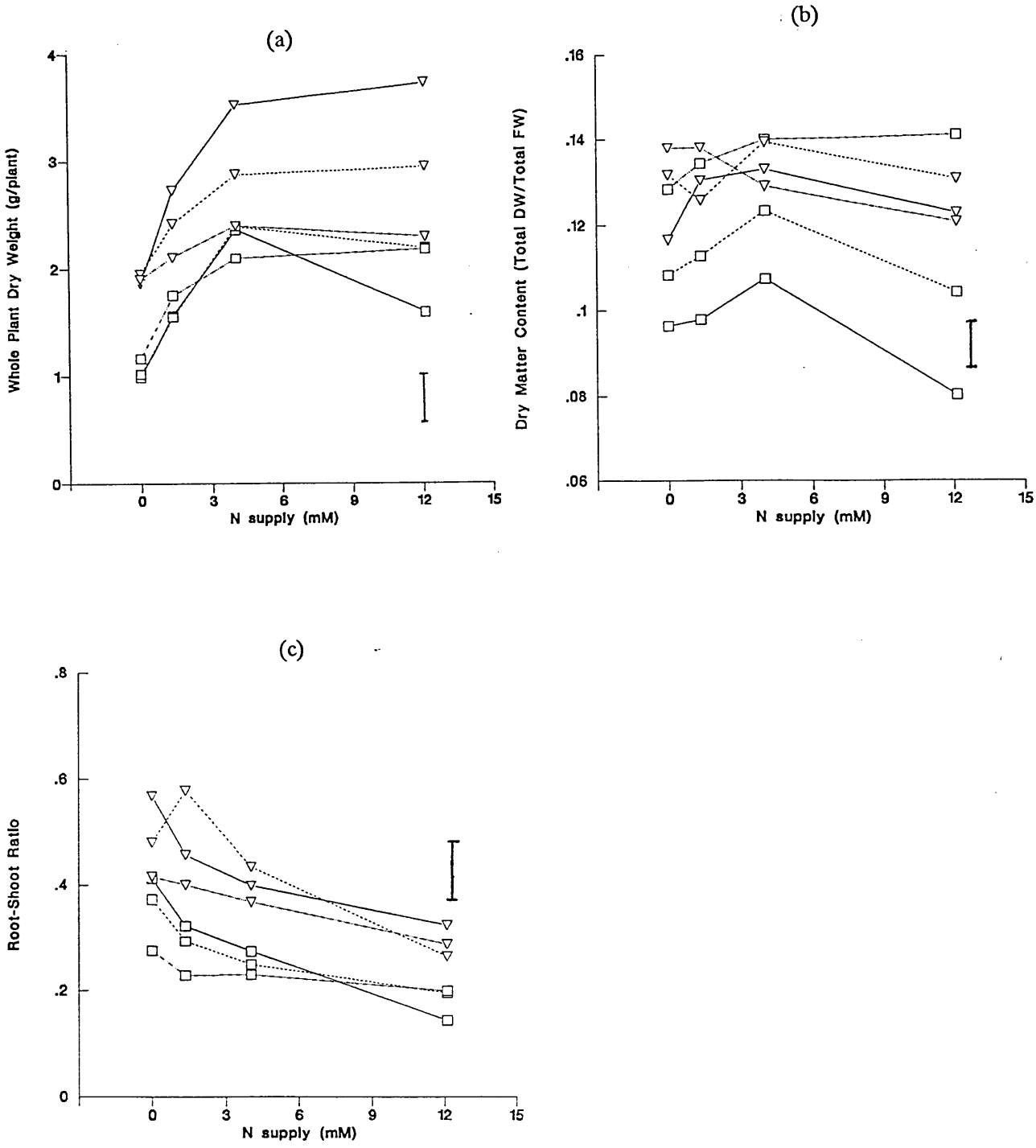



**Figure 3.1** Effects of N level on: a) Total dry weight (g/plant) b) Dry matter ratio (Total DW/Total FW) c) Root/shoot ratio, in Panicum (□) and Avena (▽), grown at different soil moisture regimes. Vertical bars represent LSD's at the 5% level.

(———) Field Capacity (FC)

(- - - - - -) 3/4 FC

(—·—·—) 1/2 FC

DW = Dry weight, FW = Fresh weight

Nutrient concentration (shoot)

The concentrations of N, K, Mg and Ca in the shoot dry weights of the two species are illustrated in Fig 3.2a to 3.2d with their anova's in Table 3.3.

The patterns of treatment effects were quite similar for all nutrients. Analysis of variance, with variance ratios calculated using the residual mean squares as denominator showed highly significant effects of N supply and species for all nutrients, and of water stress only for Mg and Ca levels. All nutrients showed significant interactions between water stress and species, however, which complicated interpretation of the main effects. Examination of Figures 3.2a to 3.2d confirms that the two species reacted to water stress in opposite ways with respect to nutrient uptake.

The shoot N concentration of both species increased with increasing N supply as might be expected (Fig 3.2a) and the interactions of N with water stress and species (Table 3.3) were not significant. As noted above, however, the interaction between water stress and species was significant, due to a consistent increase in N content with increased water stress in Avena compared with an opposite although very small response in Panicum.

Unlike N, the concentration of K of both species decreased with increasing N supply. Panicum, at the W1 moisture level showed the lowest concentration of K (Fig 3.2b). In contrast, unstressed Avena had its lowest concentration of K with full strength N, whilst, the W2 stressed plants maintained higher concentrations of K at all N levels. This again confirmed the interaction detected by the analysis of variance.

The concentration of Mg in plants of both species showed similar responses to N supply (Fig 3.2c). Panicum had a higher tissue Mg than Avena at all N levels and the difference was highly significant (Table 3.3). Avena showed no differences due to water stress but Panicum showed consistently lower uptake in the W2 stressed treatment, so producing the significant W x S interaction.

Both species showed differences in their Ca concentrations. The response of Ca content to N supply was different from the other nutrients such that there was little change or even a decline from N0 to N2 followed by a marked increase to N3. Panicum showed consistently lower concentrations as water stress increased, but Avena showed no consistent changes (Fig 3.2d).

Table 3.3 Analysis of variance of the effects of nitrogen levels on nutrient concentration (mMol/g shoot DW) of Panicum and Avena under varying soil moisture conditions experiment

| Source of Variations | df | Shoot Nutrient Concentration | | | |
|---|---|---|---|---|---|
| | | N | K | Ca | Mg |
| | | Mean Square (MS) | | | |
| Nitrogen (N) | 3 | 33.689 | 0.214 | 0.00949 | 102.692 |
| Moisture (M) | 2 | 0.0321 | 0.0126 | 0.00362 | 6.925 |
| Species (S) | 1 | 0.372 | 0.131 | 0.00641 | 162.751 |
| N x M | 6 | 0.0400 | 0.0167 | 0.000296 | 0.821 |
| N x S | 3 | 0.0722 | 0.00650 | 0.000212 | 3.852 |
| M x S | 2 | 0.282 | 0.0719 | 0.00284 | 4.351 |
| N x M x S | 6 | 0.0218 | 0.00949 | 0.000225 | 0.175 |
| Residual | 69 | 0.0378 | 0.00455 | 0.000138 | 0.546 |
| Total | 92 | 1.144 | 0.0156 | 0.000664 | 5.963 |
| | | Variance Ratio (VR) | | | |
| Nitrogen (N) | 3 | 890.566*** | 47.034*** | 68.973*** | 187.928*** |
| Moisture (M) | 2 | 0.848ns | 2.781ns | 26.318*** | 12.674*** |
| Species (S) | 1 | 9.839* | 28.722*** | 46.543*** | 297.838*** |
| N X M | 6 | 1.058ns | 3.672** | 2.151ns | 1.503ns |
| N x S | 3 | 1.909ns | 1.429ns | 1.538ns | 7.050*** |
| M x S | 2 | 7.457* | 15.822*** | 20.637*** | 7.963*** |
| N x M x S | 6 | 0.575ns | 2.087ns | 1.638ns | 0.322ns |

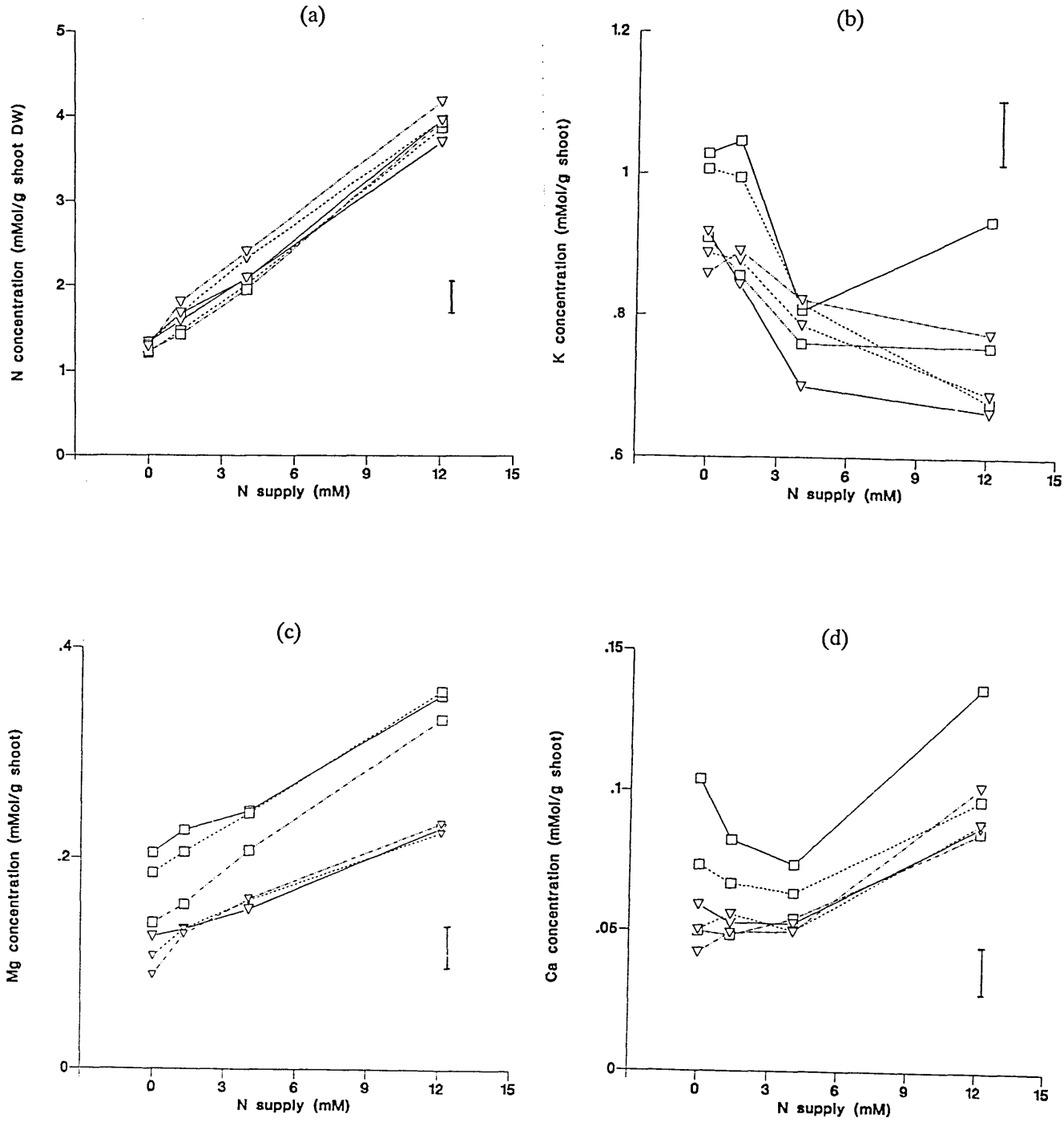



**Figure 3.2** Effects of N level on the concentration of: a) N b) K c) Mg d) Ca (mMol/g DW), in shoot of Panicum ( □ ) and Avena ( ▽ ), grown at different soil moisture regimes. Vertical bars represent LSD's at the 5% level.

(———) Field Capacity (FC)

(- - - - - -) 3/4 FC

(—·····—) 1/2 FC

Total Nutrient Uptake (shoot)

The total shoot N uptake in Panicum and Avena increased with increasing N supply. Water stress did not significantly affect N uptake in Panicum although the unstressed plants accumulated the lowest amount (Fig 3.3a). On the other hand, the unstressed plants of Avena had significantly the highest N uptake, whilst W2 stressed plants accumulated the lowest at N3.

Unlike their responses to N, the two species differed greatly in their patterns of K uptake. Apart from the low total uptake at N0 and widely scattered points at N2, Panicum showed little reponse to N supply or to water stress (Fig 3.3b). The shoot of Avena, on the other hand showed quite large and consistent effects of both N supply and water stress treatments. In general, water stress had no significant effect on the uptake of K in Panicum but caused large differences in Avena, particularly at N3 (see also the anova's in Table 3.4).

The uptake of Mg in Panicum also showed no effect of water stress and increased with increasing N supply (Fig 3.3c). The Mg uptake in Avena, followed a similar pattern to K, in which the effect of soil water stress significantly lowered the shoot K at all N levels.

The total shoot Ca in both species increased with increasing N supply and decreased with increased water stress. Consistently, the unstressed treatment of both species showed the highest accumulation of Ca (Fig 3.3d).

Efficiency of Nutrient Uptake of Roots

The efficiency of uptake for N, K, Ca and Mg by the roots in both species increased with increasing N levels (Fig 3.4a to 3.4d). Generally the efficiencies of Panicum showed little change due to water stress treatments although at N3 the unstressed consistently gave the highest values and W2 the lowest. The roots of the Avena on the other hand, generally increased in efficiency with increased water stress at all levels of N although the interaction of water stress with species was not significant except for Ca and Mg (Table 3.5). The anova table of N, K, Ca and Mg showed that the interactions of N x M x S were significant, thus confirming that the species responded differently to changed combinations of N and water stress.

Table 3.4 Analysis of variance of the effects of nitrogen levels on total nutrient uptake (mMol/plant) of Panicum and Avena under varying soil moisture conditions experiment

| Source of Variations | df | Total Nutrient Uptake N | K | Ca | Mg |
|---|---|---|---|---|---|
| | | Mean Square (MS) | | | |
| Nitrogen (N) | 3 | 193.145 | 1.529 | 0.0758 | 803.891 |
| Moisture (M) | 2 | 1.0454 | 0.141 | 0.0100 | 30.603 |
| Species (S) | 1 | 38.822 | 1.665 | 0.00291 | 50.694 |
| N x M | 6 | 0.630 | 0.0617 | 0.000416 | 3.285 |
| N x S | 3 | 4.160 | 0.0677 | 0.00294 | 9.609 |
| M x S | 2 | 3.842 | 0.111 | 0.0000284 | 21.608 |
| N x M x S | 6 | 2.396 | 0.0351 | 0.000244 | 15.289 |
| Residual | 69 | 0.478 | 0.0218 | 0.000360 | 3.981 |
| Total | 92 | 7.518 | 0.0983 | 0.00313 | 32.410 |
| | | Variance Ratio (VR) | | | |
| Nitrogen (N) | 3 | 403.773*** | 70.240*** | 210.653*** | 201.948** |
| Moisture (M) | 2 | 2.186ns | 6.495** | 27.765*** | 7.688** |
| Species (S) | 1 | 81.158*** | 76.500*** | 8.098** | 12.735*** |
| N x M | 6 | 1.316ns | 2.832* | 1.155ns | 0.825ns |
| N x S | 3 | 8.697*** | 3.110ns | 8.157*** | 2.414ns |
| M x S | 2 | 8.031*** | 5.119** | 0.079ns | 5.428** |
| N x M x S | 6 | 5.009*** | 1.612ns | 0.679ns | 3.841** |

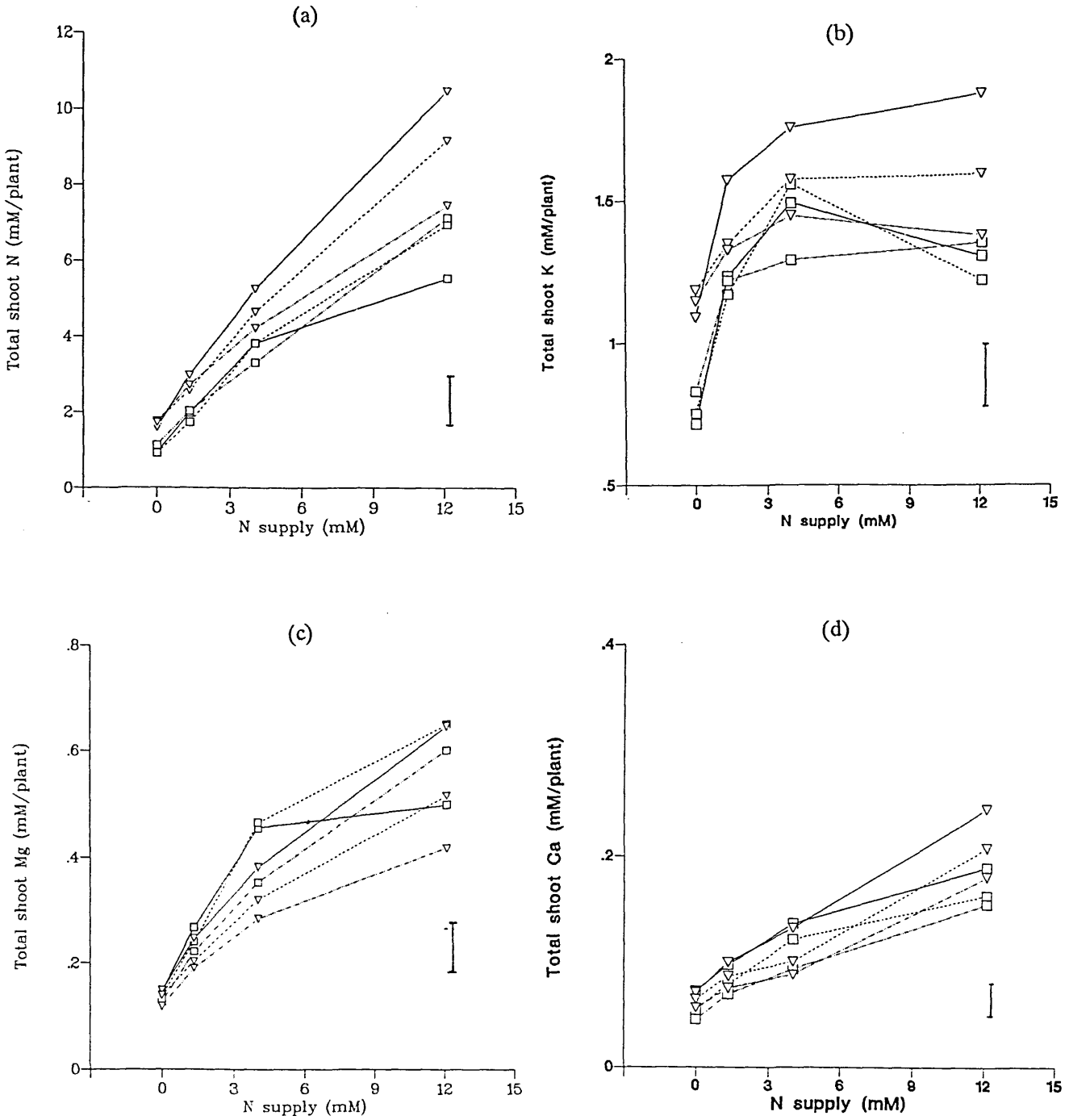



**Figure 3.3** Effects of N level on the total shoot: a) N b) K c) Mg d) Ca (mMol/g plant), in Panicum (□) and Avena (▽) grown at different soil moisture regimes. Vertical bars represent LSD's at the 5% level.

(———) Field Capacity (FC)

(- - - - -) 3/4 FC

(—·—·—) 1/2 FC

Table 3.5 Analysis of variance of the effects of nitrogen levels on efficiency of nutrient uptake (mMol/g root DW) of Panicum and Avena under varying soil moisture conditions experiment

| Source of Variations | df | Efficiency of Nutrient Uptake of Root | | | |
|---|---|---|---|---|---|
| | | N | K | Ca | Mg |
| | | Mean Square (MS) | | | |
| Nitrogen (N) | 3 | 1226.921 | 7.065 | 0.645 | 5627.109 |
| Moisture (M) | 2 | 2.627 | 0.669 | 0.0580 | 43.970 |
| Species (S) | 1 | 366.969 | 63.156 | 0.783 | 10181.522 |
| N x M | 6 | 11.044 | 1.925 | 0.0343 | 79.421 |
| N x S | 3 | 103.691 | 1.947 | 0.0823 | 1214.806 |
| M x S | 2 | 23.448 | 1.642 | 0.0973 | 136.438 |
| N x M x S | 6 | 25.796 | 2.554 | 0.0606 | 126.778 |
| Residual | 69 | 9.294 | 0.543 | 0.0159 | 44.118 |
| Total | 92 | 57.310 | 1.730 | 0.0537 | 384.037 |
| | | Variance Ratio (VR) | | | |
| Nitrogen (N) | 3 | 132.019*** | 13.003** | 40.472*** | 127.546*** |
| Moisture (M) | 2 | 0.244ns | 1.232ns | 3.642* | 0.997ns |
| Species (S) | 1 | 39.487*** | 116.237*** | 49.138*** | 230.778*** |
| N x M | 6 | 1.188ns | 3.543** | 2.152ns | 1.800ns |
| N x S | 3 | 11.157*** | 3.583* | 5.165** | 27.535*** |
| M x S | 2 | 2.523ns | 3.023ns | 6.111** | 3.093* |
| N x M x S | 6 | 2.776* | 4.700*** | 3.807** | 2.806* |

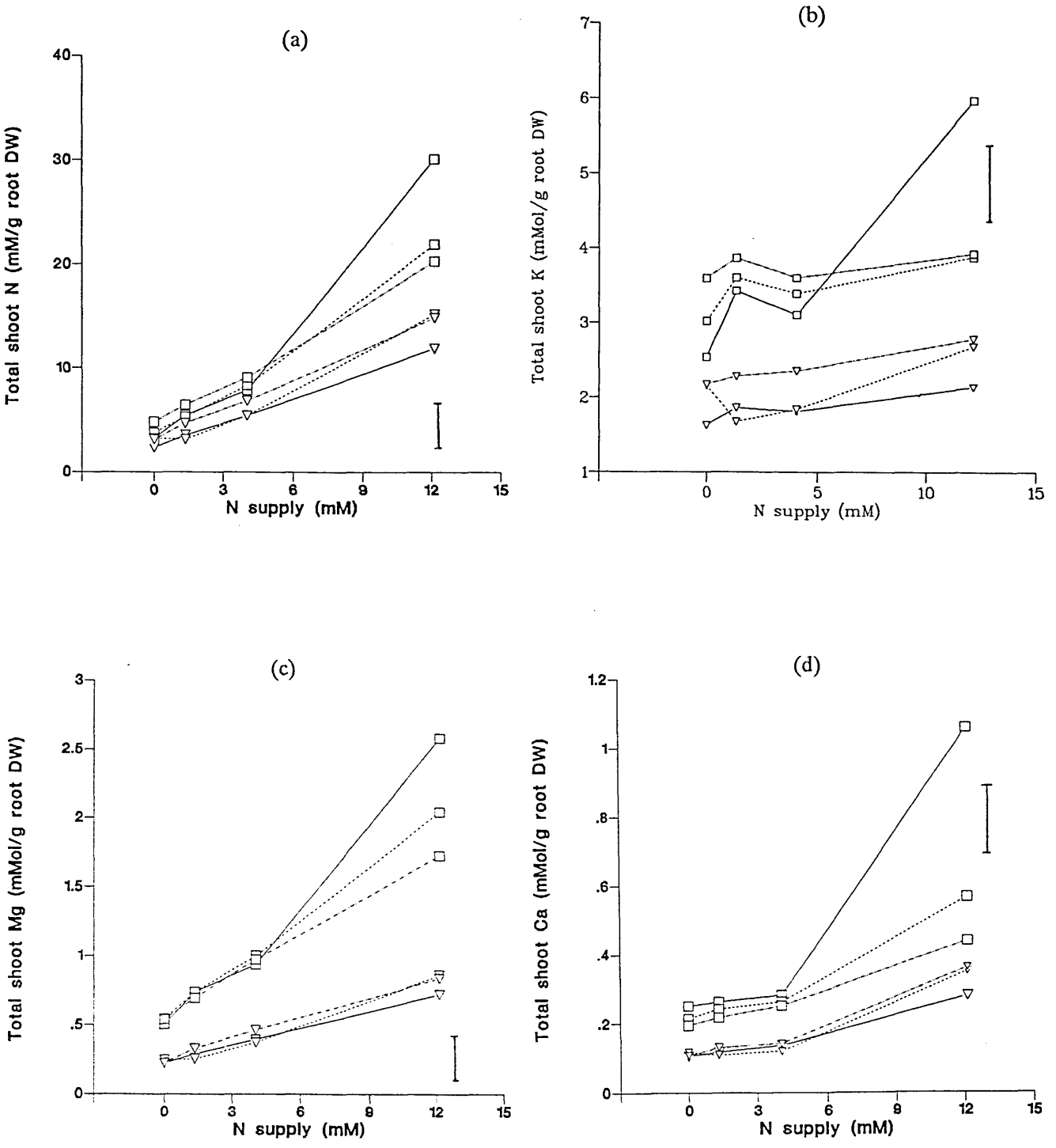



**Figure 3.4** Effects of N level on the efficiency of uptake (mMol/g root DW) of: a) N b) K c) Mg d) Ca, in Panicum (□) and Avena (▽) grown at different soil moisture regimes. Vertical bars represent LSD's at the 5% level.

(———) Field Capacity (FC)

(- - - - - -) 3/4 FC

(—·—·—) 1/2 FC

Nutrient Utilization Efficiency

N utilization efficiency in both species decreased with increased N supply so that the greatest efficiency was shown at the lowest N level. There was a slight, but significant species difference, with Panicum giving slightly higher values. Water stress showed no obvious changes, and none of the interactions were significant (Table 3.6, and Fig 3.5a).

Unlike N, K utilization efficiency differed significantly between the two species as shown by analysis of variance in Table 3.6. Values for Avena were larger than those for Panicum, particularly at the higher N levels. The efficiencies of both species increased with N supply, although the response of Panicum was rather irregular (Fig 3.5b). Both species were significantly affected by water treatments, but in opposite directions, so that the values for Panicum mostly increased with increasing water stress, whilst those for Avena were mostly highest in the controls and decreased with stress.

Both species showed a fall in Mg utilization efficiency with increasing N supply, although the values for Panicum were consistently smaller than those for Avena. At the lower N levels, both species showed significantly higher efficiencies at the lower N levels but the differences decreased or disappeared with increasing N supply. So that at the higher N levels there were no differences between the stressed and and unstressed treatments (Fig 3.5c, Table 3.6).

Differences in utilization efficiencies for Ca were greatest for N supply and species. The pattern of change with N level was complex in that Panicum rose to a maximum with increasing N and then declined, whilst Avena declined fairly consistenly from N0 to N3 (Fig 3.5d). Water stress caused quite large differences for Panicum, but not for Avena, so that all the main effects and first order interactions were significant (Table 3.6).

Commentary

Both species gave the highest root/shoot ratio as expected when N supply was limiting. This conforms with the usual adaptation to poor, infertile soils in which resources are switched from shoot to root growth. At all N levels, Avena maintained higher ratios than Panicum. The results also revealed that the stressed plants of the two species did not show higher root mass production compared with shoots, as might be expected. One explanation that should be kept in mind when studying plant responses to soil water stress is the distribution of roots in space.

Table 3.6 Analysis of variance of the effects of nitrogen levels on nutrient utilization efficiency (g DW/mMol) of Panicum and Avena under varying soil moisture conditions experiment

| Source of Variations | df | Nutrient Utilization Efficiency | | | |
|---|---|---|---|---|---|
| | | N | K | Ca | Mg |
| | | Mean Square (MS) | | | |
| Nitrogen (N) | 3 | 1.300 | 0.460 | 314.469 | 0.0743 |
| Moisture (M) | 2 | 0.00205 | 0.00958 | 152.754 | 0.00848 |
| Species (S) | 1 | 0.0324 | 0.211 | 299.865 | 0.147 |
| N x M | 6 | 0.00253 | 0.0415 | 21.454 | 0.00330 |
| N x S | 3 | 0.00589 | 0.00842 | 28.007 | 0.00612 |
| M x S | 2 | 0.0142 | 0.143 | 50.714 | 0.00111 |
| N x M x S | 6 | 0.00280 | 0.0270 | 2.179 | 0.000629 |
| Residual | 69 | 0.00521 | 0.00956 | 4.746 | 0.000561 |
| Total | 92 | 0.0475 | 0.0325 | 23.951 | 0.00512 |
| | | Variance Ratio (VR) | | | |
| Nitrogen (N) | 3 | 249.639*** | 48.080*** | 66.266*** | 132.465*** |
| Moisture (M) | 2 | 0.394ns | 1.001ns | 32.189*** | 15.768*** |
| Species (S) | 1 | 6.228* | 22.065*** | 63.189*** | 262.471*** |
| N x M | 6 | 0.485ns | 4.339** | 4.521*** | 5.878*** |
| N x S | 3 | 1.130ns | 0.880ns | 5.902** | 10.911*** |
| M x S | 2 | 2.730ns | 14.983*** | 10.687*** | 1.985ns |
| N x M x S | 6 | 0.537ns | 2.820* | 0.459ns | 1.121ns |

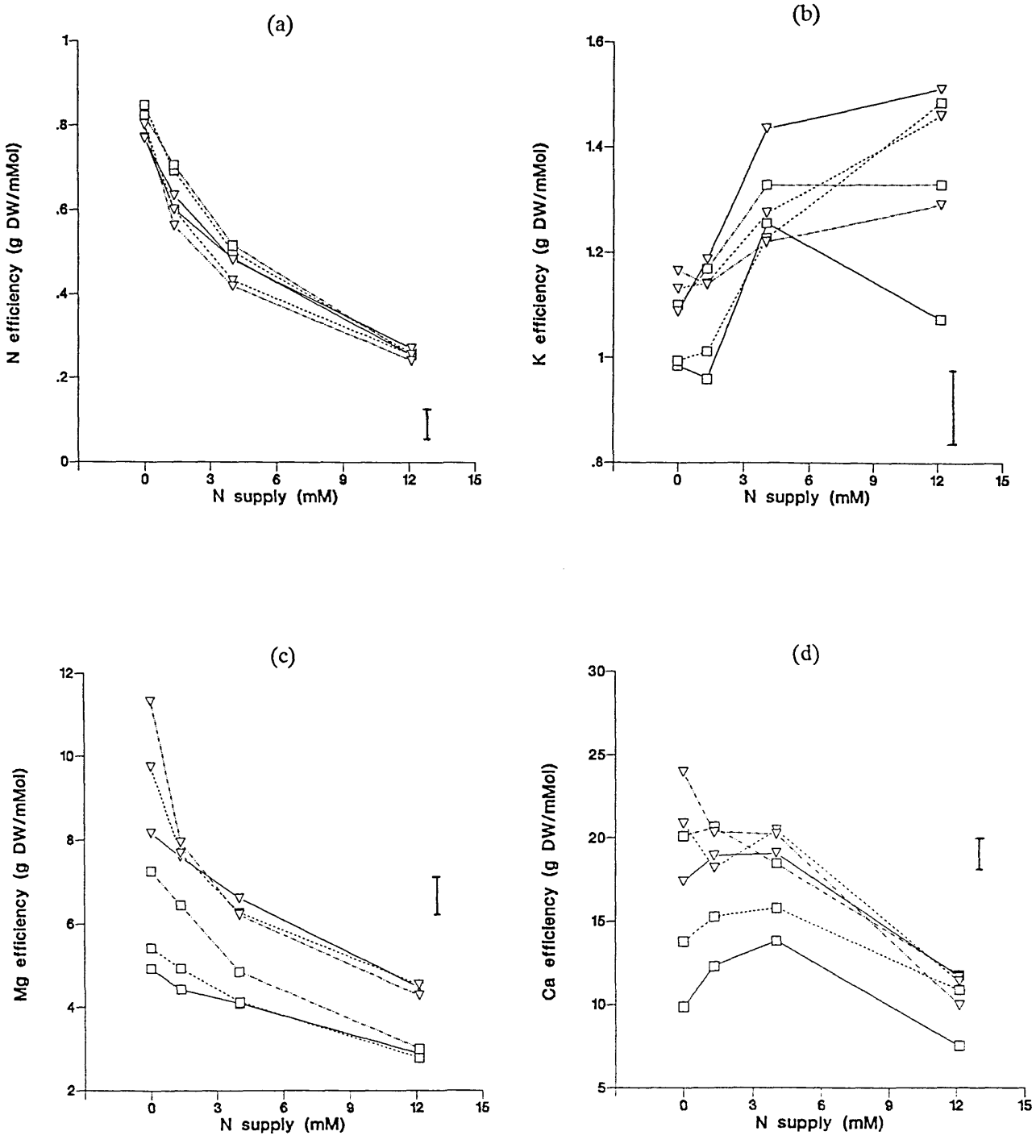



**Figure 3.5** Effects of N level on the utilization efficiency (g DW/ mMol) of: a) N b) K c) Mg d) Ca, in Panicum (□) and Avena (▽) grown at different soil moisture regimes. Vertical bars represent LSD's at the 5% level.

(———) Field Capacity (FC)

(- - - - - -) 3/4 FC

(—·—·—) 1/2 FC

When a small quantity of water is added to a mass of dry soil, it wets only the top portion. Root development is therefore restricted to this zone. The problem may be particularly severe in pot experiments.

**Experiment 3.2.2 The response of *Panicum miliaceum* (Panicum) and *Avena fatua* (Avena) to varied levels of potassium and soil water stress.**

*Materials and Methods*

Seeds of both species were sown on 15/5/88. Seedlings were transplanted after ten days into the standard soil and plants were harvested six weeks later (i.e. four weeks after treatment). The imposed treatments were three levels of K in Long Ashton nutrient solution and two levels of soil moisture:

(K1) 0.44 mM (17.3 ppm K)
(K2) 1.33 mM (52.0 ppm K)
(K3) 3.99 mM (156.0 ppm K or full strength Long Ashton solution)

(W0) FC (Field Capacity or unstressed)
(W2) 1/2FC

The different K concentrations were obtained by replacing some of the $KNO_3$ with equivalent concentrations of $NaNO_3$. A randomised complete block design with five replications was used.

*Results*

Root and shoot growth

The effects of the three levels of K and two soil moisture levels on the growth of both species are presented in Table 3.7 and Fig 3.6a to 3.6c. Panicum showed a much larger response than Avena, but this was mainly due to its much larger dry weight in the control water level. Both

species were greatly affected by the water stress treatment, although to significantly different extents.

Differences in K supply produced no significant responses, although there was a modest increase in dry weight in Panicum supplied with the higher water level (Fig 3.6a).

Averaged over all three K levels, water stress caused a large and significant increase in the root-shoot ratio of Avena, and a smaller, but still significant increase for Panicum. The different responses gave rise to a significant interaction between the two species (Table 3.7 and Fig 3.6b). The K treatments showed little effect on the root/shoot ratio of Panicum. The ratios for Avena, however, responded inconsistently in that the lowest value was observed in treatment K2 in unstressed plants, but the highest value was obtained in this treatment with stressed plants.

The dry matter contents of both species increased substantially in response to water stress (Table 3.7 and Fig 3.6c), with Avena showing a greater effect than Panicum. This caused a significant species x water stress interaction, even though the main effect of species was non-significant. Differences associated with K level were also non-significant, although the distribution of points in Fig 3.6c is almost a mirror-image of that in the previous graph.

Nutrient Concentrations (Shoot)

The concentration of N in the shoots of non-stressed Panicum was considerably lower than in the other treatments (Fig 3.7a). In both stressed and non-stressed condiditons, there were slight decreases with increasing K level. Stressed Avena, on the other hand, showed a gradual increase with increasing K supply, whilst the unstressed treatment gave a minimum with K2.

Shoot K concentration of both species increased with increasing K supply as might be expected although Avena showed a smaller response than Panicum to the highest K level (Fig 3.7b). The concentration also increased with water stress, although the increase was again smaller in Avena than in Panicum. At all K levels, Avena consistently showed the highest values and Panicum the lowest.

Unlike N, the concentration of Mg in non-stressed Panicum was much higher than other treatments (Fig 3.7c). Stressed Avena, on the other hand gave a higher concentration than the non-stressed treatment. In both stressed and non-stressed Panicum, the concentrations

Table 3.7 Effects of potassium supply on some growth variates of Panicum (PM) and Avena (WO) under different soil moisture conditions

| K Conc (K) Soil moisture levels (WS) | | Whole Plant dry wt (g/plt) | | Root/Shoot ratio (dry wt) | | Dry Matter Ratio (Total DW/Total FW) | |
|---|---|---|---|---|---|---|---|
| | | PM | WO | PM | WO | PM | WO |
| K(mM) | WS | | | | | | |
| 0.44 | FC | 4.996 | 1.800 | 0.1832 | 0.2588 | 0.1652 | 0.1472 |
| | 1/2FC | 0.940 | 0.388 | 0.2339 | 0.3584 | 0.1794 | 0.1982 |
| 1.33 | FC | 5.138 | 1.560 | 0.1763 | 0.1416 | 0.1646 | 0.1675 |
| | 1/2 | 0.880 | 0.332 | 0.2328 | 0.4749 | 0.1832 | 0.1861 |
| 3.99 | FC | 5.452 | 1.778 | 0.1763 | 0.2180 | 0.1630 | 0.1533 |
| | 1/2FC | 0.930 | 0.346 | 0.2270 | 0.4156 | 0.1861 | 0.2092 |
| Significance level | | | | | | | |
| K | | ns | | ns | | ns | |
| WS | | *** | | *** | | *** | |
| SP | | *** | | *** | | ns | |
| K x WS | | ns | | *** | | ns | |
| K x SP | | ns | | ns | | ns | |
| WS x SP | | ** | | *** | | ** | |
| K x WS x SP | | ns | | ** | | ns | |
| LSD (P< 0.05) | | | | | | | |
| K | | - | | - | | - | |
| WS | | 0.129 | | 0.0243 | | 0.00744 | |
| SP | | 0.129 | | 0.0243 | | - | |
| K x WS | | - | | 0.0422 | | - | |
| K x SP | | - | | 0.0422 | | - | |
| WS x SP | | 0.182 | | - | | 0.0105 | |
| K x WS x SP | | - | | 0.0596 | | - | |

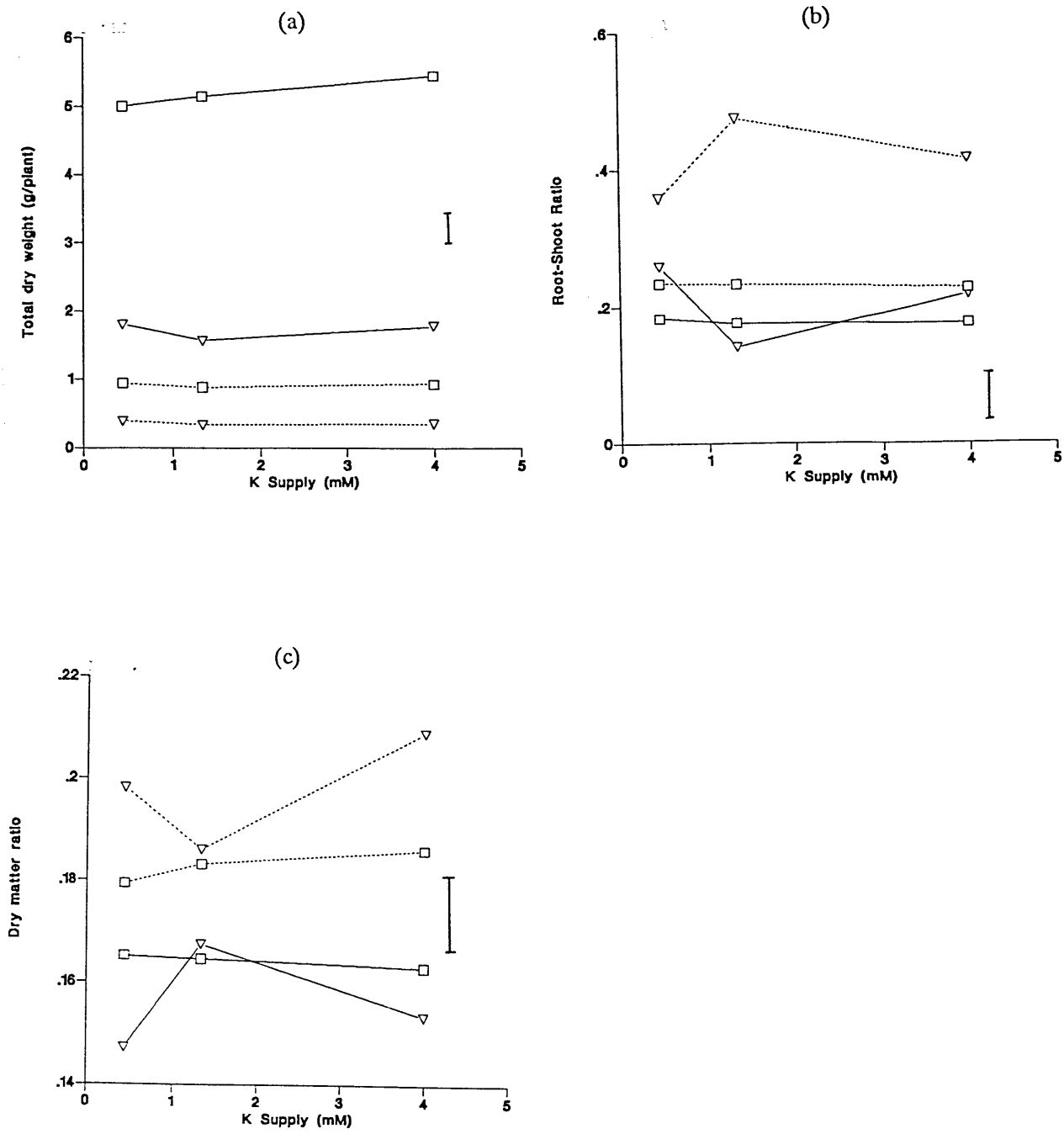



**Figure 3.6** Effects of K level on: a) Total dry weight (g/plant) b) Root/shoot ratio c) Dry matter ratio (Total DW/Total FW), in Panicum ( □ ) and Avena ( ▽ ), grown under well-watered and water-stressed conditions. Vertical bars represent LSD's at the 5% level.

(——— ) well-watered

(- - - - -) water-stressed

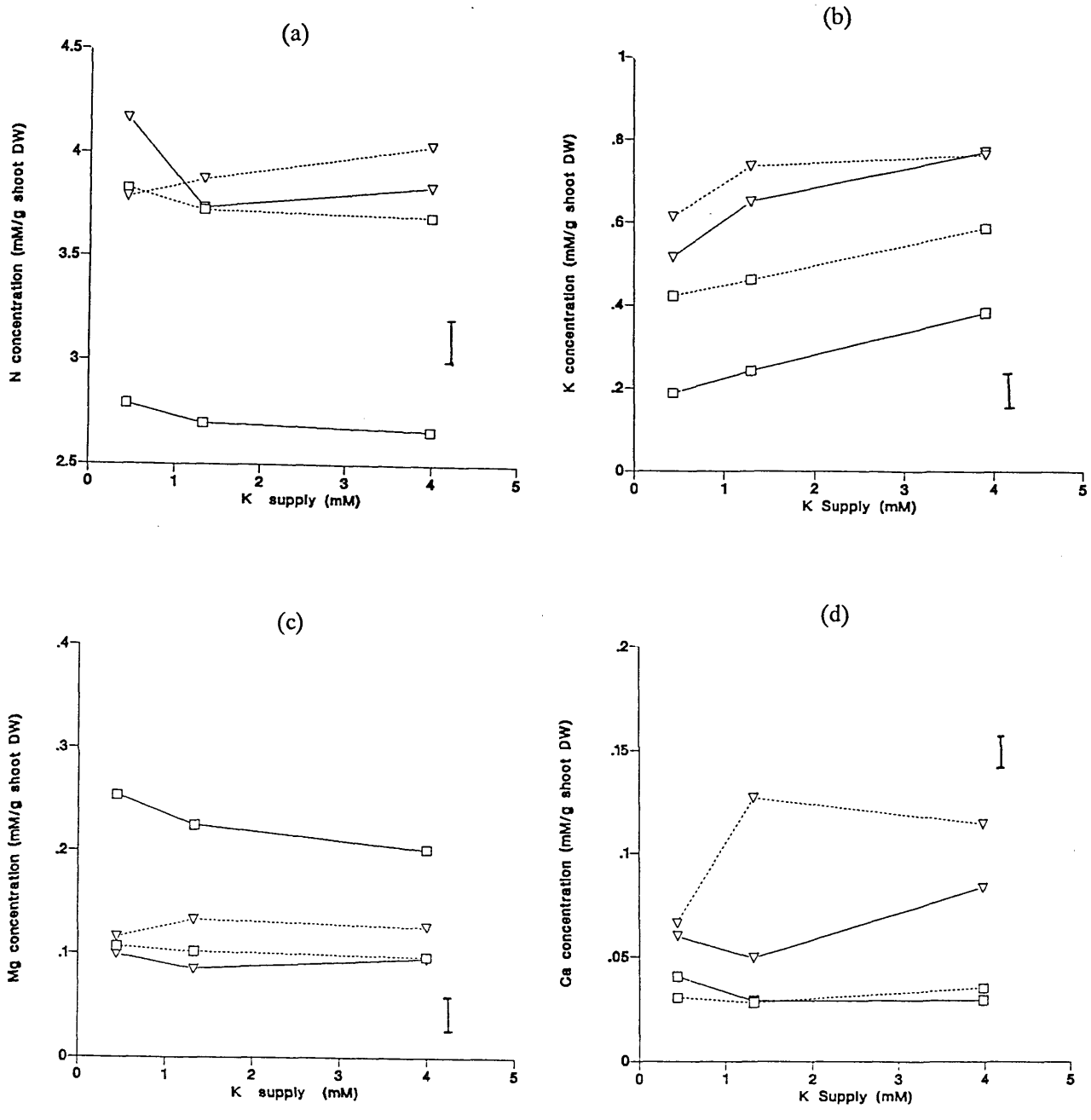



**Figure 3.7** Effects of K level on the concentration of: a) N b) K c) Mg d) Ca (mMol/g DW), in shoot of Panicum ( □ ) and Avena ( ▽ ), grown under well-watered and water-stressed conditions. Vertical bars represent LSD's at the 5% level.

(———) well-watered

(- - - - -) water-stressed

decreased with increasing K level although the stressed treatment showed a smaller response. Avena showed inconsistent responses in that the K2 treatment showed the lowest Mg concentration in unstressed plants but the highest in the stressed material.

The Ca concentration of shoots of stressed Avena was consistently higher than the non-stressed, whilst Panicum showed no effect (Fig 3.7d). Both non-stressed and stressed Avena, again showed inconsistent responses to K level, since the highest concentration was observed at K2 in the stressed treatment, but the lowest in the non-stressed. Panicum showed little change with K supply.

Total Nutrient Uptake (shoot)

The total N content of both species were greatly affected by water stress (Fig 3.8a), with Panicum showing a much larger response than Avena. Different K levels produced no significant responses, although there was a small increase for non-stressed Panicum toward the higher K level. Stressed Avena showed the lowest content of shoot N.

The uptake of K of both species increased with increasing K supply. Panicum showed a bigger response than Avena and obtained the highest content at K3. The uptake was decreased with water stress, with stressed Avena showed the lowest content (Fig 3.8b).

The shoot Mg of non-stressed Panicum was considerably higher than other treatments. In general, the Mg uptake in both species, followed a similar pattern to N, in which the effects of soil water stress lowered the shoot Mg at all K levels (Fig 3.8c).

The uptake of Ca in shoots of non-stressed Panicum was consistently higher than other treatments in response to water stress (Fig 3.8d). Whilst the stressed treatments of both species showed consistently lower uptake and again showed no obvious changes with different levels of K.

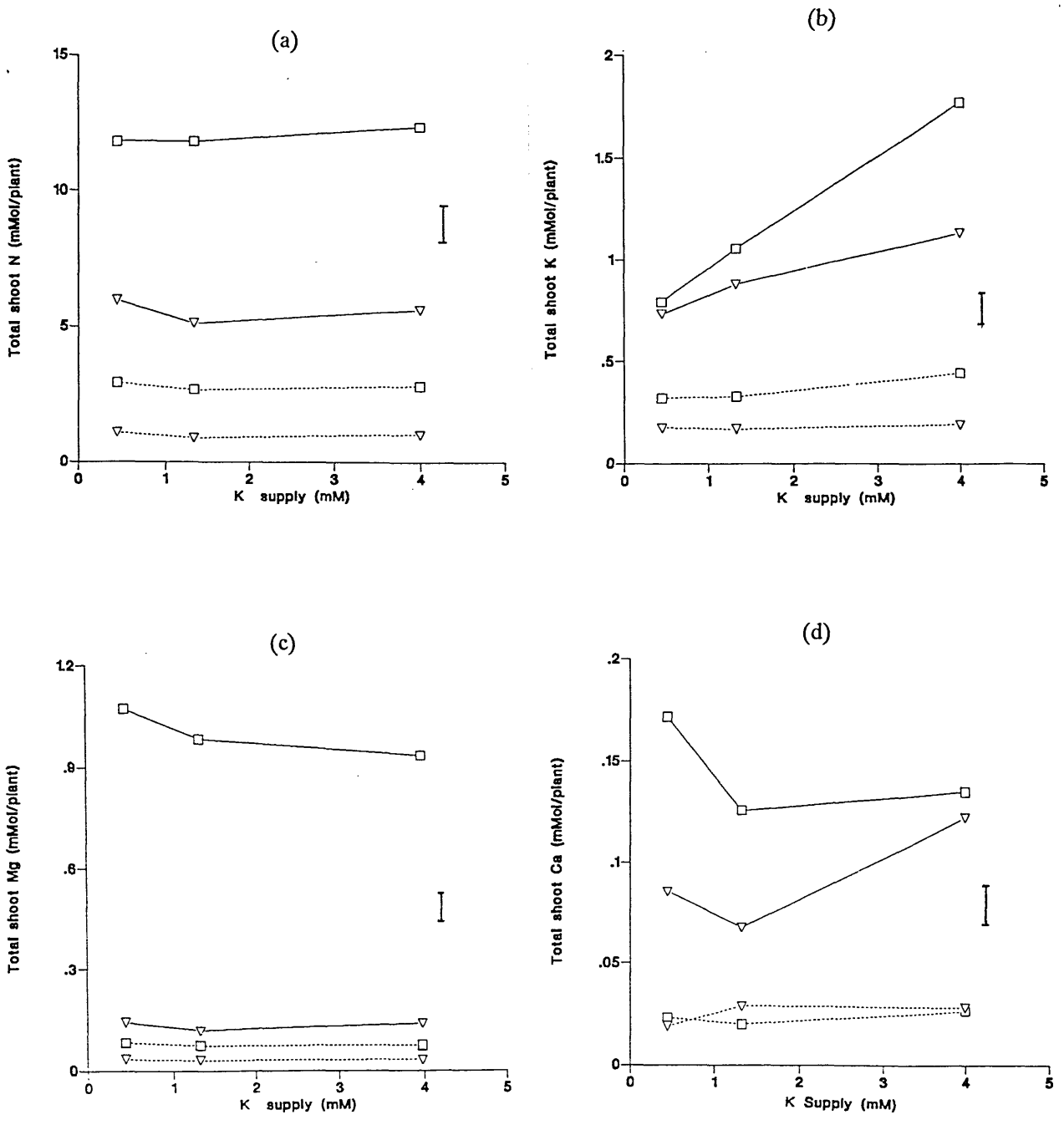



**Figure 3.8** Effects of K level on the total shoot: a) N b) K c) Mg d) Ca (mMol/g plant), in Panicum ( □ ) and Avena ( ▽ ), grown under well-watered and water-stressed conditions. Vertical bars represent LSD's at the 5% level.

(———) well-watered

(------) water-stressed

Efficiency of Nutrient Uptake of Roots

The efficiency of N uptake by Avena roots decreased markedly with water stress (Fig 3.9a), whilst the roots of Panicum increased, although the effect was not significant. There was a large, but non-consistent response of Avena to the different K levels, whilst Panicum showed no obvious changes.

Both species generally increased their K uptake efficiency with increasing K supply although non-stressed Avena showed a decline from K2 to K3 (Fig 3.9b). Panicum showed small and non-consistent changes in response to water stress whereas Avena showed large decreases in efficiency.

The Mg uptake by the roots of non-stressed Panicum was considerably higher than any of the other treatments (Fig 3.9c). In both stressed and non-stressed conditons, generally there were slight decreases in efficiency with increasing K levels except in non-stressed Avena.

The Ca uptake in both stressed and non-stressed treatments of Avena consistently showed much higher efficiencies than Panicum, and the difference increased with increasing K supply (Fig 3.9d). Values in both species decreased with increasing water stress.

Nutrient Utilization Efficiency

N utilization efficiency of non-stressed Panicum was considerably higher than other treatments (Fig 3.10a). In both stressed and non-stressed conditions, Panicum showed slight increases with increasing K level. Avena, on the other hand, showed gradual, but not consistent decreases, since the non-stressed treatment gave its lowest efficiency at K1.

As expected, K utilization efficiency of both species decreased with increased K supply, so that the greatest efficiency was shown at the lowest K level (Fig 3.10b). Non-stressed Panicum showed consistently higher values than other treatments, whilst Avena showed no significant changes.

Unlike N and K, Mg utilization efficiencies of the two species responded in opposite ways to water stress, such that non-stressed treatment of Avena gave greater values than the stressed, and vice versa for Panicum (Fig 3.10c). The efficiencies for Panicum, gradually increased with K supply but Avena showed no consistent responses.

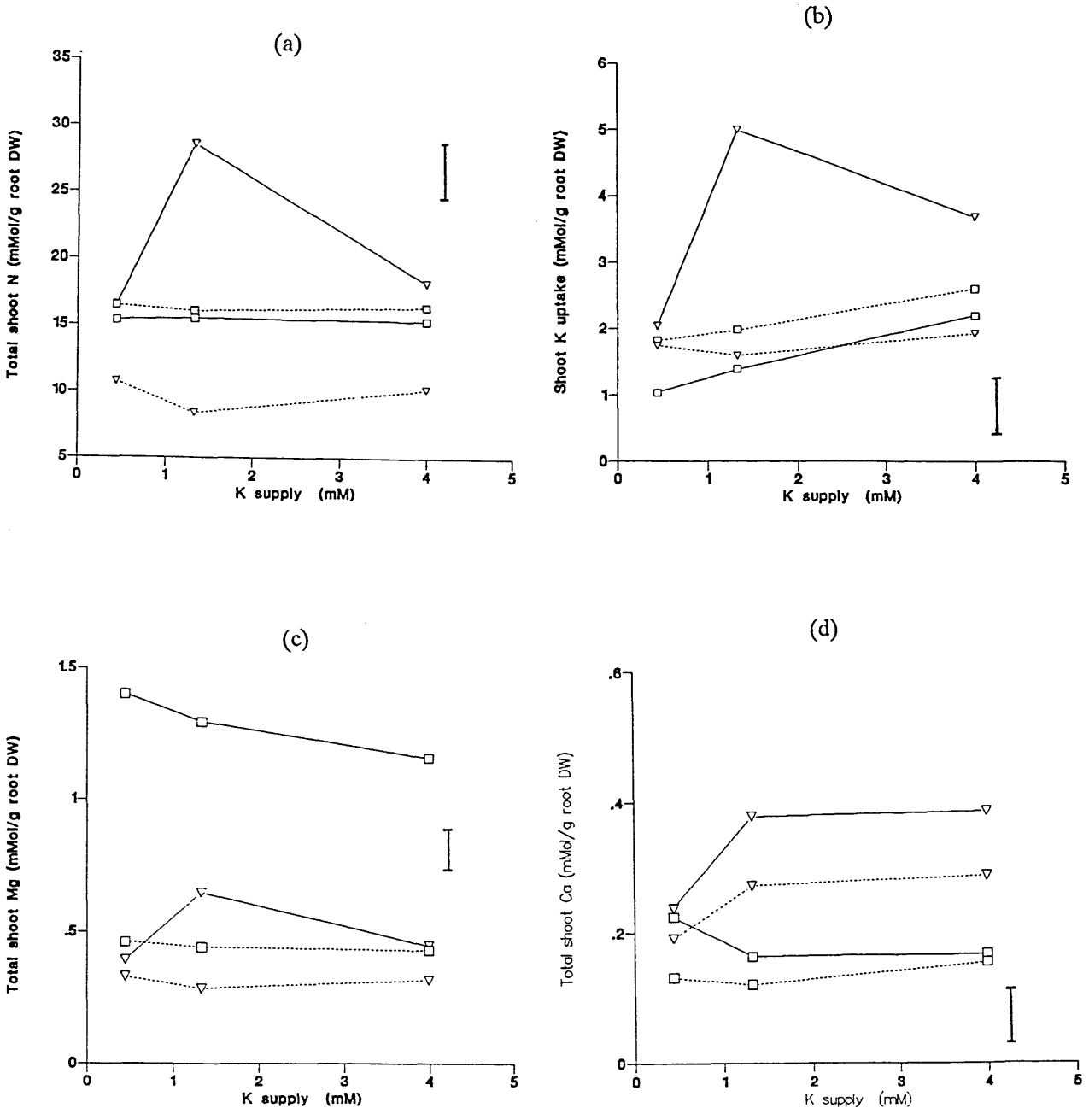



**Figure 3.9** Effects of K level on the efficiency of uptake (mMol/g root DW) of: a) N b) K c) Mg d) Ca, in Panicum ( □ ) and Avena ( ▽ ), grown under well-watered and water-stressed conditions. Vertical bars represent LSD's at the 5% level.

(———) well-watered

(- - - - -) water-stressed

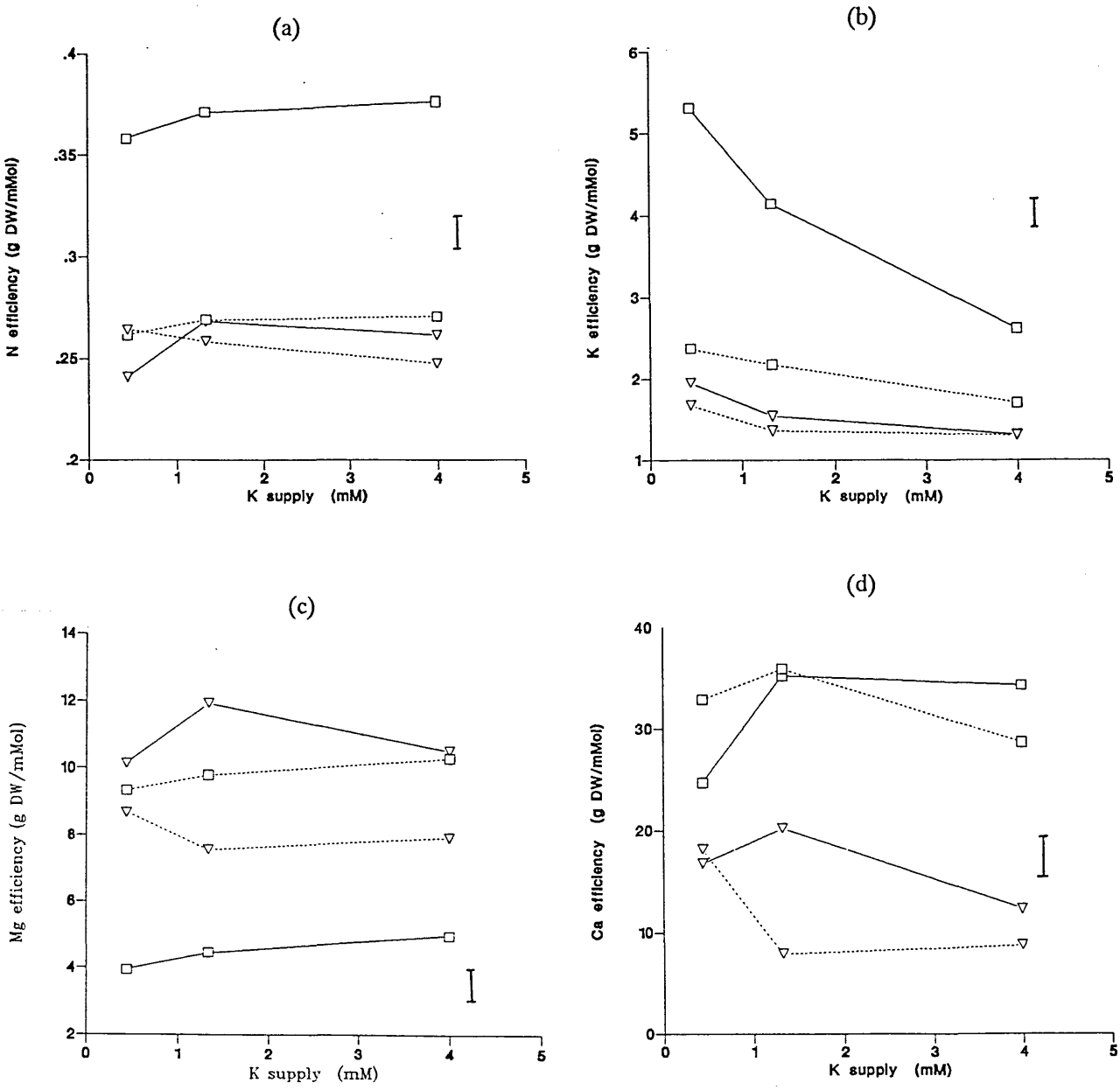



**Figure 3.10** Effects of K level on the utilization efficiency (g DW/ mMol) of: a) N b) K c) Mg d) Ca, in Panicum ( □ ) and Avena ( ▽ ), grown under well-watered and water-stressed conditions. Vertical bars represent LSD's at the 5% level.

(——————) well-watered

(- - - - - -) water-stressed

Ca utilization efficiencies for Panicum in both stressed and non-stressed conditions were consistently much higher than for Avena (Fig 3.10d). Avena, on the other, showed inconsistent responses to K supply, so that the stressed treatment gave its lowest value at K2.

Commentary

In this experiment, both species showed increases in root-shoot ratio with increased water stress as might be expected. However, in experiment 3.2.1, there were inconsistent responses amongst the two species, with stressed Panicum giving much lower ratios than non-stressed treatments particularly at lower N supplies. The most likely explanation for these conflicting results is that there were differences in the average soil water stresses experienced by plants of different absolute sizes or different rates of water loss. Similarly, different rates of water use would have given rise to differences in the total nutrient availability.

The stressed treatments in this experiment, also showed increases in concentrations of shoot N and K of both species together with that of Ca in Avena. This could be explained by water stress effects on ion uptake in which reduced soil water leads to increases in local concentrations of these ions. The low water potentials in the soil would require the plants to have low osmotic potentials in cells as well, so that turgor may be maintained and $CO_2$ fixation continued. Unless adequate osmotica were present, the stomata might close and growth be reduced. Such increases in concentration of these ions in plants tissue are known to play significant roles in osmotic adjustment to water stress in many species, although the mechanisms involved are still not fully understood. The ability to withstand such local increases in ion concentrations might be part of a plant's adaptation to water stress, and might explain the differences observed between species.

**Experiment 3.2.3 The response of *Aristida funiculata* (Aristida) and *Panicum miliaceum* (Panicum) to varied levels of potassium and soil water stress.**

*Materials and Methods*

Aristida seeds were sown on 16/4/87 and Panicum on 26/4/87. Seedlings of Panicum and Aristida were transplanted after 10 and 20 days respectively and plants were harvested six weeks later (i.e. four weeks after treatments). A randomised complete block design with three replication was used. Plants were treated with four levels of K and two levels of soil moisture:

(K0) 0 mM (0 ppm K)
(K1) 0.44 mM (17.3 ppm K)
(K2) 1.33 mM (52.0 ppm K)
(K3) 3.99 mM (156.0 ppm K or full strength Long Ashton solution)
(W0) FC (Field Capacity or unstressed)
(W2) 1/2FC

*Results*

Root and shoot growth

In this experiment, the total dry weight of Panicum increased with increasing K supply up to the highest level (Fig 3.11a), with greater increases in the unstressed than in the water stressed treatments. Aristida, on the other hand, was insensitive to both K level and water stress. This difference between the species caused a significant K x SP interaction (Table 3.8). Panicum had higher total dry weights than Aristida at all K levels and the difference was highly significant.

Water stress caused large differences in the root-shoot ratios of Panicum, whilst Aristida showed effectively no change (Fig 3.11b). Increasing K supply also showed no consistent effects on Aristida, but generally caused a decrease in the ratios of Panicum. This confirmed the significant main effects of species and water stress and their interaction. Aristida maintained the highest ratios and stressed Panicum the lowest.

The dry matter contents of both species were significantly increased by water stress, but showed little effects due to K supply (Fig 3.11c). Aristida consistently maintained higher values than Panicum.

Nurient Concentrations (Shoot)

The shoot N concentrations of the two species responded differently to changes in K supply (Fig 3.12a). Panicum showed decreases in N concentrations as the K supply incresed, except for the stressed treatment at K3. Avena, on the other hand, showed slight increases in both water treatments, with the unstressed plants having consistently higher values.

K concentration in the tissue of both species increased with increasing K supply (Fig 3.12b). The concentration of K in Aristida also increased with water stress, but not significantly. Stressed Aristida consistently showed the highest concentrations at all K levels.

The Mg concentrations in shoots of non-stressed Panicum were consistently higher than in other treatments (Fig 3.12c). Both species gave large decreases under water stress, with stressed Aristida showing the lowest concentrations. Differences in K level caused decreases in Mg concentration, although the effect was small except for non-stressed Panicum.

The shoot Ca in both species, generally followed similar patterns to Mg, in that concentrations decreased with both increasing K supply and water stress (Fig. 3.12d).

Total Nutrient Uptake (Shoot)

The shoot N of Panicum was considerably higher than that of Aristida in all treatments (Fig 3.13a). The stressed treatments of both species showed a lower values, with Panicum showing a larger response than Aristida. Increasing K levels gave inconsistent responses in Panicum, but small increases in stressed Aristida.

The uptake of K of both species increased with increasing K supply, with Panicum showing a bigger response than Aristida (Fig. 3.13b). Uptake decreased slightly with water stress in Panicum at the higher K levels, but increased with water stress in Aristida.

Table 3.8 Effects of potassium supply on some growth variates of Aristida (AR) and Panicum (PM) under soil water stress

| K Conc (K) Soil moisture levels (WS) | | Whole Plant dry wt (g/plt) | | Root/Shoot ratio (dry wt) | | Dry Matter Ratio (Total DW/Total FW) | |
|---|---|---|---|---|---|---|---|
| | | PM | AR | PM | AR | PM | AR |
| K(mM) | WS | | | | | | |
| 0 | FC | 2.987 | 1.907 | 0.2628 | 0.3179 | 0.1226 | 0.1939 |
| | 1/2FC | 3.427 | 1.893 | 0.2419 | 0.3418 | 0.1911 | 0.2982 |
| 0.44 | FC | 4.360 | 1.940 | 0.2897 | 0.3156 | 0.1339 | 0.1933 |
| | 1/2FC | 3.873 | 1.907 | 0.2172 | 0.3119 | 0.2068 | 0.2817 |
| 1.33 | FC | 4.687 | 1.980 | 0.2806 | 0.3775 | 0.1406 | 0.1797 |
| | 1/2FC | 4.067 | 1.913 | 0.1995 | 0.3764 | 0.2292 | 0.2704 |
| 3.99 | FC | 6.107 | 1.867 | 0.2430 | 0.3362 | 0.1532 | 0.1930 |
| | 1/2FC | 4.593 | 1.940 | 0.1844 | 0.3170 | 0.2179 | 0.2541 |
| Significance level | | | | | | | |
| | K | *** | | ns | | ns | |
| | WS | ns | | ** | | *** | |
| | SP | *** | | *** | | *** | |
| K x WS | | ns | | ns | | ns | |
| K x SP | | *** | | ns | | *** | |
| WS x SP | | ns | | ** | | ns | |
| K x WS x SP | | ns | | ns | | ns | |
| LSD (P< 0.05) | | | | | | | |
| | K | 0.401 | | - | | - | |
| | WS | - | | 0.0213 | | 0.00841 | |
| | SP | 0.284 | | 0.0213 | | 0.00841 | |
| K x WS | | - | | - | | - | |
| K x SP | | 0.567 | | - | | 0.0168 | |
| WS x SP | | - | | 0.0301 | | - | |
| K x WS x SP | | - | | - | | - | |

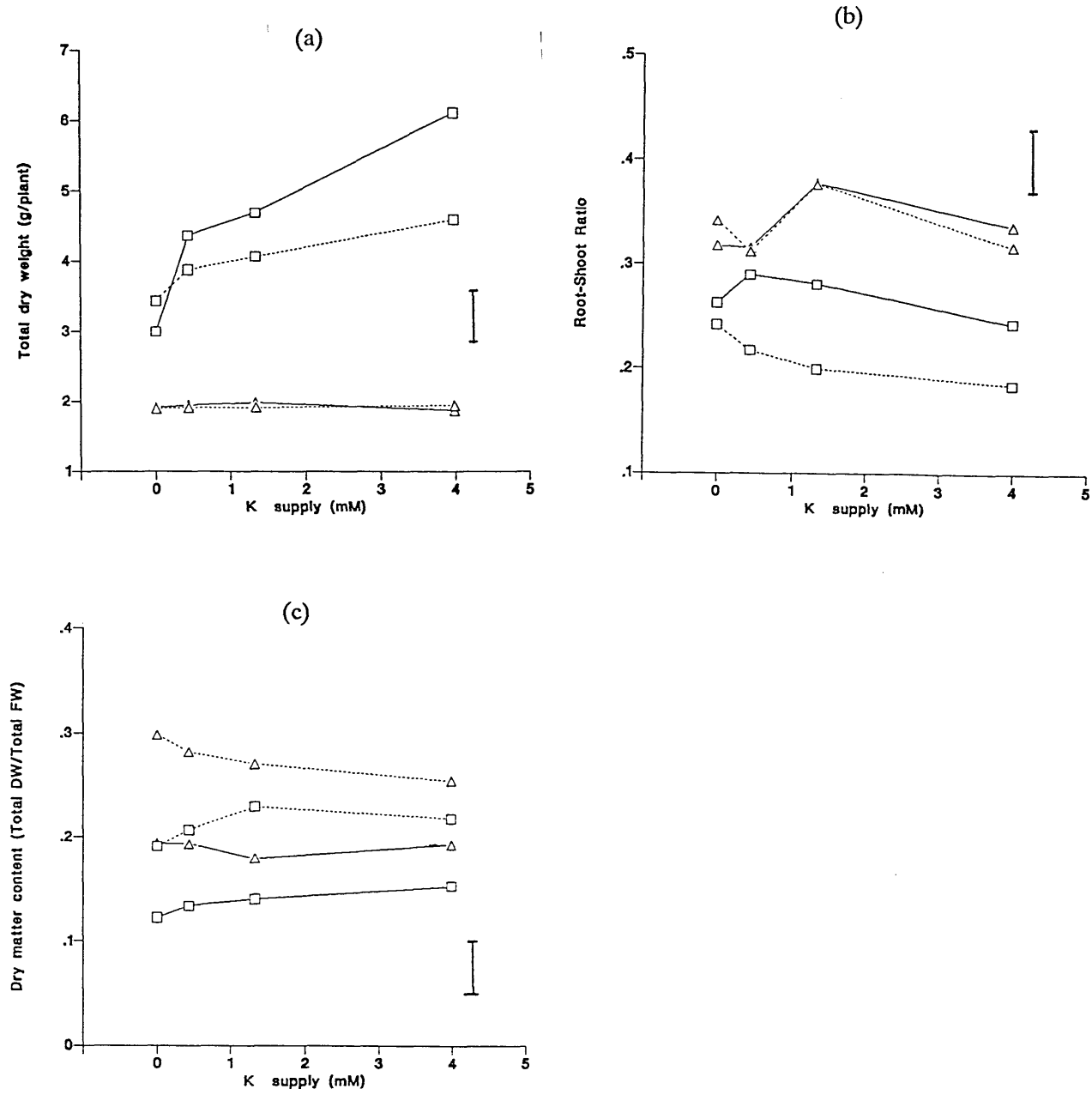



**Figure 3.11** Effects of K level on: a) Total dry weight (g/plant) b) Root/shoot ratio c) Dry matter ratio (Total DW/Total FW), in Panicum ( □ ) and Aristida ( △ ), grown under well-watered and water-stressed conditions. Vertical bars represent LSD's at the 5% level.

(———) well-watered

(------) water-stressed

The incorporation of Mg into shoots of Panicum was consistently higher than into Aristida (Fig 3.13c). Stressed treatments of both species, showed consistently lower values than the non-stressed treatments, and there were no obvious changes with different levels of K.

Ca uptake followed a similar pattern to Mg, in that Panicum possessed much larger quantities than Aristida, and water stress decreased the amounts at all K levels (Fig 3.13d).

Efficiency of Nutrient Uptake of Roots

The efficiency of N uptake by Panicum roots was significantly higher with water stress, due to the large difference at the highest K level (Fig 3.14a), whilst the roots of Aristida showed no obvious changes. In Panicum, there were decreases in efficiency with increasing K levels except for the stressed treatment which showed a marked increase from K2 to K3. Aristida, on the other hand, showed inconsistent responses.

The K uptake by the roots of all treatments increased with increasing K supply (Fig 3.14b). The effect was greatest with stressed Panicum, with no significant differences between the other treatments.

The Mg uptake of Panicum consistently showed much higher efficiencies than Aristida (Fig 3.14c), and values in both species decreased with water stress. In both stressed and non-stressed conditions, the two species showed decreases in efficiency with increasing K level, but the effect was greater in non-stressed treatments.

Unstressed treatments of Panicum significantly decreased their Ca uptake efficiency with increasing K supply, whilst the stressed plants showed small increases from K0 to K2 and a decline to K3 (Fig 3.14d). In Aristida, generally there were slight decreases in efficiency with increasing K level. Panicum showed non-consistent changes to water stress whilst Aristida gave a consistent decrease.

Nutrient Utilization Efficiency

N utilization efficiency in both stressed and non-stressed Panicum increased with increasing K level from K0 to K2, but the stressed treatment then declined sharply to K3 (Fig 3.15a).

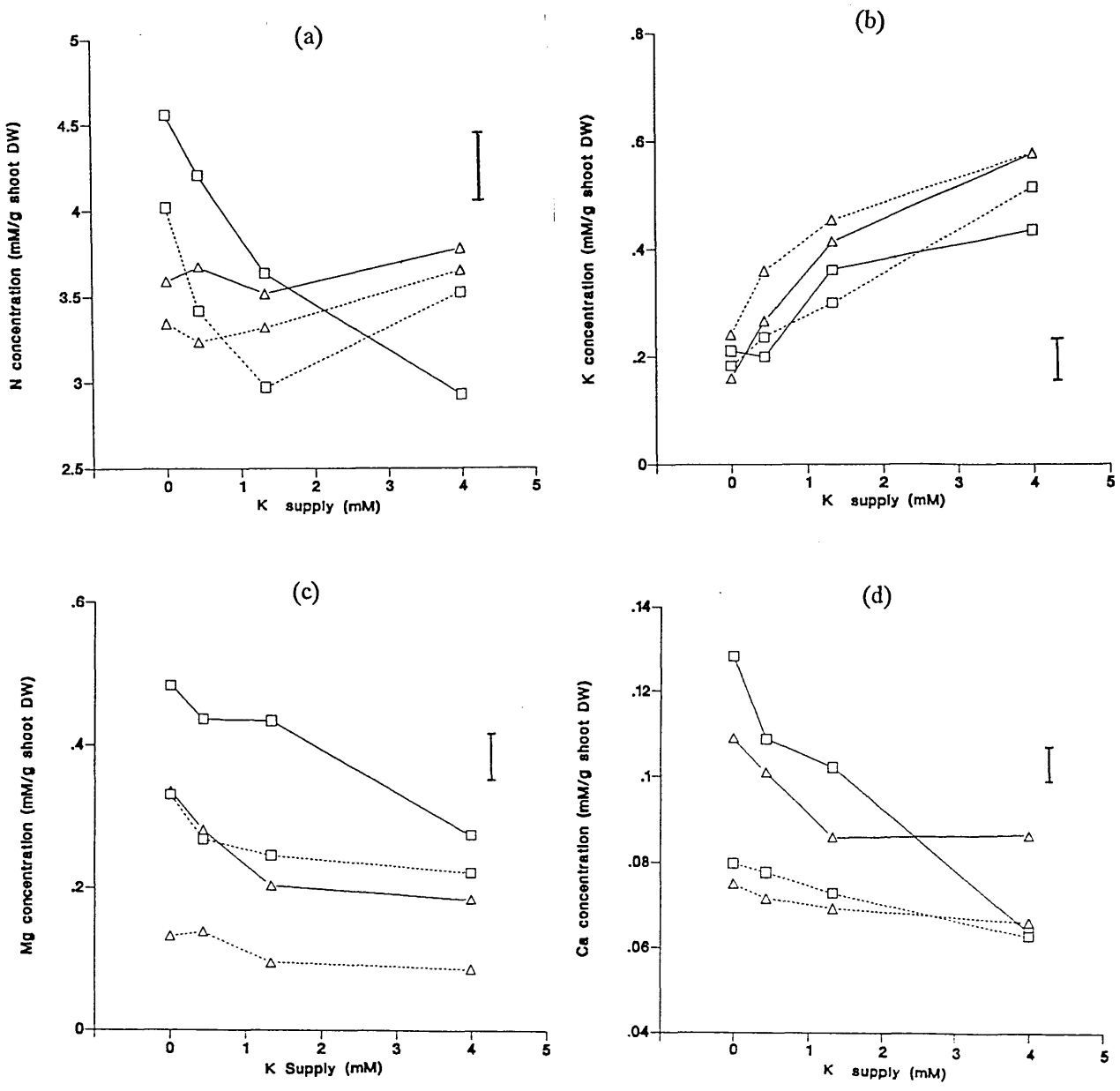



**Figure 3.12** Effects of K level on the concentration of: a) N b) K c) Mg d) Ca (mMol/g DW), in shoot of Panicum (□) and Aristida (△), grown under well-watered and water-stressed conditions. Vertical bars represent LSD's at the 5% level.

(———) well-watered

(- - - - - -) water-stressed

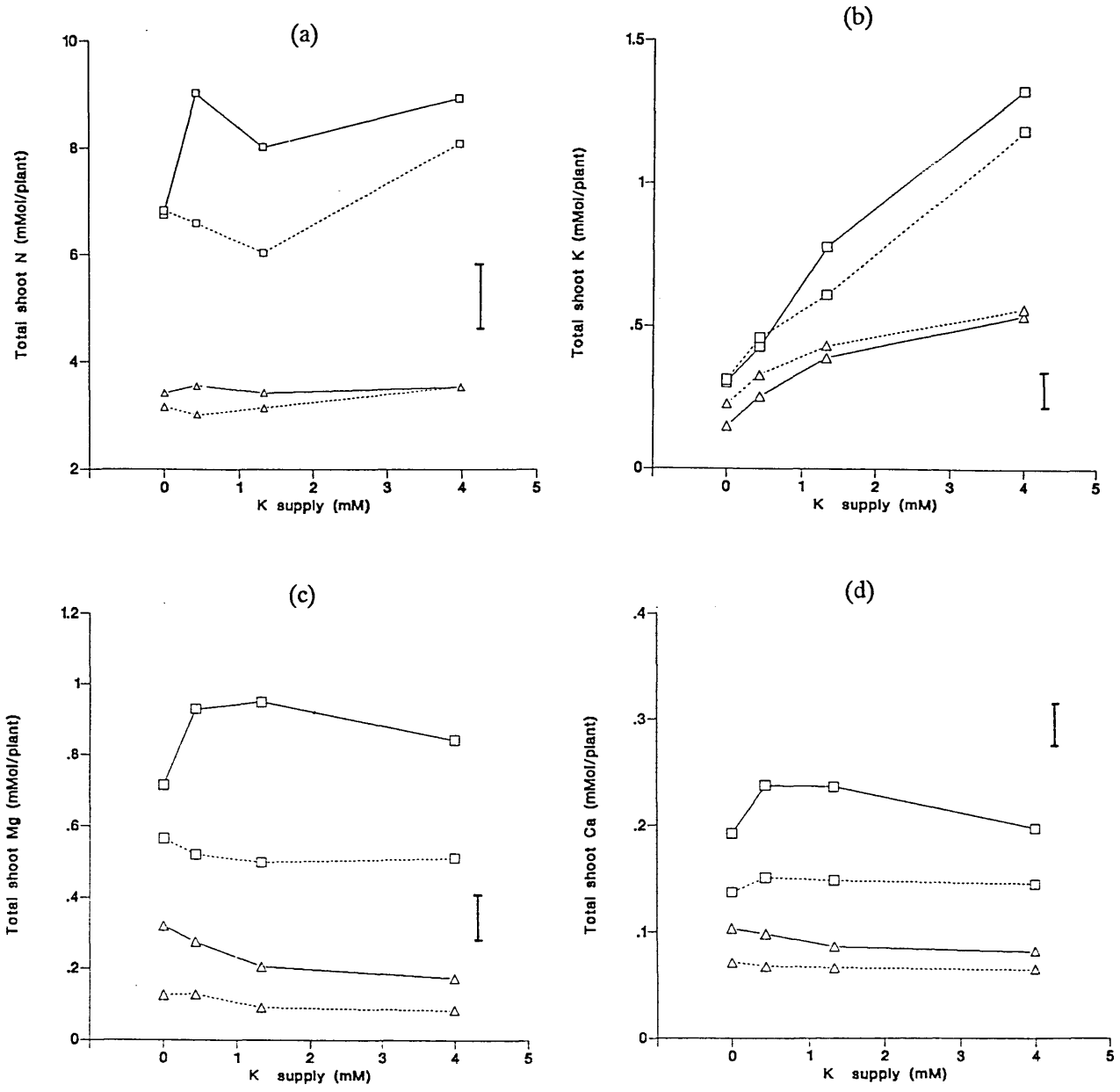



**Figure 3.13** Effects of K level on the total shoot: a) N b) K c) Mg d) Ca (mMol/g plant), in Panicum ( □ ) and Aristida ( △ ), grown under well-watered and water-stressed conditions. Vertical bars represent LSD's at the 5% level.

(———) well-watered

(------) water-stressed

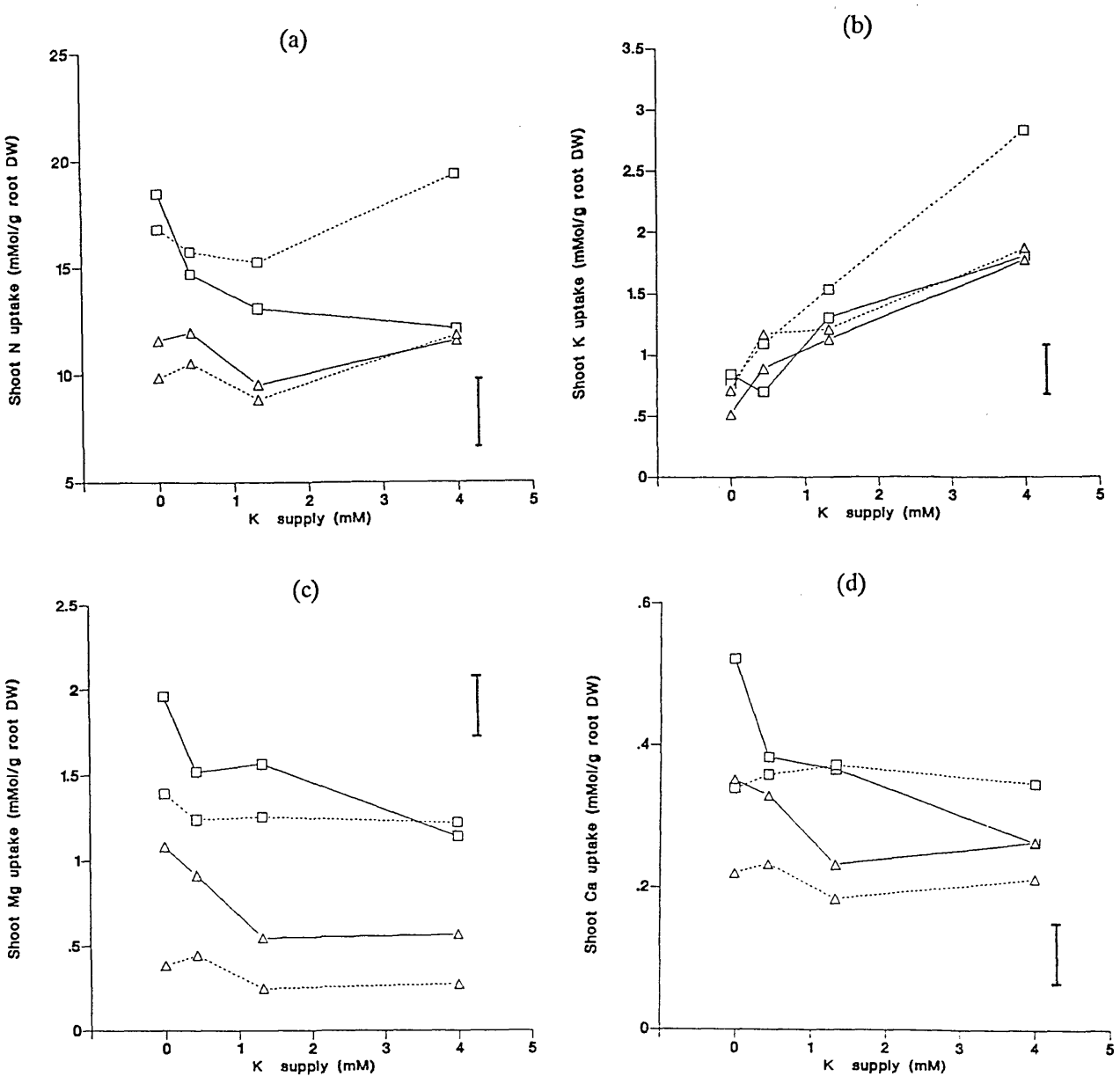



**Figure 3.14** Effects of K level on the efficiency of uptake (mMol/g root DW) of: a) N b) K c) Mg d) Ca, in Panicum ( □ ) and Aristida ( △ ), grown under well-watered and water-stressed conditions. Vertical bars represent LSD's at the 5% level.

(———) well-watered

(- - - - - -) water-stressed

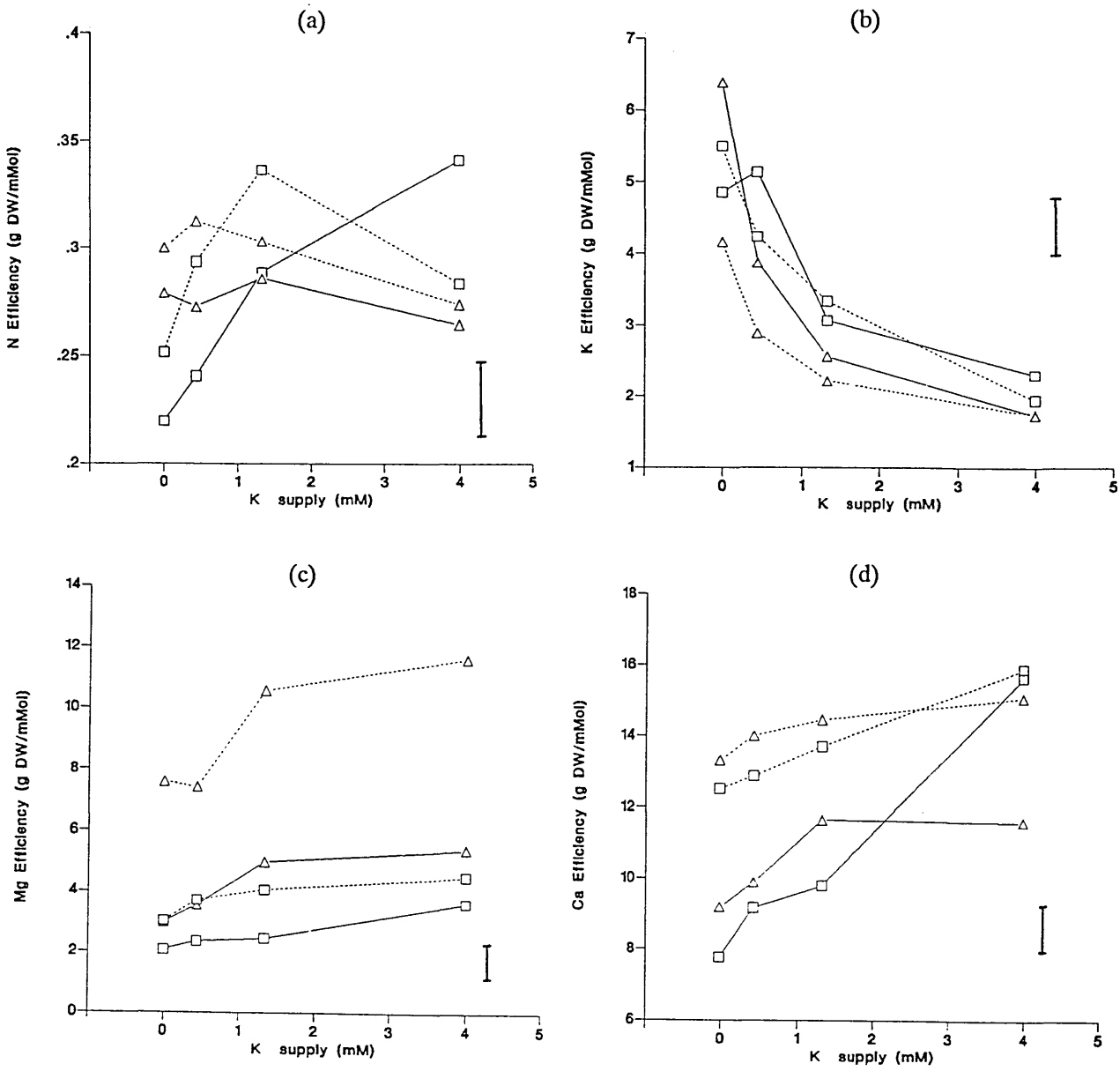



**Figure 3.15** Effects of K level on the utilization efficiency (g DW/ mMol) of: a) N b) K c) Mg d) Ca, in Panicum (□) and Aristida (△), grown under well-watered and water-stressed conditions. Vertical bars represent LSD's at the 5% level.

(———) well-watered

(------) water-stressed

Aristida, on the other hand, showed a gradual but not significant decrease. Values in both species increased with water stress except for the anomalous value for stressed Panicum at K3.

K utilization efficiency of both species decreased with increasing K supply (Fig 3.15b). The greatest efficiencies of both stressed and non-stressed treatments were shown at the lowest K level. Neither species showed any significant effect of water stress except for Aristida at the lowest K level.

Mg utilization efficiency of stressed Aristida was considerably higher than the other treatments (Fig 3.15c). In both stressed and non-stressed conditions, there were slight increases in efficiency with increasing K level with stressed Aristida showing a larger response.

The stressed treatment of both species showed consistently higher Ca utilization efficiencies than the non-stressed treatment (Fig 3.15d). In both stressed and non-stressed conditions, there were significant increases with increasing K supply, except for non-stressed Aristida which levelled off at K2.

Commentary

Once again great difficulty was experienced in maintaining uniform levels of water stress, since the plants were of different sizes even though the pots were returned to the same predetermined water content daily. Since Panicum plants were consistently larger than Aristida they would have experienced greater average water stresses.

In this experiment, the total dry weight of Panicum markedly increased with increasing K supply, however in experiment 3.2.2, it showed no significant responses. This was due to different soils being used in the two experiments, with the former most likely having a higher K content (51 ppm K). The concentrations of N, Mg and Ca in shoots of non-stressed Panicum of this experiment also showed substantial decreases with increasing K supply. This can be explained by the marked increase in total dry weight as shown in Fig 3.11a combined with the constant supply of these three nutrients. In contrast Aristida, which showed no response in total dry weight to different K levels, showed relatively little change in these concentrations. Both species showed large increases in K concentration as the supply increased, but only Aristida gave a greater response under water stress. This might suggest a difference between the species in their use of this ion for osmotic adjustment to water stress conditions.

## 3.3 Discussion

Dry Matter Accumulation

The results showed that there were marked differences in dry matter accumulation of the three species to the levels of N and/or K, and to the different soil moisture levels (Fig 3.1a, 3.6a and 3.11a). The total dry weight of Avena was significantly larger than Panicum and Aristida in all the experiments. Water stress generally caused the largest effects on the total dry weights of Avena, followed by Panicum, and there were no obvious changes in weights of Aristida.

Avena showed a continuing increase in total dry weight up to the highest N level, whilst Panicum levelled off at the second highest level. The results also showed that there were differential responses of Avena plants to soil moisture levels at different levels of N supply. In contrast, the differences in K supply produced no significant changes in weights of Avena but generally caused the total dry matter of Panicum to increase with increasing K supply up to the highest level, with greater increases in the non-stressed than in the water stressed treatments. Aristida, on the other hand, was insensitive to both K level and water stress. These differences of response between the species and the treatments caused significant K (potassium) x SP (species), N (nitrogen) x WS (water stress), WS x SP and N x WS x SP interactions.

The increase in dry matter accumulation of Avena over the full range of N supplies is to be expected because of its success as a serious weed of crops growing under relatively high soil fertility levels. Clapham *et al.* (1981) described Avena or wild oat as a weed naturalised in arable fields or disturbed soils, and often common in fertile habitats. The differential responses of Avena to water stress at different levels of N, as shown in experiment 3.2.1, may also explain the observation that although Avena is most abundant in cool climates under moist soil conditions, it is a major problem in cereals over a broad range of soil and climatic zones including arid and semiarid regions (Sharma and Born 1978). Pavlychenko and Harrington (1935) determined that wild oat developed a more extensive root system than wheat under dryland farming conditions and concluded that this was one of the reasons why wild oat was highly competitive in areas of limited rainfall.

The total dry weight of the stressed Avena (50% FC) at the highest N levels was about half of that of the well-watered controls, a result similar to that of Sharma *et al* (1977) who found dry weight reductions of 20 and 45% for wild oats grown in soil with moisture contents cycled

between 50 and 100% field capacity (FC) and 25 and 100% FC, respectively, compared with a variation from 75 to 100% FC. Akey and Morrison (1984), who found that water stress initiated at the four-leaf stage resulted in approximately an 80% increase in the stomatal diffusion resistance of wild oat. However, dry matter accumulation in the non-stressed wild oat increased from jointing to maturity even though leaf senescene during the same period resulted in a loss of about 45% of the assimilating area, and they suggested that the reductions in dry-matter accumulation in the stressed wild oat plants were primarily due to an inhibition of photosynthesis through stomatal closure. In contrast, Hsiao and Acevedo (1974) reported that leaf expansion is more sensitive to water stress than is $CO_2$ assimilation and, consequently, dry matter production can be reduced even when the plant water deficit is insufficient to cause stomatal closure and restrict $CO_2$ uptake. Furthermore, Hsiao (1973) determined that photosynthesis was not only affected by stomatal aperture, but also by the assimilating area available for light interception. In all the experiments reported here, plants of Avena generally showed similar responses, although the photosynthetic parameters were not analysed.

The results also showed that water stress caused very little effect on the total dry weight of Panicum in experiment 3.2.1. The response to N supply ceased above 4.04 mM N. This is consistent with the known ability of Panicum or proso millet to produce a greater yield than other cereals under low N levels and under the hot, dry conditions in which it is usually grown. Cobley (1956) reported that it has the lowest water requirement of any grain crop and grows quickly, requiring a growing season of as little as 60 days, depending on variety. However, it has also become a serious weed problem in corn-growing areas. Rodriguez *et al.* (1990) studied the dry matter accumulation of the proso millet cultivars Cope and Dawn at optimum nutrient and water availability. They found that the total dry matter accumulation followed a sigmoid pattern. After emergence, dry matter accumulation increased linearly up to maturity and then progressed at a diminishing rate due to leaf senescene during ripening. Similar patterns of dry matter accumulation were observed for corn, wheat and sorghum (Karlen *et al.* 1988 and Karlen *et al.* 1987).

The insensitivity of Aristida to variation in nutrient and soil moisture levels could be a very important feature of this weed, explaining its ability to grow and to reproduce, even at a low level, in infertile soils and water stress conditions. Fitter and Hay (1983) reported low relative growth rate as a typical characteristic of low nutrient species. Such species may not outstrip the available nutrient supply and would therefore grow more or less normally. Aristida, and weeds in general which live on soils of low nutrient potential, may have a selective advantage since they do not create high metabolic demands on the limited resources, and at the same time can metabolise normally. It therefore seems likely that Aristida reflects this ability, for example,

by being able to flourish and complete its life cycle under relatively poor external environment conditions. This is a necessity if these plants are to compete with crops which have been selected by man for vigorous growth and high yields.

Other worker have also observed differential responses of various plant species to nutrient levels and attributed such differences to the natural habitats of the species. For example Tarawali (1983) studied the responses of *Alopecurus myosuroides* and wheat to various levels of P, K, Ca and Mg in nutrient film culture and observed that the weed was relatively insensitive to P, Ca and Mg supply whilst the crop was markedly responsive to all these. He concluded that the observed difference between the two species could be related to the evolutionary background or the the native habitats of the species. Similarly, Redai (1989) observed the differential responses of wheat, wild oat and *Bromus sterilis* to varied levels of N, P, K, Ca and Mg in nutrient film culture and suggested the same reasons for the differences in their responses.

It is therefore suggested that the insensivity of Aristida to soil moisture and K, and the marked responses of Avena to N and water stress when both were compared with Panicum may be related to the evolutionary backgrounds or the native habitats of these species. It is thus possible that Aristida has developed the ability to produce maximum growth at very low levels of nutrients compared with Panicum. The agronomic or ecological implication is that if Aristida, Panicum and Avena were allowed to compete at low soil fertility and limited supply of water, the growth of Aristida would be more nearly normal than Panicum followed by Avena.

Root-Shoot Ratio

The partitioning of dry matter between roots and shoots, as revealed by the root/shoot ratios showed that both Panicum and Avena gave the highest ratios when N supply was limiting. This conforms with the usual adaptation to poor, infertile soils in which resources are switched from shoot to root growth. The ratios of Panicum and Avena in experiment 3.2.1 were affected most by variation in N level, although the smaller effects of water stress and species were still significant. These effects of N level upon root/shoot ratio are similar to those reported by Heitholt, (1989); Campbell and Davidson, (1979) and Smith *et al.*, (1983). However, the different levels of K produced little effect on the root/shoot ratios of Panicum or Aristida, whilst the ratios for Avena responded inconsistently.

The different responses of Panicum, Avena and Aristida in root/shoot ratios to N and K in these experiments may indicate differences between these species in their adaptability to nutrient stress. Many workers have observed changes in root/shoot ratios with changes in

environmental stresses. For example, Chapin (1980) stated that plants of infertile habitats maximise nutrient intake more through high root/shoot ratios and mycorrhizal associations than through high root absorption capacities, as predicted by Nye (1977), and Nye and Tinker (1977). Grime and Curtis (1976) also reported that rapidly growing species from high-nutrient habitats showed considerable phenotypic plasticity in root/shoot ratios and generally had higher ratios in low availability and lower ratios in high availability environments than did species adapted to low-nutrient habitats. Similarly, the high root/shoot ratios found in species from infertile habitats were considered in part as phenotypic responses to reduced nutrient availability (Christie and Moorby, 1975; Loneragan and Asher, 1967 and White, 1972). However, in general, Grime (1977) concluded that species characteristic of favorable habitats show greater plasticity in allocation pattern than do species from stressful environments. Thus in this present investigation, it is possible that Aristida, an arid zone wild $C_4$ plant, showed a contrasting response compared with Panicum or Avena, and this is most probably due to their different native habitats. The root/shoot ratios of Aristida were insensitive to enviromental stress, and this may be explain as a tolerance mechanism, whilst the ratios for Panicum and Avena showed greater plasticity reflecting adaptation to richer environments.

The results from these experiments also revealed that the effects of water stress on the root/shoot ratios of Panicum and Avena were variable although they generally increased with stress, whilst Aristida showed effectively no change. This is in agreement with the results presented by many other workers (e.g. Gales, 1979; Jackson, 1971) who found that drought caused either an increase or decrease in the root/shoot ratio of *Lolium perenne*, and the results from Jupp (1986) who stated that drought had no effect on the ratios of the same species. In a literature survey of numerous species, Gales (1979) found that the root/shoot ratio was increased by drought in nineteen, was reduced in four, did not change in three, and was variable in two. Where an increase has been found it has commonly been regarded as an adaptive response to water stress, and when both shoot and root weights were unaffected as shown by Aristida in this study (Fig 3.11b), it has been suggested that the species was tolerant to water stress.

Other workers have usually found that root/shoot ratios in most species increase in response to water stress, and some have regarded it as an adaptive change. For example, Christie (1975), studied the responses of mulga (*Thyridolepis mitchellina*) and buffel grass (*Cenchrus ciliaris*,) and found that the root/shoot ratios increased both with increasing water stress, and with increasing temperature to 30° C, but decreased with high P. In contrast, Gales (1979), studied the responses of *Lolium perenne* to water stress in fertile loamy soil and concluded that the relative growth rate of the root system was reduced more than that of the shoot, which lead to

a decrease in the root/shoot ratio. He further suggested that when water supply is restricted the root/shoot ratio may respond to an accompanying decrease in the availability of mineral nutrients, particularly phosphate, rather than the availability of water. Therefore, at high levels of phosphate fertilization, plant requirements may be satisfied during water stress, but at low phosphate levels, the deficiency might be exacerbated by water stress. Well fertilized clay soils were, therefore, less likely to show deficiencies than sandy soils. It is thus possible that the increased root/shoot ratios of Panicum and Avena reported here are similar to those reported by others (Atkinson 1973; Brewster, Bhat and Nye, 1976; Loneragan and Asher, 1967), which are typical responses to nutrient deficiency, particularly phosphorus. These observations have been supported by Davidson (1969), who studied the responses of *L.perenne* to three levels of soil moisture and three levels of nitrogen and phosphorus in a combined factorial experiment found that the root/shoot ratio was inversely proportional to the availability of water, nitrogen, and phosphorus but with interactions between these factors.

Two main factors are the most probable reasons for the inconsistent experimental results reported in this chapter, namely the types of soil used as the growing medium and variability of soil moisture content (and thus soil matric potential). Because of this the analysis and interpretation of the results become complicated. For example, there is the great difficulty in maintaining uniform levels of soil water stress within and between the experiments, since the plants were of different sizes even though they were returned to the same predetermined total pot water content daily. Christie (1975) reported that the total water content of a pot may not give an accurate assessment of the water potential within it, because of differences in root exploitation by different species, as well as variation with depth in soil water potential in the pot. Therefore the total pot soil moisture content as discussed relates only to an average potential. For example, when a plant withdraws water from soil, the lowering of the soil water potential is not uniform throughout the volume of the soil. The lowest potentials occur in the uppermost layers, where rooting density is greatest and water evaporates directly to the atmosphere. This problem may be particularly severe in pot experiments using this method of inducing soil water stress, since when a small quantity of water is added to a mass of dry soil, it wets only the top portion. It is thus possible that root development is restricted to this zone. Secondly, the soil which was used for experiment 3.2.3 was entirely different from that used in the other two experiments. Caution should therefore be used when interpreting specific responses, especially those related to soil water conditions.

Nutrient Uptake and Accumulation

The results in experiment 3.2.1 revealed that the concentrations of N in the shoots of Panicum and Avena at various levels of N supply were not affected by water stress. However, both species showed differences in their K concentrations in response to water stress. Panicum showed consistently lower concentrations as water stress increased, whilst Avena showed increases. This caused the interactions of water stress with nitrogen and species to be significant. On the other hand, N and K concentrations in experiment 3.2.2 increased with water stress in both Panicum and Avena at all levels of K supply although the increase was smaller in Avena. Furthermore, the results in experiment 3.2.3 showed that N concentration decreased with water stress in Aristida but the responses in Panicum were inconsistent. These variable responses are in agreement with those found by many other workers, who have reported both decreases (e.g. Dunham and Nye, 1974; Fawcett and Quirk, 1962; Frota and Tucker, 1978; Jenne, *et al.*, 1958; Mattson, 1973; Mengel and Braunschweig, 1972) and increases (e.g. Abdel Rahman, *et al.*, 1971; Gerakis, *et al.*, 1975) in mineral element concentrations with increased water stress.

Plant species vary in their responses to nutrient uptake and these variations might explain some of the differences observed here. For example, the concentration of Mg in tissue of Avena in experiment 3.2.1, showed no differences due to water stress but the uptake by stressed Panicum was greatly reduced. Similarly, both species showed differences in their Ca concentrations, in that the concentration in Panicum was lowered as water stress increased, but Avena showed no consistent changes. This is in accordance with Wimmer, *et al.* (1944), who found non-consistency of K uptake with moisture stress in two varieties of sugar beet, in that under equally increased water stresses, K concentration increased in one variety and decreased in the other. Similarly, William and Shapter (1955) also observed that the relative amounts of K, Ca, and Mg increased considerably more in barley than they did in rye when water stress was imposed.

A number of authors (e.g. Bradshaw *et al.*, 1958, 1960a, 1960b, 1964; Crossley and Bradshaw, 1968; Davies and Snaydon, 1973a, 1973b) have compared the responses of species to different levels of nutrients and concluded that the observed differences were related to their evolutionary background and distribution in the field. Similarly, others have claimed that weeds or plants characteristic of infertile soils are able to take up nutrients better than crops or plants of high-nutrient habitats, when the levels of certain nutrients in the media are low (Johnson, 1987; Qasem, 1987). In the current study, Panicum had generally higher tissue concentrations of K, Mg and Ca than Avena at all levels of N supply in experiment 3.2.1. This indicates that Panicum's requirement for these elements is greater than that of Avena, and/or its ability to take up is superior. If this was so, then the competition between Panicum and

Avena, where the supply of K, Mg and Ca in the soil is limited, one might expect Panicum to dominate because of its ability to absorb more of these elements than Avena.

The effects of water stress in experiment 3.2.2 were similar to those in experiment 3.2.1, in that both showed increases in concentrations of shoot N and K in both species together with those of Mg and Ca in Avena, at all levels of K supply. Soil moisture level has a marked effect on the absorption of solutes by the plants and the interpretation of these effects is extremely complicated because it involves both the transport of soil solutes, and complex plant physiological responses, particularly of different species as in this experiment (Nye and Tinker, 1977). These complications were also investigated by Pitman (1981), who considered the effects of reductions in soil moisture on the relative concentrations of solutes. Ions such as $Cl^-$ or $NO_3^-$, which are poorly adsorbed on to soil particles, would increase in concentration, with equivalent increases in the total concentration of cations, such $K^+$, $Na^+$, $Ca^{2+}$ and $Mg^{2+}$. These changes might then affect uptake since the water content of the soil affects the rate of nutrient transport to the root surface. Apart from nutrient diffusion from the soil immediately around the root, there could be bulk flow of solution, in response to transpiration from the plant. In practise at low soil water contents, Pitman (1981) showed that flow in transpirational water is less important than diffusion. Thus, it is possible that the increased concentrations found here for Mg and Ca in the tops of stressed Avena might be partly due to selective uptake or to adaptation to water stress, since the root/shoot ratio also showed a similar increase. This larger, more extensive root system of stressed Avena might reduce the necessity to transport these ions to the root sufaces by water flow, through providing a larger surface area for diffusion. A further complication is that in dry soil, it is possible that movement of water and solutes to the root could have been blocked by air gaps between root and soil (Newman, 1974; Nye and Tinker, 1977).

The results in experiments 3.2.3, revealed that both Panicum and Aristida showed large increases in K concentration as the supply increased, but only Aristida gave a greater response under water stress. The increases in K reported here are similar to those studied by Tanguilig, *et al.* (1987), who found that the ability of maize and soybean to grow better than rice under water stress conditions might have been due to their ability to maintain turgor, partly through increased uptake of K relative to growth, which must have allowed osmotic adjustment to occur. Increased K uptake in Aristida in this experiment suggests that, under water stress conditions, there is a difference between the species in their use of this ion for osmotic adjustment.

Regardless of water stress, uptake of N and K in Aristida, Avena and Panicum reported here increased with increasing supplies of each element. This is consistent with the observations by Fawcett and Quirk (1962), who reported that P uptake increased with increased P levels in the soil. Similarly Olsen *et al.* (1961) found that P uptake depended on the soil P level as much as on the magnitude of water stress. This was also noted for many other elements (Broyer and Hoagland, 1943). Mengel and Braunschweig (1972) also found that higher levels of mineral elements such as K counter-balanced many of the negative effects of water stress. Viets (1972), however, reported that under some field conditions fertilization in dry seasons impaired plant performance and suppressed yields. This could happen since increased size led to an increased rate of water use such that there was insufficient available soil water to carry the crop through periods of stress. The present study provided no similar evidence, but these instances of reduced yields are not common even though they do occur, particularly under field conditions.

It is well known that water availability affects growth, but it is often difficult in published work to separate effects of water stress on ion uptake on the one hand and on dry matter production on the other. For instance, in many plants the rate of accumulation of N, P and K is proportional to the rate of growth, so that relative contents tend to be constant. It has been suggested that this proportionality in uptake and growth can be attributed variously to increasing root size, to feedback processes in the plant, or to rate of supply of sugar to the root (Pitman and Cram, 1977). Where uptake and growth are not rigidly proportional, as in this work, it is possible to separate them by comparing the proportionate changes caused by water stress, as in Table 3.9. For example, in experiment 3.2.1, the changes in N, K and Mg content of stressed Panicum were similar to the changes in growth, whilst the reduction in Ca content was much greater. In comparison, for Avena the reductions in growth and uptake of all four elements were of similar size.

In experiment 3.2.2, where all the reductions were much greater than in experiment 3.2.1, Panicum showed greater reductions in growth than in uptake of N and K, whilst Avena again showed proportionate changes. The results for uptake of Mg and Ca were not consistent with the earlier experiment.

Similarly, in experiment 3.2.3, the changes for Panicum in accumulation of N and K were similar to the changes in growth whilst the accumulation of both Mg and Ca was reduced. Aristida responded similarly, except for an enhanced uptake of K.

Overall, these three experiments gave fairly consistent trends, in that the accumulation of divalent cations in both Panicum and Aristida was reduced under water stress, whilst that of Avena was unaffected (or possibly increased under severe stress) (Table 3.9). The uptake of monovalent ions was consistently proportional to growth, except for the enhanced uptake of K in Aristida, which might well be an adaptive feature of this species. The data reported by others (i.e. Clark, 1981) confirmed the general trends found in this study. For example, Gerakis *et al.* (1975), observed that dry matter yields usually decreased faster than nutrient uptake. Similarly, Jenne *et al.* (1958), showed that mature maize plants grown with inadequate water, accumulated P, N, Mg, K and Ca at 40, 50, 65, 71 and 91% respectively, of those found in mature plants grown with adequate water, whilst dry matter yields of the mature water-stressed plants were 44% those of the unstressed controls.

Efficiency of Uptake and Utilization

Panicum showed a higher efficiency than Avena in nutrient uptake (i.e. mMol/g root dry weight) for all four elements in experiments 3.2.1 and 3.2.2 at the various levels of soil moisture. The efficiencies of uptake in all three species also increased with increasing N or K supplies. Similar effects have been reported by Johnson (1987), who found that under low P levels blackgrass was relatively more efficient in absorbing nutrient than wheat. At higher P levels the opposite was true. He suggested that the higher values for blackgrass under low P could have been due to its greater density of roots. Root morphology, density and distribution are important characteristics affecting the uptake of nutrients. Species whose roots can exploit a greater volume of soil might be expected to be more efficient. Drew (1979) and Drew and Saker (1975) showed that lateral roots and root hairs enhanced the absorption of nutrients. The lower root/shoot ratios of Panicum compared with Avena in this study, together with their greater efficiency on a weight basis could be explained on the basis of a finer root system, causing the more efficient uptake of nutrients from the soil.

There were marked differences in the efficiencies of nutrient uptake amongst the three species in response to water stress. The results of all the experiments showed that the efficiencies of Panicum generally increased with water stress whilst Avena showed inconsistent responses. On the other hand, the results in experiment 3.2.3 showed that the efficiency of Aristida showed no obvious changes with water stress. The higher efficiency of Panicum in response to water stress may suggest the adaptation of this species by producing more finer roots in order to exploit a greater volume of soil in searching for nutrients and moisture. The inconsistent responses of

Table 3.9 Summary the effects of water stress on growth and nutrient uptake in Panicum, Avena and Aristida.

| | Percentage relative to control (well-watered plants) | | | | |
|---|---|---|---|---|---|
| | | Total Shoot Content | | | |
| | Growth | N | K | Mg | Ca |
| Experiment 3.2.1 (averaged over N levels) | | | | | |
| Panicum | 103.84 (SE=15.3) | 100.86 (SE=14.8) | 98.74 (SE=11.9) | 95.31 (SE=13.8) | 73.06 (SE=8.8) |
| Avena | 76.13 (SE=11.7) | 79.38 (SE=15.2) | 84.19 (SE=6.9) | 71.10 (SE=9.3) | 72.93 (SE=10.1) |
| Experiment 3.2.2 (averaged over K levels) | | | | | |
| Panicum | 16.90 (SE=1.4) | 23.35 (SE=2.1) | 30.16 (SE=2.2) | 7.65 (SE=0.6) | 16.10 (SE=2.0) |
| Avena | 17.87 (SE=2.9) | 17.81 (SE=2.4 ) | 19.32 (SE=2.4) | 23.77 (SE=3.2) | 27.56 (SE=4.2) |
| Experiment 3.2.3 (averaged over K levels) | | | | | |
| Panicum | 90.85 (SE=12.5) | 88.51 (SE=14.8) | 94.38 (SE=11.7) | 64.39 (SE=11.5) | 70.33 (SE=8.1) |
| Aristida | 108.57 (SE=16.4) | 101.0 0 (SE=13.5) | 131.54 (SE=13.1) | 46.54 (SE=7.7) | 78.89 (SE=11.2) |

Avena again suggest the great variability of soil moisture content between the experiments, which was discussed earlier and which also caused the similar variations in root/shoot ratios. On the other hand, the efficiency of Aristida in taking up nutrients was little affected possibly confirming the tolerance of this species to water stress.

In the present investigation the species also differed in their utilization efficiencies (i.e. the inverse of tissue concentration) of various nutrients. Panicum was generally higher than Avena in utilizing N (low in tissue concentration) at all N levels and soil moisture regimes in experiment 3.2.1, whilst Avena was higher than Panicum for K, Mg and Ca. N utilization efficiency in both species however, decreased with increased N supply so that the greatest efficiencies were shown at the lowest N level. This result agrees with Chapin (1989), who reported that all plants exhibit an increase in nutrient use efficiency under conditons of nutrient stress because a larger proportion of plant biomass is allocated to tissues with low nutrient concentrations, nutrient stores in the vacuoles decline and fibre and carbohydrate concentrations increase.

In experiment 3.2.2, Panicum was more efficient than Avena in the utilization of N, K and Ca at any K level or soil moisture regime. Water stress greatly reduced the utilization efficiencies of Panicum except for Mg, whilst the stressed Avena produced the lowest dry matter for every unit of all elements absorbed except for Mg. The lower efficiency in Avena in response to water stress may suggest that it absorbed these nutrients even if they were not required (luxury consumption). Rorison (1968) also found excess P uptake in *Scabiosa columbaria* and concluded that the ability to take up more than the immediate requirement is ecologically valuable and the accumulation of a tissue mobile element at flush times may be adequate to ensure steady growth during times of stress. It may therefore be suggested that the low utilization efficiencies in Avena may be explained as an ability to guard itself against times of deficiencies of these elements.

The results of experiment 3.2.3 showed that Aristida under water stress produced more dry matter for every unit of Mg and Ca absorbed, whilst stressed Panicum showed greater efficiency in utilizing N and K. Although stressed Aristida showed higher content and uptake efficiency for K, its efficiency in utilizing this element was the lowest. This low use efficiency for K in Aristida may again indicate some luxury consumption leading to storage in the tissue and may also indicate an adaptation to water stress, through osmotic adjustment. Similarly, Vose (1963) found in ryegrass that different genotypes varied in their efficiency of N utilization, some tending towards high dry matter yield and relatively low N content (termed efficient), or lower

yielding types with high N content, regarded as inefficient converters of N. Johnson (1987) reported that blackgrass was more efficient than wheat in utilizing P, K, and Ca at the highest P concentrations whilst the opposite was true for Mg. Similarly, Tarawali (1983) noted that blackgrass was significantly more efficient than wheat in utilizing P, K, Ca and Mg.

The present investigation has shown that there are differences in both uptake and utilization efficiencies in Panicum, Avena and Aristida in relation to N and/or K supply, and soil moisture regimes. In general, water stress caused the largest effects on the total dry weighs of Avena, followed by Panicum, and there was no obvious changes in weights of Aristida. Whilst water stress had varied effects on nutrient concentrations and contents, dry matter yields generally decreased faster than Mg and Ca uptake although a number of differences were observed. More investigations are clearly neccessary to examine both the nature and extent of these differences, since they could have important implications for adaptation to water stress between the three species. In Chapter Four, more detailed experiments are described, in which stress was applied by completely withholding water, and growth followed in much greater detail by means of frequent harvests as the soil dried out.

# CHAPTER FOUR

## Some Studies Relating Growth and Nutrient Uptake Patterns of *Avena funiculata*, *Avena fatua* and *Panicum miliaceum* With Time Under Conditions of Soil Water Stress

# CHAPTER FOUR

## SOME STUDIES RELATING GROWTH AND NUTRIENT UPTAKE PATTERNS OF *A.funiculata* (Aristida), *A.fatua* (Avena) and *P.miliaceum* (Panicum) WITH TIME UNDER CONDITIONS OF SOIL WATER STRESS.

### 4.1 Introduction

Nutrient uptake from the soil solution is closely linked to soil water status. However, plant response to water stress varies with species. These differences of plant responses explain why one species survives or yields better than another under a limited water supply. Thus to fully explain these adverse effects of water stress on nutrient uptake, study should focus on nutrient movement in the soil as well as the plant responses to nutrient supply and water stress.

The basis for the experiments in this Chapter, however, is that one-off harvests are not all that informative in revealing the diferent nutrient uptake efficiency of the three species. Therefore, the purpose of the experiments in this chapter, is to determine and compare the response of *A.funiculata* (Aristida), *A.fatua* (Avena) and *P.miliaceum* (Panicum) to soil water stress, specifically the water consumption, growth rate and the nutrient uptake patterns of N, K, Mg and Ca at various growth stages.

### 4.2 Experiments

#### Experiment 4.2.1 Effect of soil water stress on growth and nutrient uptake by Aristida, Avena and Panicum.

##### *Materials and Methods*

The seeds of Avena and Panicum were sown in trays on 11/9/88, whilst plants of Aristida had been derived by vegetative propagation from a single parent, starting about a fortnight earlier. The precise timing was adjusted by trial and error such that plants of all species reached similar

leaf areas by the beginning of the experimental treatments. Three small plants of each species were transplanted into pots containing standard soil and thinned to a single plant after establishment. A randomised complete block design with five replications was used. Plants were grown under well-watered conditions (Field Capacity) until they all had leaf areas of approximately 100 $cm^2$. Long Ashton Complete nutrient solution was supplied twice a week until the beginining of the experimental period. Water was withheld from all species on the same day, which was 10 days after transplanting (DAT) for Avena and Panicum, and 23 DAT for Aristida. Control treatments were returned to field capacity by watering daily with distilled water. The previous day the soil surface of all pots were covered with perlite and white polythene sheets to minimise soil water evaporation.

Destructive sampling was used to assess the responses as soil water stresses developed, with samples taken at 0, 2, 4, 6, 8, 9, 10 and 11 days after water was withheld. Transpiration rates were determined as the weight change of each pot over 24-hour periods. Soil moisture contents were determined gravimetrically and expressed as percentage on an oven-dry weight basis. Fresh and dry weights of shoot and root were determined, and their nutrient contents analysed.

All data were statistically analysed using polynomial constrasts. Genstat was used for fitting the orthogonal polynomials (Genstat 5 Reference Manual, 1988), where the coefficient of this polynomial examine the linear and quadratic effects of all the factors involved, for example Harvest, Species, Stress and it interactions as in Table 4.1. The growth analysis was carried out from fitted polynomial regression equations of the form:

$$W = a + bt + ct^2$$

where, W was the total dry weight (dry matter content) and t time in days. Differentiation of this equation gave instantaneous values of the absolute growth rate (GR);

$$\frac{dW}{dt} = b + 2ct$$

### *Results*

The soil drying curves for all the treatments decribed in this experiment are shown in Fig 4.1a. In all the three species, the pattern of the decreases in soil moisture content of the stressed plants were quite similar, with Aristida reaching a slightly lower moisture level by the end of the experiment. The greatest water loss in all treatments occurred between days 4 and 6, in response to particularly warm, bright conditions.

As percentage soil moisture is not a good measure of the water available to plants due to variation in properties between different soils, the relationship between moisture content and matric potential of the soil was investigated. The first trials with the ceramic pressure plate (for the range of pressure 0 to 1.5 MPa matric potential) failed to produce consistent results (data not shown), presumably due to faulty apparatus. A subsequent test gave the curve shown in Appendix IV.

Plant Growth and Water Use

The changes in total dry weight, dry matter content and water consumption of all three species in both stress treatments vs the number of days water since was withheld are plotted in Fig 4.1b to 4.1d. A pooled regression analysis (Table 4.1) was used to select the best polynomials to fit regression curves to the raw data. In ANOVA, the sum of squares of factors are partitioned into the amount that can be explained by linear relationship (Lin), the extra amount that can be explained if the relationship is quadratic (Quad), and the amount represented by deviation from the quadratic polynomial.

Analyses of all three variables showed that the data averaged over all treatments would need a high power polynomial (significant deviations from the quadratic regression). However, these overall means are of little interest. Significant differences between treatments, particularly those involving water stress (the Stress x Harvest and Stress x Species x Harvest interactions) were confined to the linear and quadratic regression terms. It was therefore decided that quadratic regressions gave the most appropriate description of the observed growth curves. When regressions were fitted to data for the individual treatments (Table 4.2a and 4.2b), some of the quadratic terms were not significantly different from zero. However, it was decided to use similar equations for all treatments, so as to simplify later interpretation.

For all three species, the growth of the control treatments was almost linear (Fig. 4.1b), whereas the stressed material showed curvilinear responses due to reduced growth rates as the period of stress extended. Plants of Aristida were substantially heavier than the other two species throughout, despite the preliminary selection for uniform leaf area. Water stress caused a reduction in growth of all three species, which expressed itself as a reduction in the quadratic term of the regression equation for Avena and Aristida, but as a reduction in the linear term for Panicum. There were differences in the responses of the three species as the water stress developed (Species x Water stress highly significant, Table 4.1). Stressed Aristida and Avena

Table 4.1 Analyses of variance of the effects of withholding water on total dry weights, dry matter contents, water consumption and root-shoot ratios of Panicum, Avena and Aristida

| Source of variation | df | Total dry weight (g/plt) | Dry matter content | Water consumption (g/plt/day) | Root-shoot ratio |
|---|---|---|---|---|---|
| | | Mean Square (MS) | | | |
| Harvest (H) | 7 | 6.815 *** | 0.0783 *** | 344206*** | 0.00673*** |
| Linear (Lin) | 1 | 45.705*** | 0.519 *** | 2392438*** | |
| Quadratic(Quad) | 1 | 0.0692ns | 0.00463 ** | 5442.5*** | |
| Deviations(Dev) | 4 | 0.387 *** | 0.00476*** | 2312.5*** | |
| Species (SP) | 2 | 103.894*** | 0.853 *** | 104318*** | 0.663 *** |
| Stress (WS) | 1 | 7.260 *** | 0.176 *** | 930732*** | 0.00599 * |
| H x SP | 14 | 0.608 *** | 0.00532*** | 5583.5*** | 0.00500*** |
| Lin | 2 | 3.544 *** | 0.0310 *** | 38056*** | |
| Quad | 2 | 0.126 ns | 0.000582ns | 466.5 ns | |
| Dev | 10 | 0.117 * | 0.00115 * | 112.4 ns | |
| H x WS | 7 | 0.897 *** | 0.0184 *** | 92887*** | 0.00214ns |
| Lin | 1 | 5.469 *** | 0.119 *** | 608376*** | |
| Quad | 1 | 0.528 *** | 0.00776 ns | 35767*** | |
| Dev | 5 | 0.0564 ns | 0.000312ns | 1213.5** | |
| SP x WS | 2 | 0.573 *** | 0.0128 *** | 94890*** | 0.00889** |
| H x SP x WS | 14 | 0.0828 ns | 0.00112 ns | 7471.9*** | 0.00312* |
| Lin | 2 | 0.416 *** | 0.00473 ** | 50640*** | |
| Quad | 2 | 0.0251 ns | 0.0000959ns | 1127.1* | |
| Dev | 10 | 0.0277 ns | 0.000599ns | 107.1 ns | |
| Residual | 188 | 0.0563 | 0.000584 | 289.7 | 0.00129 |
| Total | 235 | 1.236 | 0.0118 | 19685.3 | 0.00752 |

Dry matter content = Total dry weight/Total fresh weight

**Figure 4.1** a) Soil drying curves for Experiment 4.2.1. b) Total dry weight (g/plant) c) Dry matter ratio (Total DW/Total FW) d) Total water consumption (g/plant) e) Root/shoot ratio. All plants were grown under well-watered: Panicum (□——□), Avena (▽-------▽) and Aristida (△-·-·-·-△), and water-stressed conditions: Panicum (■——■), Avena (▼--------▼) and Aristida (▲-·-·-·-·-▲).
Lines are fitted quadratic regressions (Fig 4.1b, c & d - see Table 4.2).
Vertical bars are pooled SE's

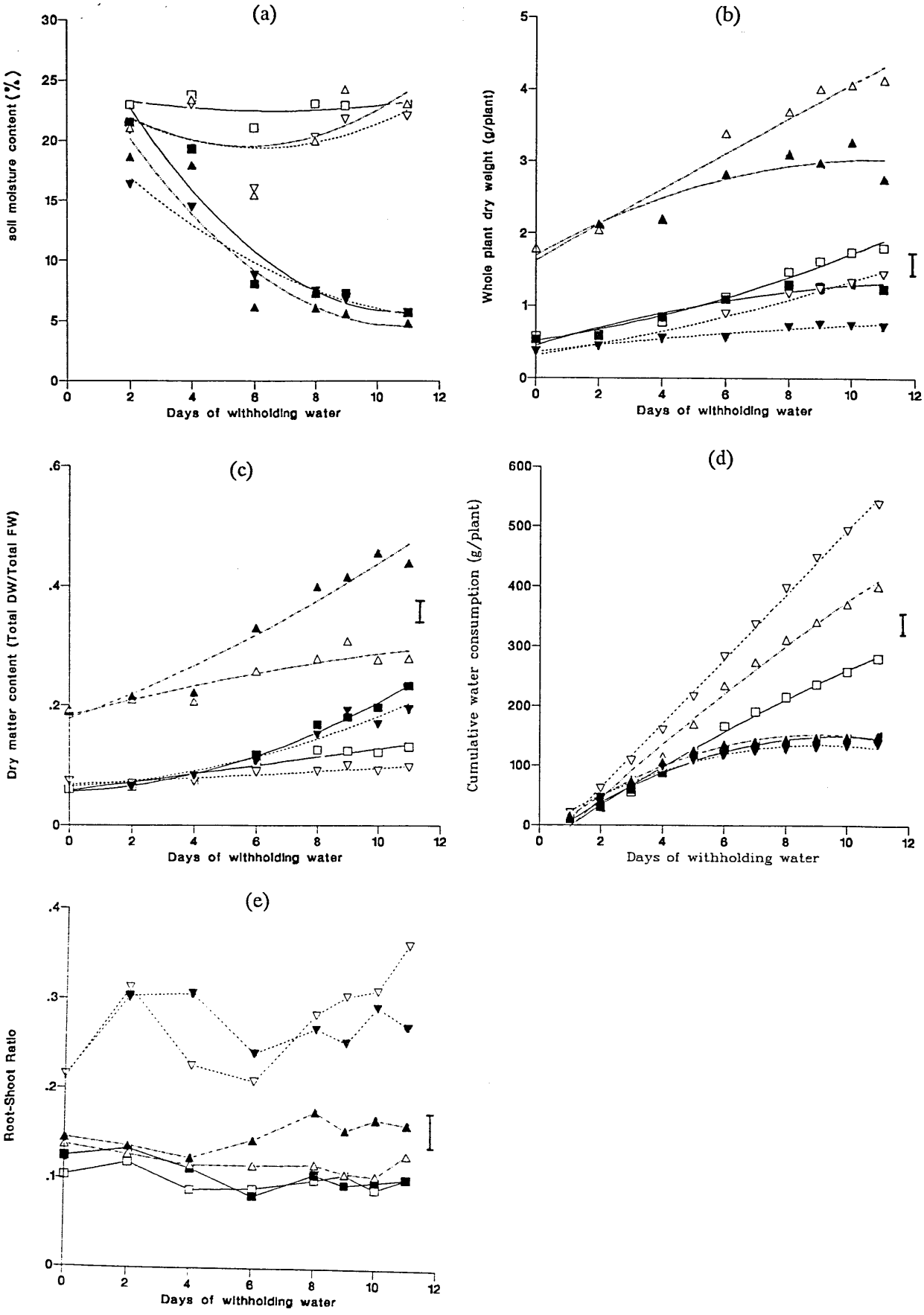

(a)
soil moisture content (%)
Days of withholding water
(b)
Whole plant dry weight (g/plant)
Days of withholding water
(c)
Dry matter content (Total DW/Total FW)
Days of withholding water
(d)
Cumulative water consumption (g/plant)
Days of withholding water
(e)
Root-Shoot Ratio
Days of withholding water

Table 4.2a Polynomial regressions on the number of days of withholding water for total dry weight and dry matter content of Panicum, Avena and Aristida

| Variables | Regression Equation | $R^2$ (%) |
|---|---|---|
| Total dry weight (TDW) | | |
| Panicum, control | $Y = 0.514 + 0.0673t + 0.00532t^2$ (0.062) (0.026) (0.0023) | 90.77 |
| Panicum, stress | $Y = 0.450 + 0.132t - 0.00484t^2$ (0.044) (0.019) (0.0016) | 88.34 |
| Avena, control | $Y = 0.317 + 0.0697t + 0.00326t^2$ (0.055) (0.023) (0.0020) | 89.60 |
| Avena, stress | $Y = 0.363 + 0.0526t - 0.00159t^2$ (0.026) (0.011) (0.00096) | 80.68 |
| Aristida, control | $Y = 1.630 + 0.248t - 0.00038t^2$ (0.18) (0.076) (0.0066) | 80.79 |
| Aristida, stress | $Y = 1.697 + 0.243t - 0.0111t^2$ (0.15) (0.063) (0.0054) | 61.44 |
| Dry matter content (DMC) | | |
| Panicum, control | $Y = 0.0581 + 0.00705t - 0.000003t^2$ (0.0046) (0.0019) (0.00017) | 84.38 |
| Panicum, stress | $Y = 0.0566 + 0.00236t + 0.00125t^2$ (0.0065) (0.0027) (0.00024) | 93.91 |
| Avena, control | $Y = 0.0685 + 0.00262t + 0.000014t^2$ (0.0034) (0.0014) (0.00012) | 60.81 |
| Avena, stress | $Y = 0.0649 + 0.0030t + 0.000874t^2$ (0.0083) (0.0035) (0.00030) | 85.07 |
| Aristida, control | $Y = 0.183 + 0.0139t - 0.000365t^2$ (0.011) (0.0048) (0.00042) | 63.58 |
| Aristida, stress | $Y = 0.178 + 0.0196t + 0.000638t^2$ (0.020) (0.0086) (0.00075) | 79.88 |

Values in parenthesis = S.E. (df=39)

Table 4.2b Polynomial regressions on the number of days of withholding water for water consumption of Panicum, Avena and Aristida

| Variables | Regression Equation | $R^2$ (%) |
|---|---|---|
| Water consumption(WU) | | |
| Panicum, control | $Y = -8.35 + 24.95t + 0.275t^2$ (8.82) (3.72) (0.32) | 95.91 |
| Panicum, stress | $Y = -6.45 + 28.75t - 1.305t^2$ (5.90) (2.49) (0.21) | 93.51 |
| Avena, control | $Y = -8.75 + 39.02t + 1.288t^2$ (9.78) (4.12) (0.36) | 98.58 |
| Avena, stress | $Y = 2.20 + 30.91t - 1.660t^2$ (7.62) (3.21) (0.28) | 87.64 |
| Aristida, control | $Y = -13.54 + 35.02t + 0.479t^2$ (9.35) (3.94) (0.34) | 97.74 |
| Aristida, stress | $Y = -3.74 + 32.44t - 1.690t^2$ (6.02) (2.54) (0.90) | |

Values in parenthesis = S.E. (df=39)

were significantly smaller than the controls after 6 days of withholding water, whilst Panicum became significantly different only after 8 days.

Unlike total dry weight, the total dry matter content of the stressed treatments of all the three species were higher than the controls (Fig 4.1c). All treatments showed increases with time, but these were greater with water stress.

Total water consumption of the control Avena consuming the most water than other treatments (Fig 4.1d). The plants of all the three species were significantly higher than the stressed treatments. The control treatments all showed marked increases with time, whilst the stressed treatments showed gradual but not significant increases.

The root-shoot ratios showed highly significant main effects of species, stress and harvest, and all their interactions except for Harvest x Water Stress (Table 4.1). However, trends with time were neither large nor consistent, and polynomial regression calculations were not considered worthwhile (Fig. 4.1e). Avena had consistently the highest ratio amongst the three species. The ratio of both Avena and Panicum showed no consistent changes with increasing water stress, whereas the stressed Aristida consistently increased.

Absolute growth rates were calculated from the fitted curves of total dry weight. As expected, the control treatments of the three species showed increased growth rates with time except for Aristida which showed a small decrease (Fig 4.2a). On the other hand, the absolute growth rates for the stressed treatments of all species declined markedly with time as the severity of water stress accumulated. Aristida, which had the highest growth rate at the beginning, had declined to the lowest by the end of the stress period (day 11).

In contrast to the dry weight, the rates of change of total dry matter content of all the three species were greatly increased with increasing stress (Fig 4.2b). Whilst the control plants of Aristida consistently decreased with time, Avena and Panicum showed gradual increases. Stressed Aristida had the highest rate throughout, whilst the control plants of Avena had the lowest.

The rate of water consumption by the control treatments of the three species, again consistently increased with time, presumably as an effect of increased size, and the rates showed consistent differences between species (Fig 4.2c). The stressed treatments, on the other hand, declined with time as the water availability decreased, with little detectable effect of species.

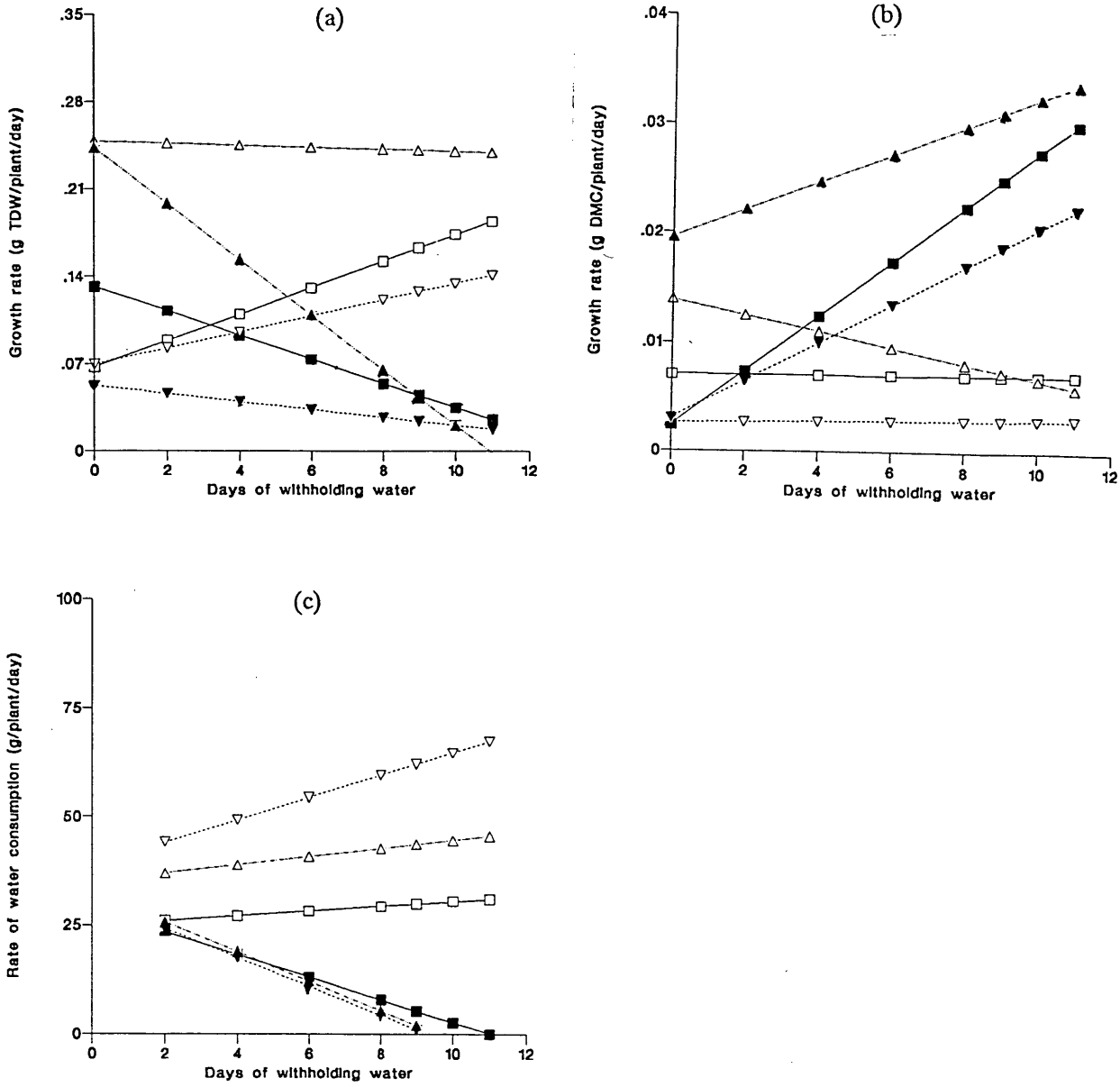



**Figure 4.2** a) Absolute growth rate (g TDW/plant/day) b) Absolute growth rate (g DMC/plant/day) c) Rate of water consumption (g/plant/day) in plants grown under well-watered: Panicum (□——□), Avena (▽- - - - - -▽) and Aristida (△·····△), and water-stressed conditions: Panicum (■——■), Avena (▼- - - - - -▼) and Aristida (▲·····▲).

TDW = Total dry weight, DMC = Dry matter content

The relationships between water use and growth, as revealed by the ratios of water consumption to total dry weight showed that the amount of dry weight produced per unit of water consumed was consistently higher for Aristida was consistently higher than for Panicum or Avena under both control and stressed conditions (Fig 4.3c). In all the three species, the stressed treatments showed greater water use efficiency (WUE) than control, with a much larger difference for Aristida. Avena had the lowest WUE due to having the greatest transpiration and the lowest total dry weight accumulation under both conditions.

Nutrient concentration

The concentrations of N, K, Mg and Ca in both root and shoot dry weights of all the three species are illustrated in Fig 4.4a to Fig 4.4h.

The patterns of change of shoot nutrient concentrations with time of all the species were quite similar, and there were no significant changes associated with water stress, although the stressed treatments of all species showed slightly higher concentrations than the controls from 8 days after withholding water. The shoot N in Avena was consistently the highest of all the species. Panicum and Avena both showed decreasing concentrations over the experimental period, but Aristida showed little change (Fig 4.4a).

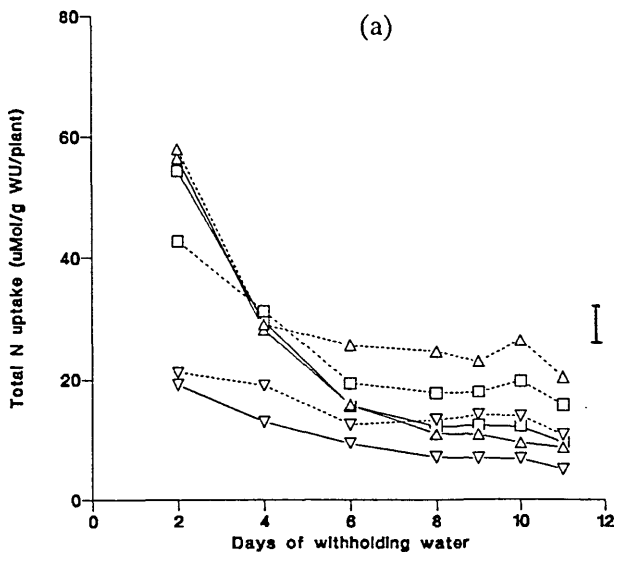
Unlike N, the shoot K concentration of all species showed no obvious changes with time (Fig 4.4b). Similarly, the stressed treatments of the three species showed consistently, but non-signicantly higher concentrations than the controls during the latter half of the experiment. At all times, Aristida consistently showed the lowest shoot K.

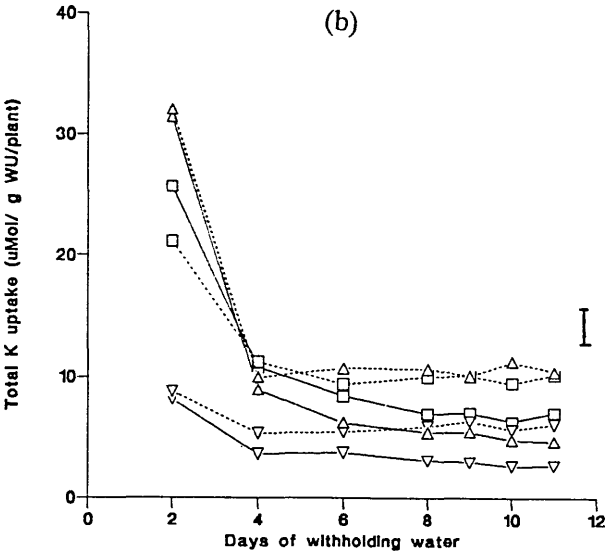
The shoot Mg of Avena and Panicum again showed no obvious changes with increasing stress, whilst Aristida showed a slight decrease (Fig 4.4c). The concentration of Ca of all the three species showed inconsistent responses with time and treatment (Fig 4.4d).
In contrast to shoots, the concentration of N in the roots of stressed treatments for all species were different from the controls (Fig. 4.4e). The stressed treatments all showed slight increases with time, whilst the controls showed decreases. Stressed treatments of all species thereby achieved higher concentrations than the controls by the end of the experiment.

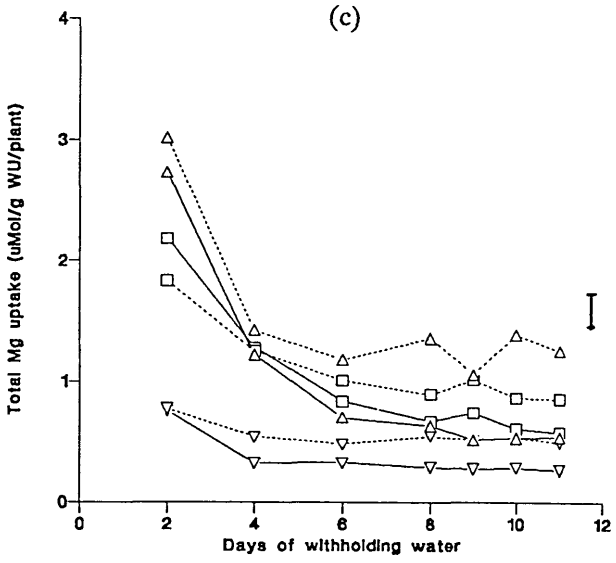
The concentrations of root K, Mg and Ca showed no consistent changes with time in any species. Increased concentrations in stressed treatments were fairly common, but not significant (Figs 4.4f to 4.4h).

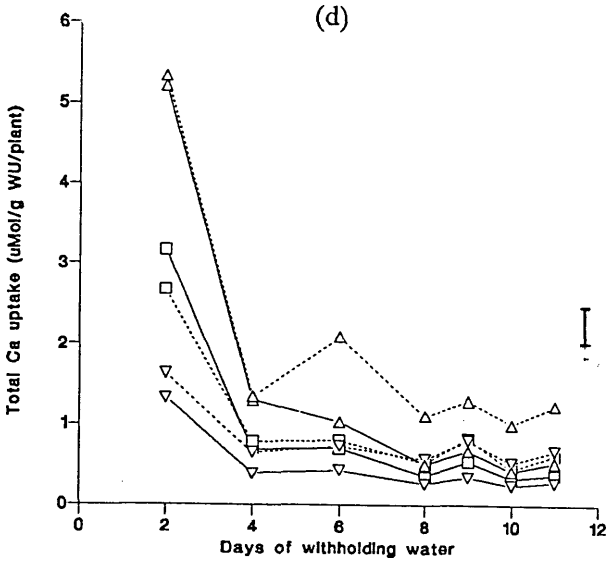
**Figure 4.3** Efficiency of uptake (μMol/g WU/plant) of: a) N b) K c) Mg d) Ca, in Panicum ( □ ), Avena ( ▽ ) and Aristida ( △ ), grown under well-watered ( ——— ) and water-stressed ( - - - - - - ) conditions.
Vertical bars are pooled SE's
WU = Water use

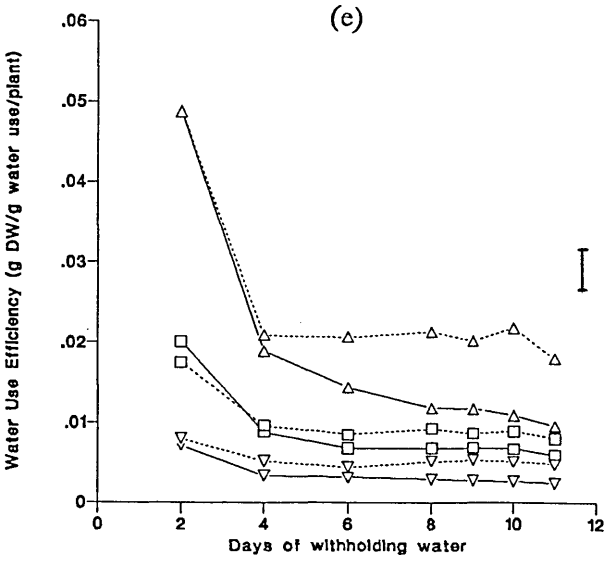
e) Water Use Efficiency (g DW/g water use/plant)


(a)
Total N uptake (uMol/g WU/plant)
Days of withholding water


(b)
Total K uptake (uMol/ g WU/plant)
Days of withholding water


(c)
Total Mg uptake (uMol/g WU/plant)
Days of withholding water


(d)
Total Ca uptake (uMol/g WU/plant)
Days of withholding water


(e)
Water Use Efficiency (g DW/g water use/plant)
Days of withholding water

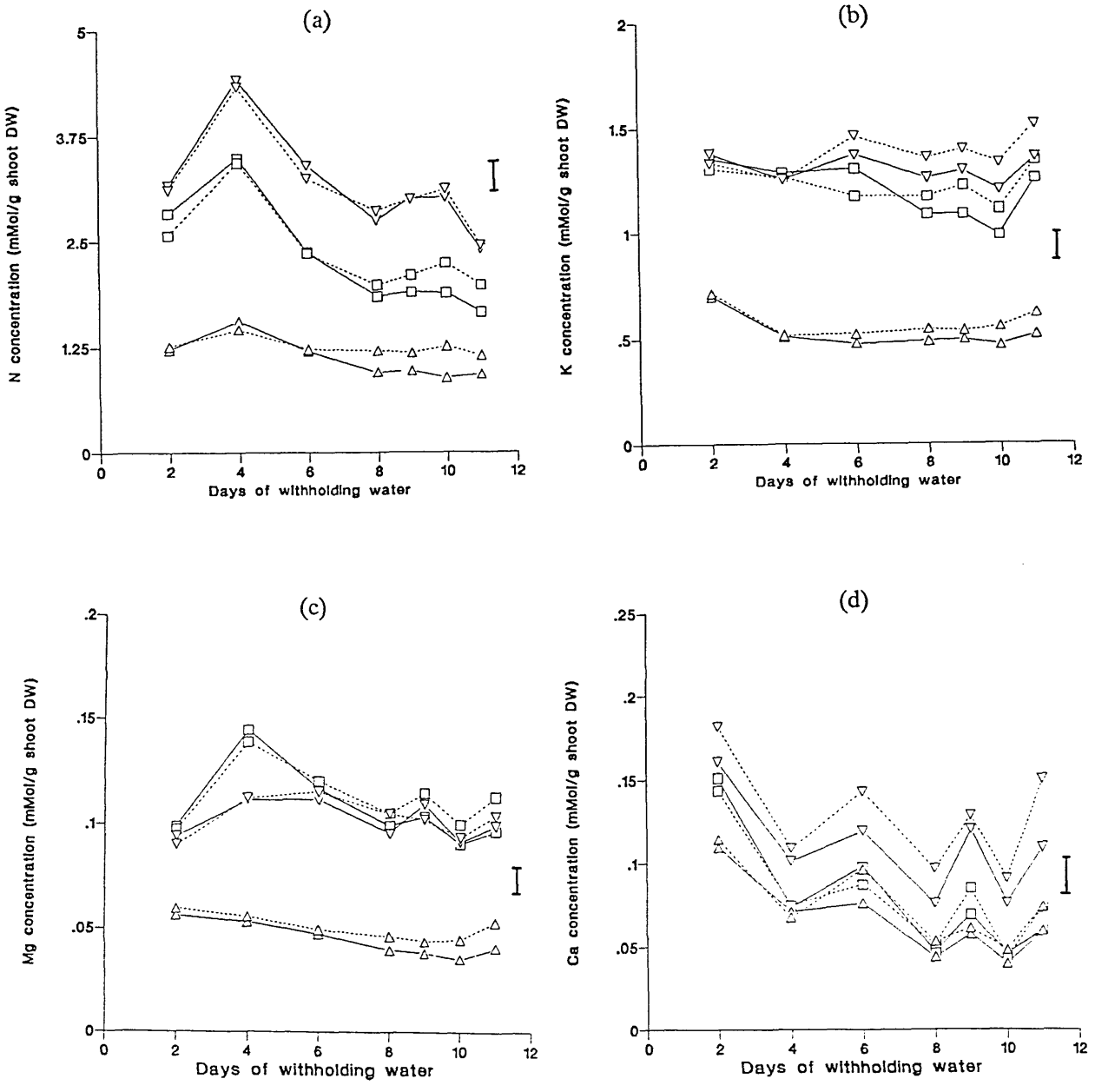



**Figure 4.4 (a-d)** The concentration of: a) N b) K c) Mg d) Ca (mMol/g DW), in shoot of Panicum ( □ ), Avena ( ▽ ) and Aristida ( △ ), grown under well-watered (——————) and water-stressed (- - - - - -) conditions.
Vertical bars are pooled SE's

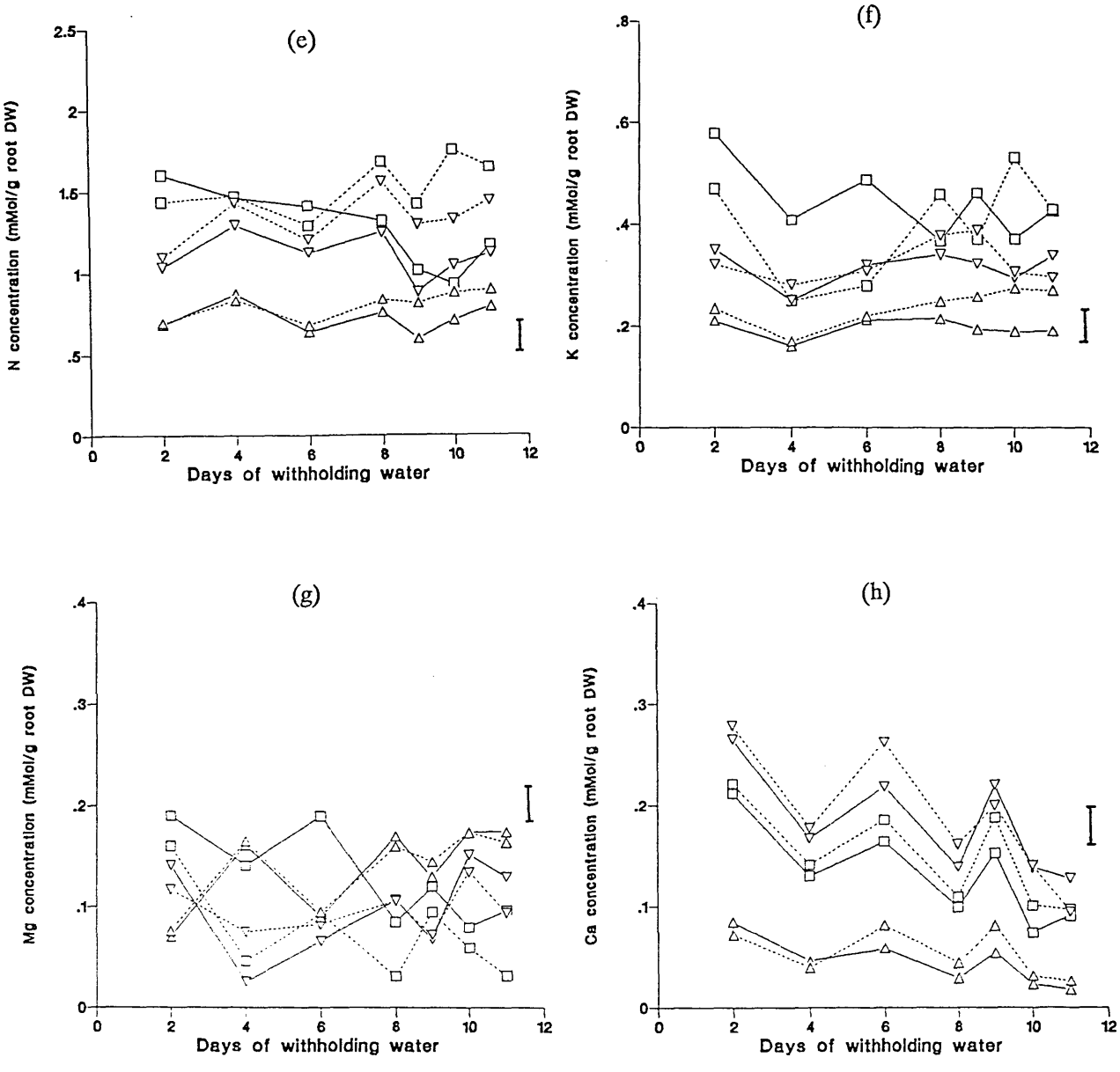



**Figure 4.4 (e-h)** The concentration of: e) N f) K g) Mg h) Ca (mMol/g DW), in root of Panicum ( □ ), Avena ( ▽ ) and Aristida ( △ ), grown under well-watered ( ——— ) and water-stressed ( - - - - - - ) conditions. Vertical bars are pooled SE's

Nutrient Uptake

The shoot N contents of Avena were greatly affected by water stress, and stressed plants showed large decreases earlier than Panicum or Aristida (Fig 4.5a). The data for Aristida showed no consistent differences between control and stressed plants despite their growth being very much reduced under stress conditions. Values for Aristida were consistently higher than the other species throughout, consistent with their increased total dry weights.

The N contents of stressed Avena roots declined significantly after 6 days of withholding water (Fig 4.5e), whilst those of Aristida increased and Panicum was not consistently affected. The N content of Avena roots was initially lower than Aristida despite having the highest root-shoot ratio.

The K contents of shoots of the control treatment of all species were higher than the stressed from day 4 onwards (Fig 4.5b), with Avena showing much larger differences than Panicum or Aristida. In contrast, the root K contents of Panicum and Aristida were not affected by water stress (Fig 4.5f), whilst that of stressed Avena was again lower than its control from day 6 onwards. All treatments of the three species increased with time.

The shoot Mg of the control plants of Panicum markedly increased with time and gave the highest uptake by the end of the stress period (Fig 4.5c). In general, the shoot Mg uptake in all three species, followed a similar pattern to K, in which the total uptake was reduced as the water stress progressed. In contrast to the shoot, the root Mg contents of all species were not significantly affected by stress (Fig 4.5g).

The amounts of Ca in shoots of control Aristida was much higher than other treatments although it showed inconsistent responses with time (Fig 4.5d), whilst those of both Avena and Panicum showed slight increases with time. Effects of stress were small, but generally showed reduced contents in stressed treatments. On the other hand, the root Ca content of Avena was greatly decreased by water stress, and that of Aristida was increased (Fig 4.5h), a pattern similar to that of N content.

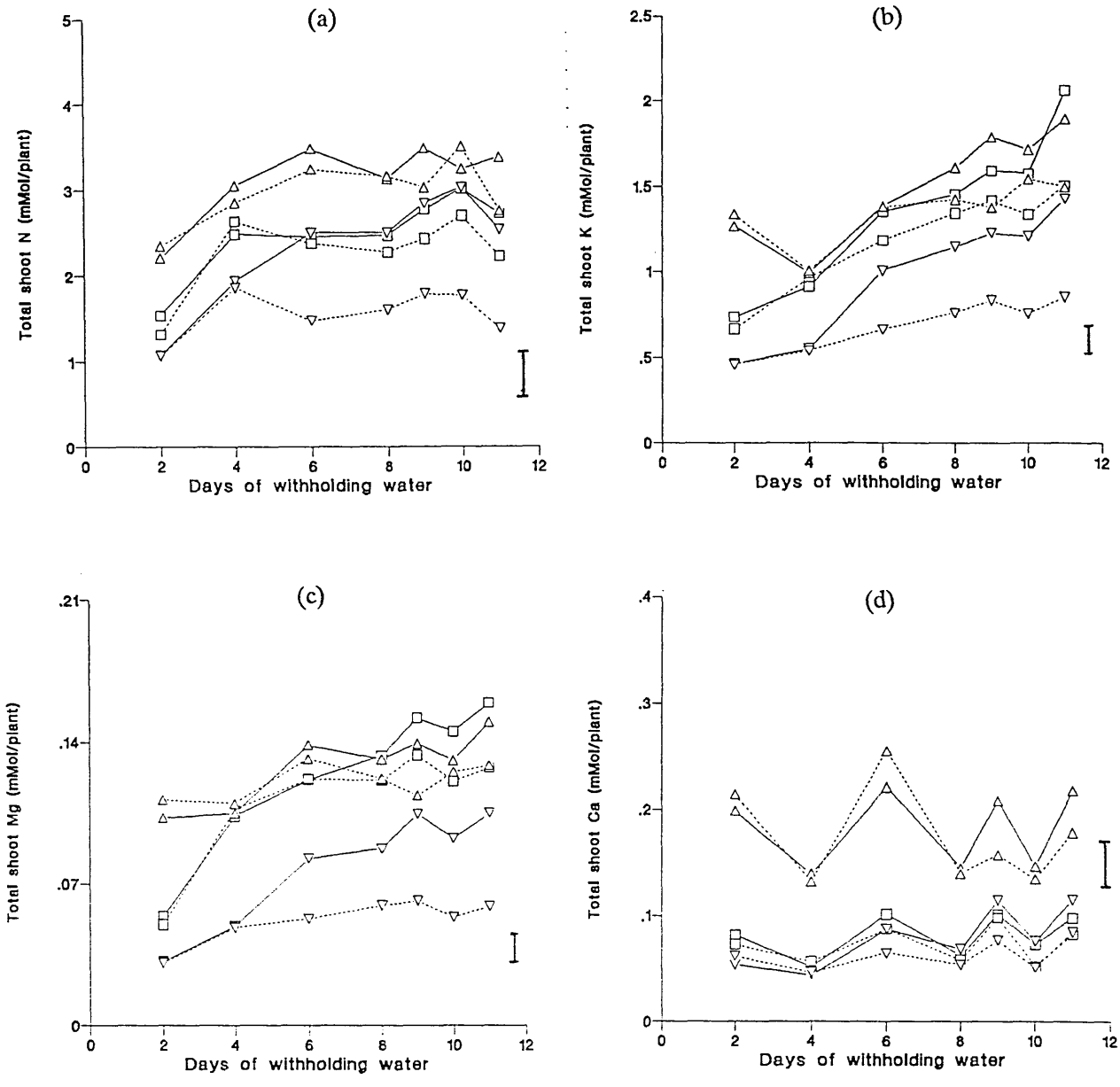



**Figure 4.5 (a-d)** The total shoot: a) N b) K c) Mg d) Ca (mMol/plant), in Panicum ( □ ), Avena ( ▽ ) and Aristida ( △ ), grown under well-watered ( ——— ) and water-stressed ( - - - - - - ) conditions. Vertical bars are pooled SE's

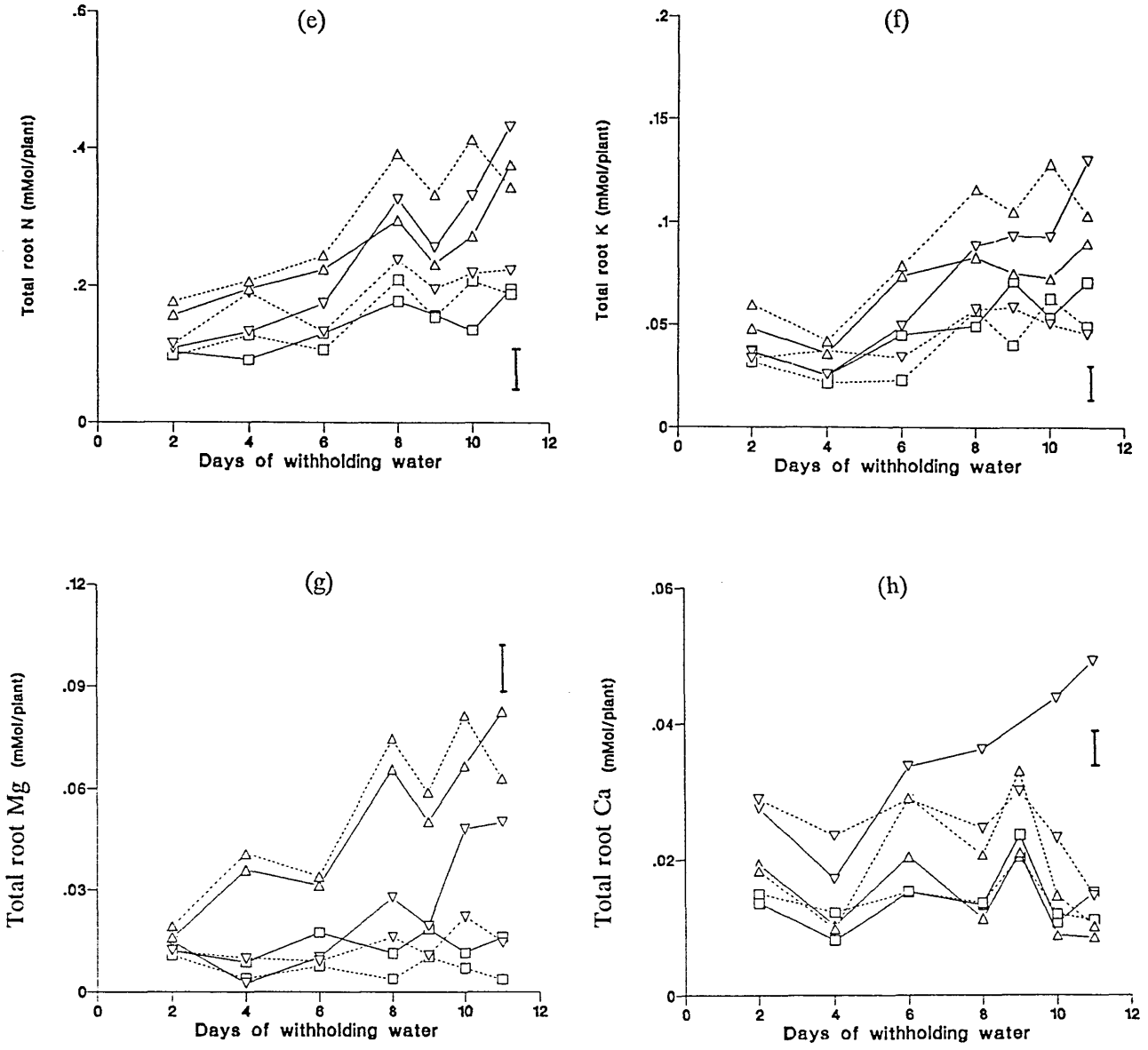



**Figure 4.5 (e-h)** The total root: e) N f) K g) Mg h) Ca (mMol/plant), in Panicum ( □ ), Avena ( ▽ ) and Aristida ( △ ), grown under well-watered ( ——— ) and water-stressed ( - - - - - ) conditions. Vertical bars are pooled SE's

Commentary

The plants of Aristida were substantially heavier than Panicum and Avena, even after a preliminary trial to study timing, and the attempt to produce uniform leaf areas for all species at the beginning of the experiment. Since leaf area in this case was determined solely as leaf lamina only, and that the Aristida leaf was very small, narrow and finely pointed compared to the other two species. This obviously created a lower leaf area ratio in aristida, in that the plants matched for leaf area were not matched for leaf number and weight.

This experiment is reported before earlier ones, such as Experiment 4.2.2 and several other experiments reported in this thesis, so as to ease the grouping of experiments and simplify later interpretation of the results.

In most of the experiments, the plants of Aristida were derived by vegetative propagation from a single parent. This was necessary to overcome the problem of very low germination rate of the seeds.

The larger control plants of Aristida used more water than those of Panicum but less than those of Avena. They also possessed lower nutrient concentrations in their shoots, but still had larger total contents for all the treatments.

The results showed that the general trends with time of nutrient concentrations in all the three species were quite similar, and there were no significant changes associated with water stress. The most likely explanation is that the experimental period was relatively short (11 days stress period) and the nutrients required were readily available from the soil reserves.

The increased root-shoot ratios of Aristida with stress suggests an adaptive mechanism. The values for the greater efficiencies of water use and the greater uptake of K shown by the roots of Aristida might also be similarly explained.

**Experiment 4.2.2 The response of Panicum and Avena at various growth stages under adequate and inadequate supplies of nitrogen and water.**

### *Material and Methods*

Seeds of both species were sown on 26/8/87 and seedlings transplanted after ten days into pots of soil. A randomised complete block design with four replications was used. The imposed treatments were two levels of N in Long Ashton nutrient and two levels of soil moisture:

(N1) 1.35 mM (18.9 ppm N)
(N2) 12.13 mM (170 ppm N)
(W0) FC (Field Capacity or control)
(W1) 1/2FC

Destructive sampling was used to assess the responses to the treatments used. Samples were taken at 7, 14, 21, 28, 35 and 42 days after transplanting. The different N concentrations were obtained by replacing some of the $KNO_3$ and $Ca(NO_3)_2$ with equivalent concentrations of KCl and $CaCl_2$ respectively, and were applied weekly. The soil moisture contents and the amount of distilled water required to maintained the predetermined soil moisture levels were estimated by weighing the pots daily. Fresh and dry weights of shoot and roots were determined, and shoot nutrient contents analysed.

### Results

Plant Growth

The effects of the two levels of N at various growth stages of Panicum and Avena under soil water stress on growth are presented in Figs 4.6a to 4.6c. The growth curves of all treatments were nearly linear, with the exception of the high N Panicum with no water stress. This particular treatment showed a complex growth curve, partly as the result of presumed sampling error at the beginning of the experiment, but mainly because this treatment flowered early and had begun to senesce during the course of the experiment. This then caused many of the interaction terms within the polynomial regression stages of the analysis of variance to become significant (Table 4.3).

Table 4.3 Analyses of variance of the effects of two nitrogen levels on total dry weight, dry matter content and root-shoot ratio of Panicum and Avena at various growth stages under soil water stress.

| Source of variation | df | Total dry weight (g/plt) | Dry matter content | Root-shoot ratio |
|---|---|---|---|---|
| | | Mean Squares (MS) | | |
| Harvest (H) | 5 | 15.389*** | 0.0287*** | 0.0633*** |
| Lin | 1 | 75.672*** | 0.141*** | |
| Quad | 1 | 0.776 *** | 0.00102** | |
| Dev | 3 | 0.166 ** | 0.000536** | |
| Nitrogen (N) | 1 | 32.480*** | 0.0120*** | 1.948*** |
| Stress (WS) | 1 | 5.204*** | 0.0234*** | 0.0539*** |
| Species (SP) | 1 | 1.282*** | 0.0526*** | 4.300*** |
| N x H | 5 | 2.513*** | 0.00329*** | 0.0705*** |
| Lin | 1 | 11.350*** | 0.104*** | |
| Quad | 1 | 0.282 ** | 0.0000104ns | |
| Dev | 3 | 0.310 *** | 0.00199*** | |
| N x WS | 1 | 0.192** | 0.00387*** | 0.0457*** |
| N x SP | 1 | 0.166* | 0.0000586 ns | 1.0803*** |
| WS x SP | 1 | 2.0646*** | 0.0108*** | 0.00373 ns |
| H x WS | 5 | 0.656*** | 0.00200*** | 0.0171** |
| Lin | 1 | 2.539*** | 0.00900*** | |
| Quad | 1 | 0.176* | 0.000650* | |
| Dev | 3 | 0.189** | 0.000115ns | |
| H x SP | 5 | 0.357*** | 0.0116*** | 0.00763 ns |
| Lin | 1 | 0.321** | 0.0574*** | |
| Quad | 1 | 0.929*** | 0.000345ns | |
| Dev | 3 | 0.178** | 0.0000419ns | |
| N x H x WS | 5 | 0.117** | 0.000882*** | 0.00797 ns |
| Lin | 1 | 0.0277ns | 0.00276*** | |
| Quad | 1 | 0.179 * | 0.000642* | |
| Dev | 3 | 0.127 ns | 0.000337* | |
| N x H x SP | 5 | 0.159*** | 0.000768*** | 0.00776 ns |
| Lin | 1 | 0.0683ns | 0.00220*** | |
| Quad | 1 | 0.281** | 0.000382ns | |
| Dev | 3 | 0.149* | 0.000420** | |
| N x WS x SP | 1 | 0.129* | 0.00335*** | 0.0753*** |
| H x WS x SP | 5 | 0.556*** | 0.00125*** | 0.00779 ns |
| Lin | 1 | 1.516*** | 0.00388*** | |
| Quad | 1 | 0.673*** | 0.000716** | |
| Dev | 3 | 0.197*** | 0.000550** | |
| N x H x WS x SP | 5 | 0.293*** | 0.000445*** | 0.00646 ns |
| Residual | 146 | 0.02701 | 0.00009659 | 0.004243 |
| Total | 188 | 0.7741 | 0.0019384 | 0.048126 |

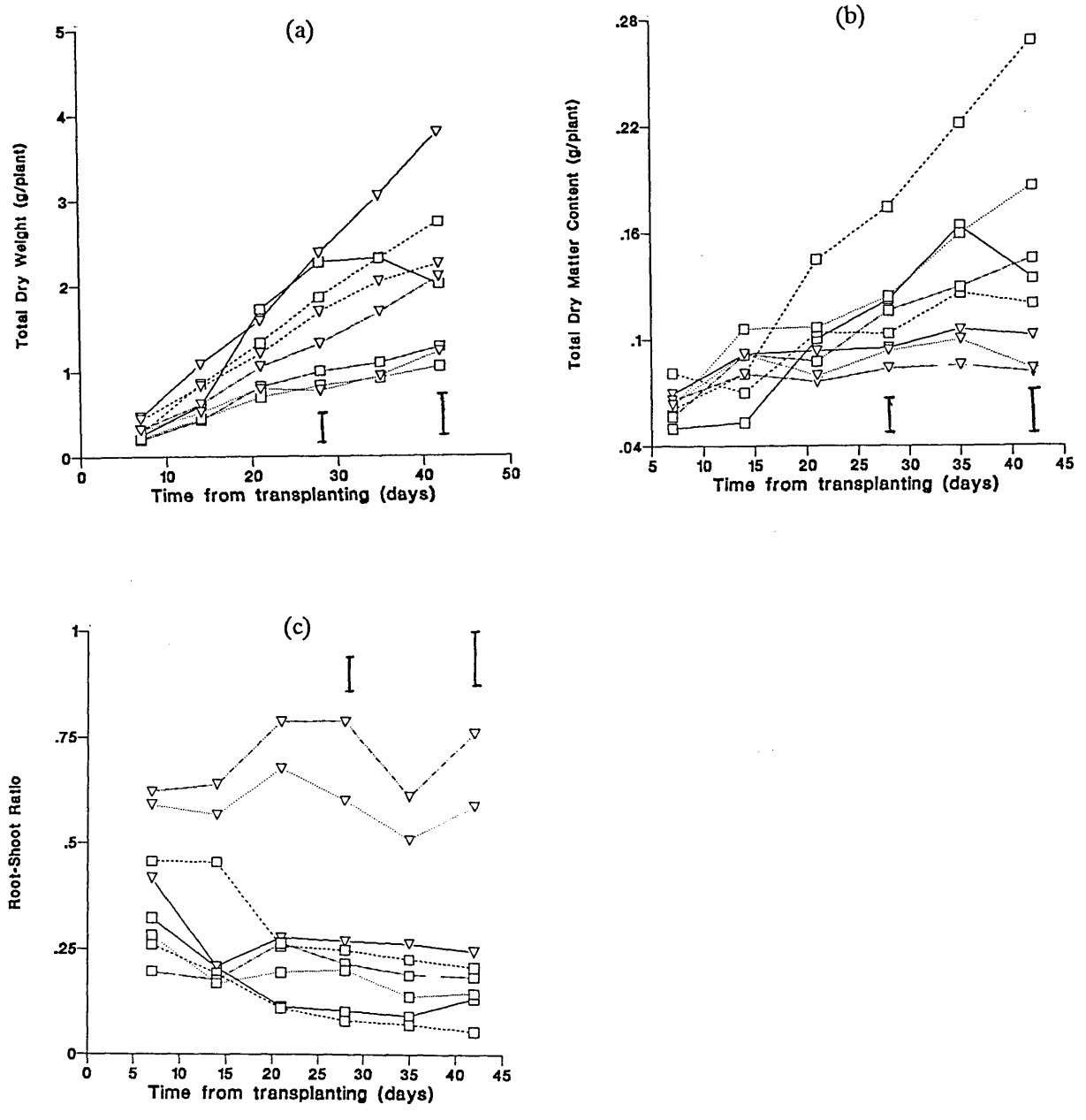



**Figure 4.6** a) Total dry weight (g/plant) b) Dry matter content (Total DW/Total FW) c) Root/shoot ratio, of Panicum ( □ ) and Avena ( ▽ ) with time, grown under well-watered and water-stressed conditions at low and high N levels.
Vertical bars are pooled SE's at the selected means.

( ——— ) High N (170.00 mM) under well-watered
( - - - - - - - ) High N (170.00 mM) under water-stressed
( —·—·—·— ) Low N (1.35 mM) under well-watered
( ····· ) Low N (1.35 mM) under water-stressed

The growth in total dry weight of both species was reduced both by water stress and by reduced N supply, and these effects were cumulative (Fig. 4.6a). Avena showed much larger responses to water stress than Panicum, with the differences between the two species being significant under high, but not under low N supply, as shown by the interaction effects of Nitrogen x Stress x Species in Table 4.3.

The dry matter contents of both species increased substantially in response to water stress, with Panicum showing a greater effect than Avena (Table 4.3 and Fig 4.6b). This caused all the main and interactions effect to reach significance, except for the Nitrogen x Species interaction.

The root-shoot ratio of Avena was consistently higher than Panicum (Fig 4.6c). The ratios of both species showed no consistent changes with time under low N supply but declined quite strongly with adequate N, and decreased with water stress except for Avena under high N. This caused all the main effects and the interactions of Nitrogen x Species and Nitrogen x Stress x Species to be significant.

Nutrient Concentration

The concentrations of N, K, Mg and Ca in the shoot dry weight of Avena and Panicum at various growth stages under two levels of N and water stress are illustrated in Fig 4.7a to 4.7d.

The concentration of N in the shoot of Avena under high N supply was consistently higher than other treatments (Fig 4.7a), and the concentrations in both species, as expected, were reduced with the lower N supply. The concentrations in Panicum decreased with water stress under high N, but showed no obvious changes under low N. Avena, on the other hand showed an opposite pattern of response.

Shoot K concentration of both species decreased with time although the stressed treatments of the high N Avena showed a smaller response than Panicum (Fig 4.7b). The concentrations in Avena also increased with water stress whilst Panicum showed inconsistent responses.

The shoot Mg of control Panicum was generally higher than Avena and the concentrations of both species showed slight decreases with time, although the treatments of high N Avena showed a gradual increase (Fig 4.7c). The concentration in the control Panicum was higher than the stressed at both levels of N supply, whilst Avena showed no obvious changes at the lower N.

The shoot Ca in the high N supply of Avena showed inconsistent responses with time (Fig 4.7d), whilst other treatments generally decreased with time and all treatments showed inconsistent responses to water stress.

Total Nutrient Uptake (shoot)

Patterns of nutrient uptake were broadly similar to those for dry weight change, in that there were differences between species and differences due to water stress only under high N conditions. The anamalous growth curve for Panicum under high N and no water stress was also observed for all nutrients.

The total N contents of both species were greatly reduced with the lower N supply. Under high N, the stressed plants showed larger decreases than the controls (Fig 4.8a). The total N in shoots of the control high N Avena was consistently higher than the other treatments.

The amounts of K in shoots of the two species were again greatly affected both by water stress and low N supply (Fig 4.8b). Avena showed much larger response to water stress than Panicum under both high and low N supply.

The shoot Mg in the controls of both species under high N supply markedly increased with time (Fig 4.8c). The contents of both species were greatly lowered with water stress, with Avena showing consistently larger responses than Panicum under both levels of N.
The shoot Ca of Avena in both control and stressed plants under high N supply showed large increases with time, whilst those in low N showed no obvious changes (Fig 4.8d). Stressed plants of the two species also showed lower contents than the controls, with greater effects under high N.

Commentary

It is common knowledge that plant growth is reduced when N or water become limiting. However, in this particular experiment plants grown under low N supply were less affected by water stress than the high N plants. This suggests that N stressed plants changed in several of the morphological and physiological characteristics which are associated with adaptation or resistance to water stress. The increased size of the high-N plants would obviously increase

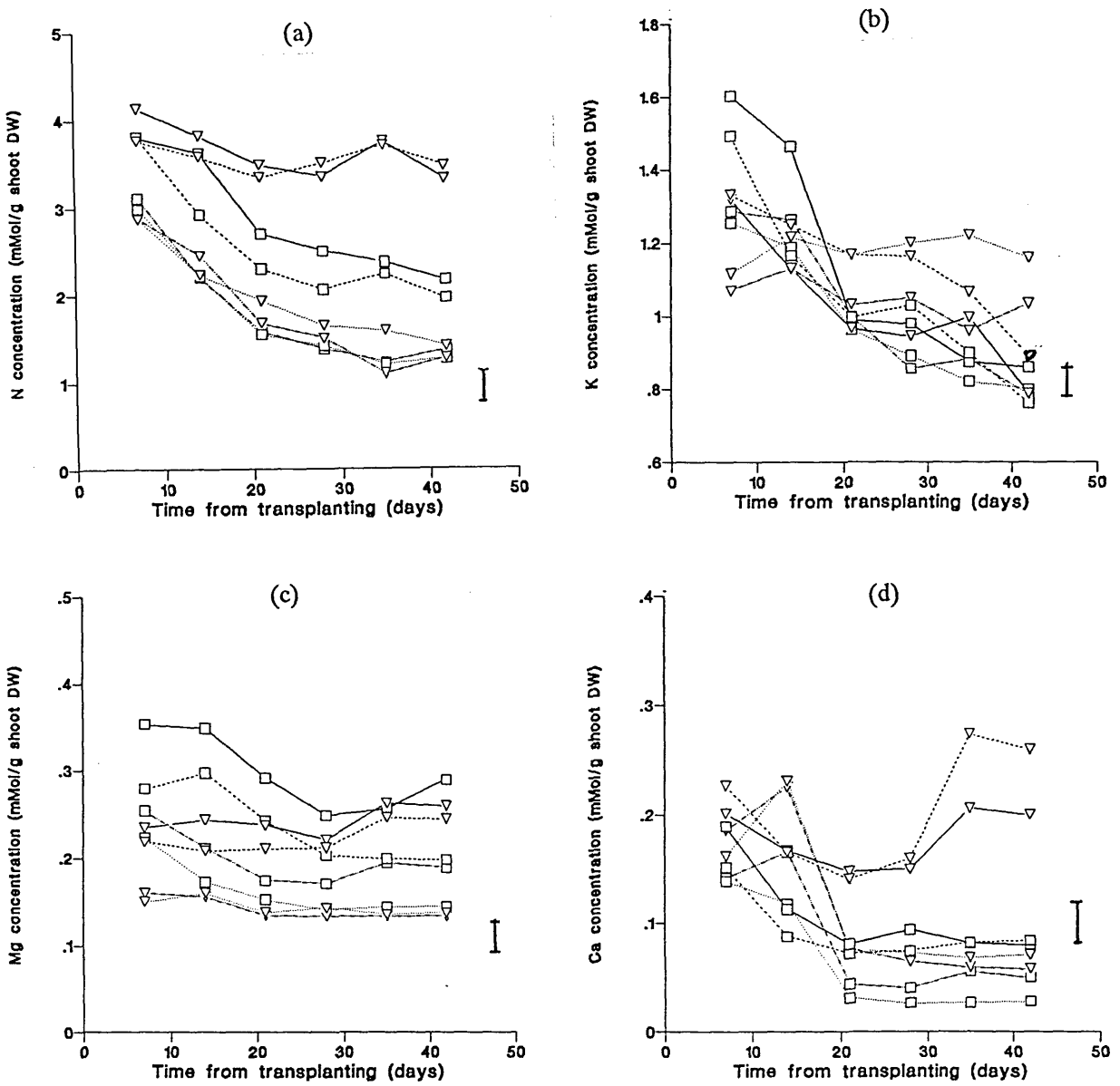



**Figure 4.7** The concentration of: a) N b) K c) Mg d) Ca (mMol/g DW), in shoot of Panicum ( □ ) and Avena ( ▽ ) with time, grown under well-watered and water-stressed conditions at low and high N levels.
Vertical bars are pooled SE's

(————) High N (170.00 mM) under well-watered
(- - - - - - -) High N (170.00 mM) under water-stressed
(—·—·—·—) Low N (1.35 mM) under well-watered
(·····) Low N (1.35 mM) under water-stressed

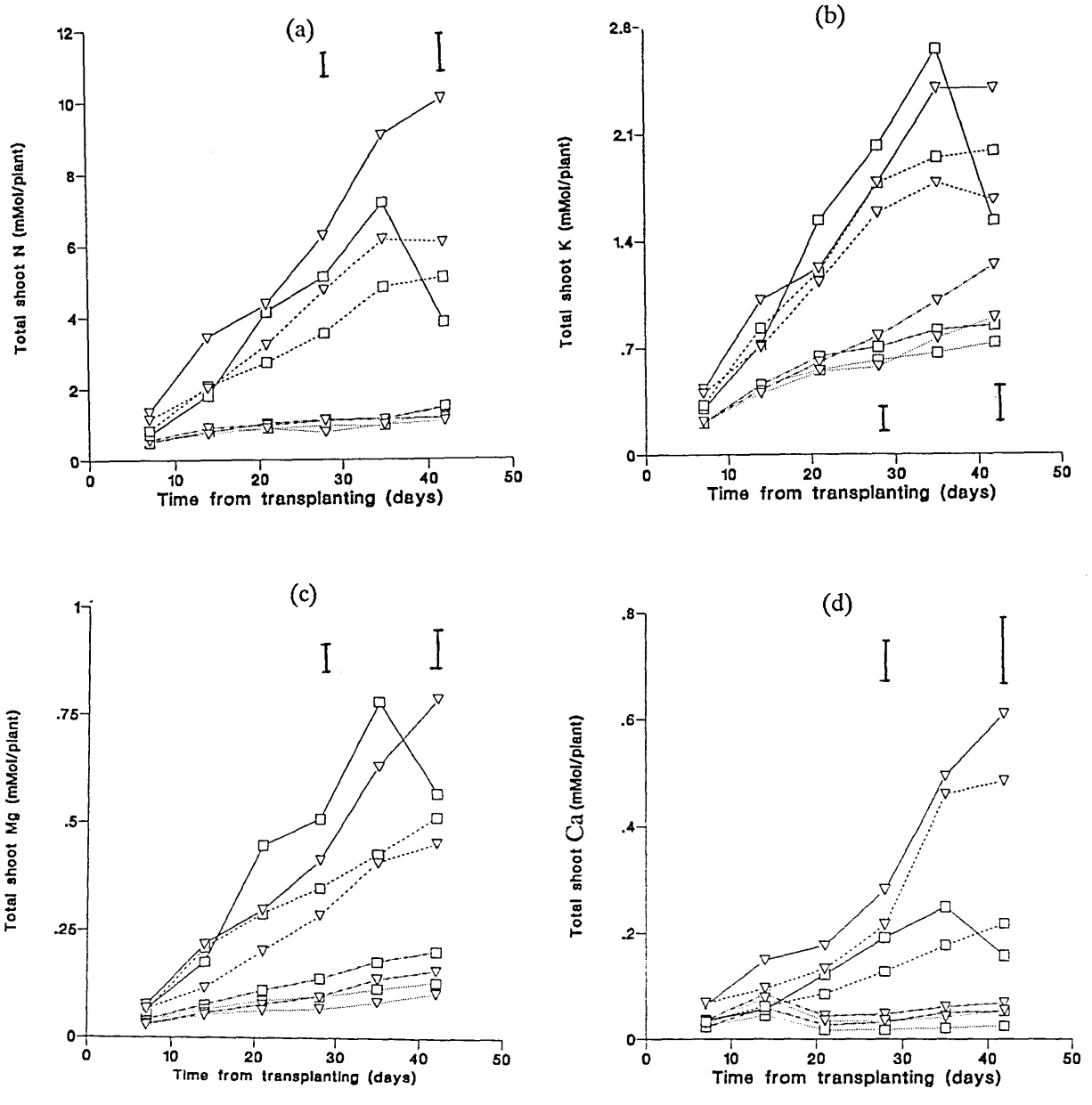



**Figure 4.8** The total shoot: a) N b) K c) Mg d) Ca (mMol/g plant) in shoot of Panicum ( □ ) and Avena ( ▽ ) with time, grown under well-watered and water-stressed conditions at low and high N levels.
Vertical bars are pooled SE's at the selected means.

(———) High N (170.00 mM) under well-watered
(- - - - - -) High N (170.00 mM) under water-stressed
(—·—·—·—) Low N (1.35 mM) under well-watered
(·····) Low N (1.35 mM) under water-stressed

their rates of water use, leading to a more rapid onset of internal water stress. In contrast, the higher root-shoot ratios of the N deficient plant may both reduce the transpiration and increase the roots' capability to extract water and nutrients so as to delay the onset of water stress, although there is no data to support this argument (water-use was measured).

It was also expected that the concentration of nutrients in the shoots of both species would decrease with increasing time. This explains why the larger plants possessed the lower concentrations. These decreases were not proportionate to the changes in weight however, so that the larger plants still accumulated more nutrient per plant except for Ca under low N conditions.

**Experiment 4.2.3 The response of Panicum and Avena to soil water stress at various growth stages.**

*Materials and Methods*

Seeds of Avena and Panicum were sown on 19/11/88. The seedlings were transplanted ten days later. A randomised complete block design with four replication was used. The plants were maintained at two levels of soil moisture: Field Capacity (FC) and 1/2 FC, and plants were harvested at 10, 20, 30, 40 and 50 days after transplanting. Long Ashton nutrient was used and applied weekly. Distilled water was used to maintain the predetermined soil moisture levels, following daily checks on water use. Fresh and dry weights of shoot and roots were determined, and shoot nutrient contents analysed.

*Results*

Plant Growth

The growth curves of the control treatments of the two species were almost linear, whereas the stressed were curved downward (Fig. 4.9a). This then caused the interaction of Harvest x Stress treatment to be highly significant. The differences were confined to the linear and quadratic regression terms only (Table 4.4).

Table 4.4 Analyses of variance of the effects of soil water stress on total dry weight, dry matter content and root-shoot ratio of Panicum and Avena at various growth stages.

| Source of variation | df | Total dry weight (log) (g/plant) | Dry matter content (log) | Root-shoot ratio (log) |
|---|---|---|---|---|
| | | Mean Squares (MS) | | |
| Harvest (H) | 4 | 18.610*** | 0.948*** | 0.188* |
| Lin | 1 | 73.307*** | 3.582*** | 0.0504ns |
| Quad | 1 | 1.114*** | 0.0560*** | 0.0650ns |
| Dev | 2 | 0.0104ns | 0.0779*** | 0.318** |
| Stress (WS) | 1 | 1.0247*** | 0.450*** | 9.309*** |
| Species (SP) | 1 | 2.487*** | 0.252*** | 1.304*** |
| H x WS | 4 | 0.333*** | 0.0870*** | 0.0878ns |
| Lin | 1 | 1.100*** | 0.145*** | 0.0276ns |
| Quad | 1 | 0.175*** | 0.187*** | 0.193* |
| Dev | 2 | 0.0250ns | 0.00824ns | 0.0653ns |
| H x SP | 4 | 0.403*** | 0.109*** | 0.143* |
| Lin | 1 | 1.328*** | 0.257*** | 0.140* |
| Quad | 1 | 0.274*** | 0.0753*** | 0.0107ns |
| Dev | 2 | 0.0054ns | 0.0521*** | 0.210* |
| WS x SP | 1 | 0.147** | 0.0069ns | 0.438** |
| H x WS x SP | 4 | 0.0122ns | 0.0109ns | 0.222** |
| Lin | 1 | 0.0104ns | 0.0121ns | 0.380** |
| Quad | 1 | 0.0104ns | 0.0127ns | 0.181ns |
| Dev | 2 | 0.0141ns | 0.00942ns | 0.164* |
| Residual | 57 | 0.01528 | 0.00488 | 0.04776 |
| Total | 76 | 1.07840 | 0.0738 | 0.21493 |

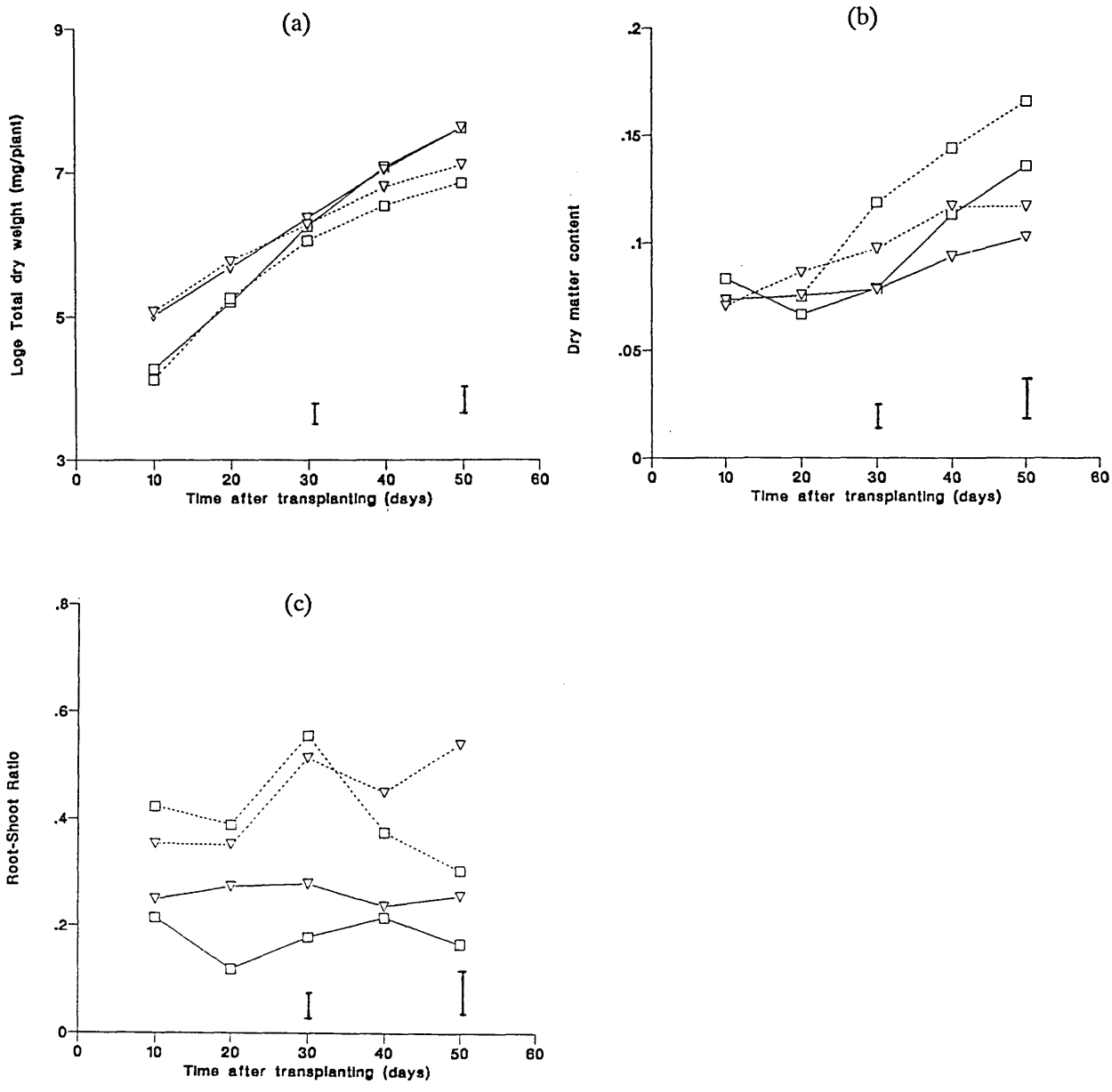



**Figure 4.9** a) Total dry weight (g/plant) b) Dry matter content (Total DW/Total FW) c) Root/shoot ratio, of Panicum and Avena with time, grown under well-watered and water-stressed conditions.

Vertical bars are pooled SE's at the selected means.

( □——□ ) Panicum under well-watered condition
( ▽——▽ ) Avena under well-watered condition
( □- - - - - - - □ ) Panicum under water-stressed condition
( ▽- - - - - - - ▽ ) Avena under water-stressed condition

The total dry weights of all increased with time and the increases were obviously greater with no water stress (Fig 4.9a). The effect of water stress in this experiment was greater for Panicum than Avena. This caused all the main effects and the interactions with water stress (Harvest x Stress and Species x Stress) to be significant.

The dry matter:fresh weight ratios of the stressed treatments of both species were higher than the controls, although the values during the first 20 days after transplanting were not consistent (Fig 4.9b). Panicum showed a slightly greater effect than Avena and all treatments increased with time.

The root-shoot ratios of the stressed treatments of both species were consistently higher than the controls (Fig 4.9c), and there were no consistent changes with time.

Nutrient Concentration

The concentration of N in the shoot of Avena was consistently higher than Panicum and showed no significant differences due to water stress (Fig 4.10a). Panicum on the other hand showed consistently lower concentrations with water stress, and the concentrations of both species decreased with time.

Shoot K concentrations of the controls of both species were consistently higher than the stressed treatments, with Panicum showing much larger differences than Avena (Fig 4.10b). The shoot K of both species increased with time up to 20 days after transplanting and then declined.

The shoot Mg of Avena increased with time, but Panicum decreased. This response in Avena was greater in the control treatment, whilst the response in Panicum was greater with stress (Fig 4.10c). The concentrations in both species were greatly reduced by water stress after 20 days from transplanting.

The concentration of Ca in the shoot of Avena was again markedly increased with time (Fig 4.10d). In contrast, the shoot Ca of Panicum decreased whilst both species showed consistently lower concentrations with water stress.

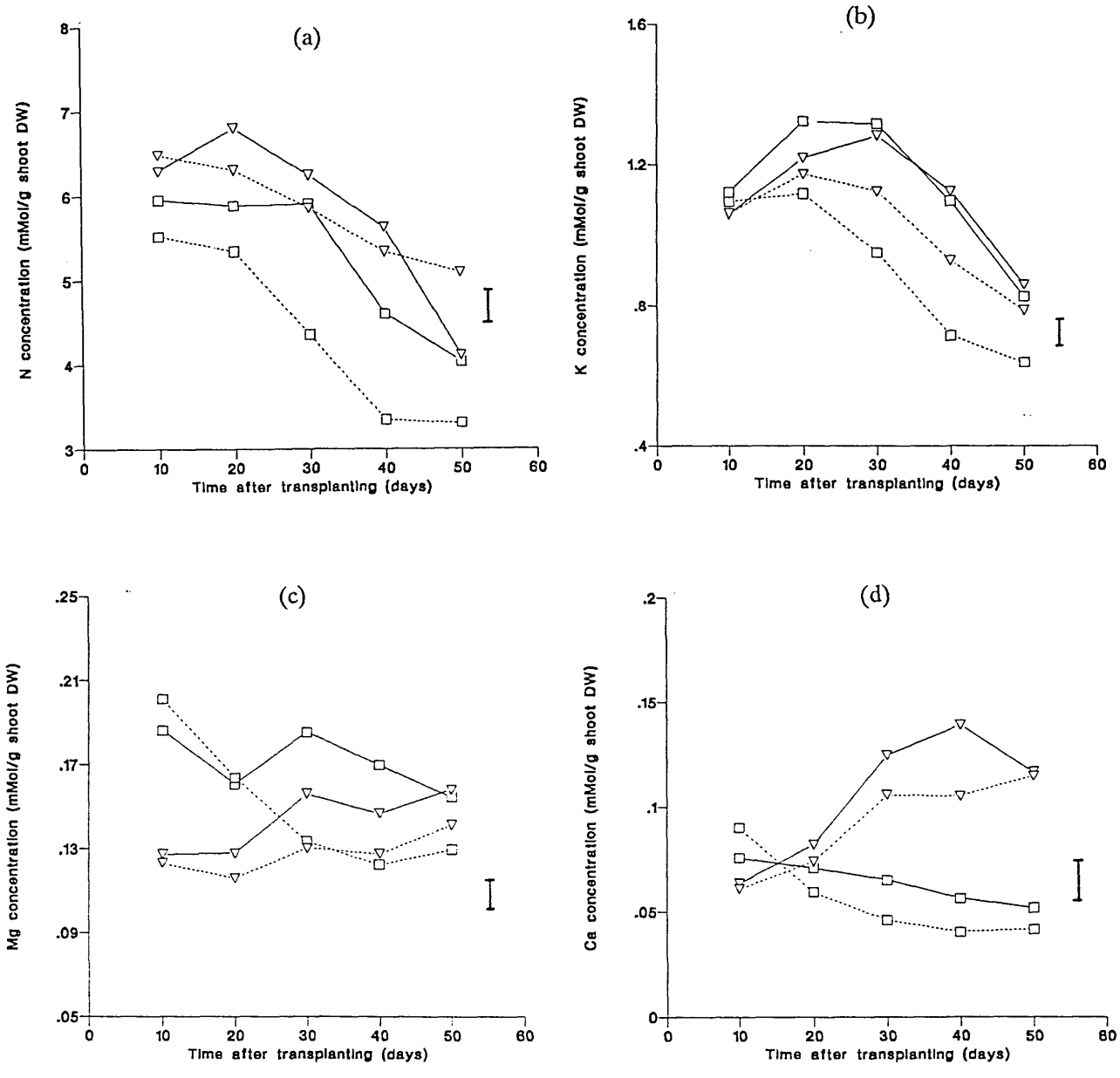



**Figure 4.10** The concentration of: a) N b) K c) Mg d) Ca (mMol/g DW), in shoot of Panicum and Avena with time, grown under well-watered and water-stressed conditions. Vertical bars are pooled SE's

Panicum under well-watered condition
Avena under well-watered condition
Panicum under water-stressed condition
Avena under water-stressed condition

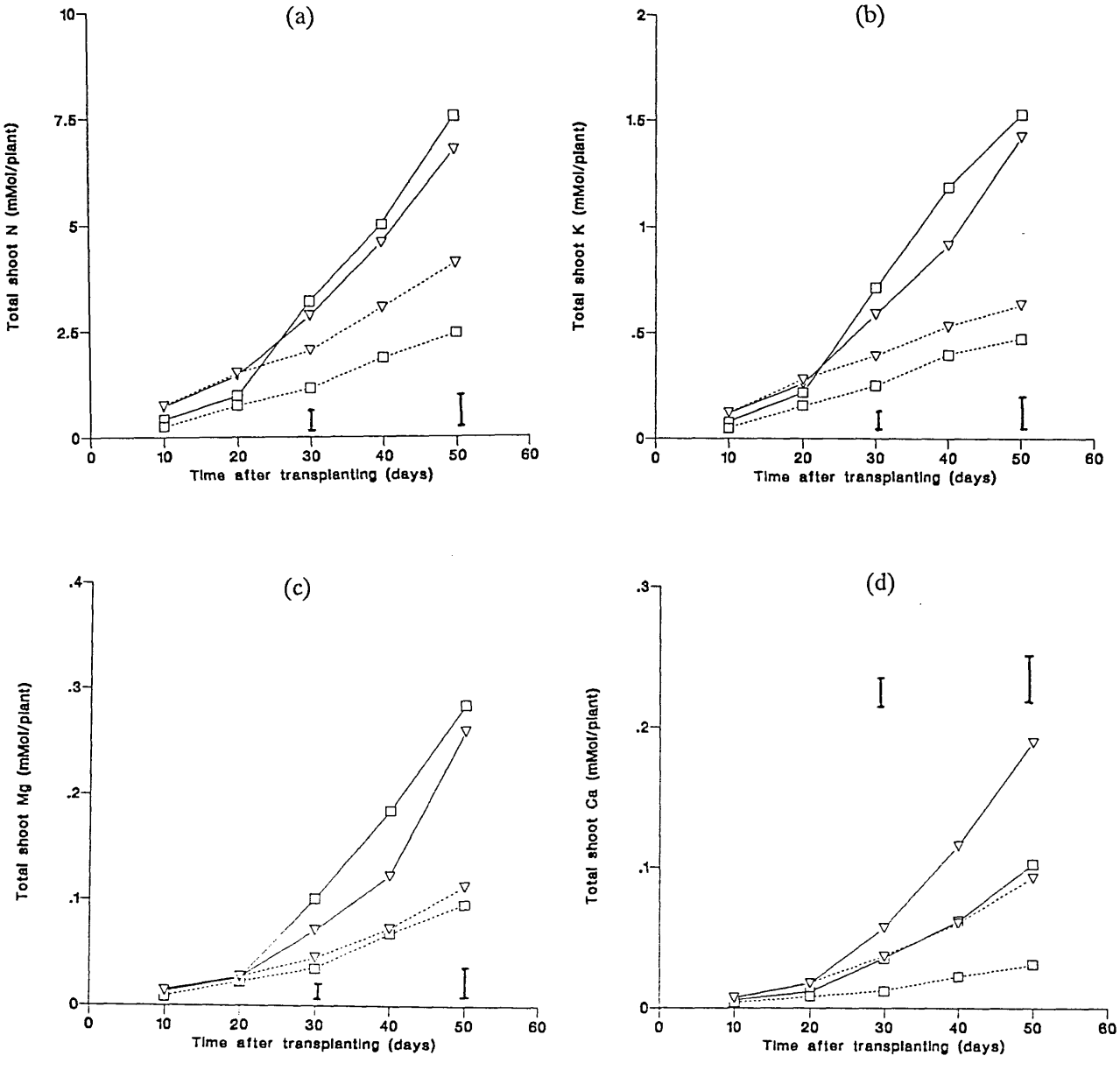



**Figure 4.11** The total shoot: a) N b) K c) Mg d) Ca (mMol/g plant) in shoot of Panicum and Avena with time, grown under well-watered and water-stressed conditions. Vertical bars are pooled SE's at the selected means.

Panicum under well-watered condition
Avena under well-watered condition
Panicum under water-stressed condition
Avena under water-stressed condition

Total Nutrient Uptake

The total N contents of the controls of both species were markedly increased with time, consistently with their increased total dry weights (Fig 4.11a). The shoot N in both species was greatly reduced by water stress, with Panicum showing much larger responses than Avena.

The K content in both species followed a similar pattern to N, in which the shoot K of both species was greatly reduced with water stress, and with Panicum showing the larger responses (Fig 4.11b).

The shoot Mg of both species again followed the same pattern, although the responses of the stressed treatments were not significantly different between the species (Fig 4.11c).

The shoot Ca in the control of Avena was consistently higher than other treatments, and showed larger increases with time (Fig 4.11d). The stressed plants of both species had much lower contents than the controls with Panicum showing the lowest value.

Commentary

The total dry weights of both species in this experiment were less affected by water stress than in previous experiments, with Panicum showing a much larger effect than Avena. In contrast, in experiment 4.2.2 there were no consistent differences between these species. The most likely explanation is the different growing seasons which caused differences in the rates of water loss and therefore in the average soil water stresses experienced by plants. This particular experiment was carried out during the winter months of November to January, so the plants experienced lower water stress due to the lower water loss through evapotranspiration. Another possible explanation might be the reduced light received during this time of year, although the natural irradiance was supplemented with artificial light throughout the experiment.

## 4.3 Discussion

Plant growth and Water Consumption

The results of experiment 4.2.1 showed that water stress increased the root/shoot ratio in Aristida, whilst the ratios of Panicum and Avena showed no consistent changes with time. The data therefore did not strongly support the previous observations that ratio of Aristida was insensitive to water stress. Such variability between experiments has been observed by many other workers. One example dicussed earlier is that of *Lolium perenne*, in which water stress either increased, decreased or caused no changes on the ratio (Gales, 1979; Jackson, 1971 and Jupp, 1986).

The different responses of Panicum, Avena and Aristida ratios in this experiment indicate the differences between these species in their adaptability to water stress. As already mentioned earlier, many workers have observed the changes in root/shoot ratios with changes in environmental stresses. For example, Blum *et al.* (1983) considered high root mass production in wheat to be associated with drought resistance. Similar finding have also been reported by Tanguilig *et al.*(1987) in maize plants. It thus suggested that the increased root relative to shoot weights for Aristida in this experiment, may be considered to be an adaptive feature of this species to resist water stress.

The control treatments of Panicum and Avena showed increasing absolute growth rates with time, but Aristida had a constant growth rate (Fig 4.2a). On the other hand, the absolute growth rates for the stressed treatments of all the three species declined with time as the severity of water stress increased, with Aristida showing the greatest change. This may suggest several possible advantages of having a low growth rate, particularly under limited water supply as shown in this experiment. However, the different response of Aristida was not surprising since the initial plants were substantially larger than the other two species. Chapin (1980) has suggested that plants which are adapted to environmental stresses such as water stress, also grow slowly and share many of the characteristics of plants adapted to infertile soils. This is in agreement with the results of experiment 4.2.2 which showed that the growth of Avena and Panicum under low N supply were less affected by water stress than the high N plants. One possibility is that the increased root/shoot ratios of N-deficient plants may have reduced water use and simultaneously increased the water supply. Other workers have also found modifications in leaf structure in N-deficient plants which act to resist water stress. For example, Radin and Parker (1979) showed that alterations in cell size and wall properties of N-deficient cotton plants

mimicked the structural changes which developed during adaptation to water stress. Radin and Ackerson (1981) also reported that stomata of N-deficient plants were more sensitive to water stress.

Water use efficiency (WUE) is another important physiological characteristic which is related to the ability of species to cope with water stress. In the present study, WUE (presented in Fig 4.3e), for Aristida was higher than Panicum or Avena throughout. In all the three species, however, the stressed treatments showed greater WUE's than well-watered plants. This is in agreement with the reports of Singh and Kumar (1981), who reported that water stress increased WUE in wheat and barley, but it conflicts with the reports of Johnson *et al.* (1984) and Heitholt (1989), who reported that water stress decreased WUE in wheat. More detailed investigations (e.g. Cowan, 1982; Raschke, 1979; Delucia and Heckathorn, 1989) have shown that the increase in WUE is due to the non-linear relationship between photosynthesis and transpiration whereby stomatal closure causes proportionately greater decreases in transpiration than photosynthesis. This therefore suggests that the maintenance of high WUE by the stressed plants in this experiment, especially Aristida, may be largely due to decreasing stomatal conductance causing smaller decreases in the rates of net photosynthesis than in transpiration.

In dry habitats greater WUE in plants using the $C_4$ photosynthetic pathway is well established (e.g. Osmond *et al.*, 1982), and this was reflected in the response of Aristida in the present study. For instance, in Aristida along with the greatest WUE compared with the other species was a greater allocation of photosynthates below ground (i.e. a high root/shoot ratio). This created a relatively smaller shoot biomass subject to moisture loss which may have further contributed to increased WUE. Izzo *et al.*(1989) observed that reduced rates of water use caused the onset of more severe stress to be delayed. Thus the success of Aristida in dry habitats, as with other perennial $C_4$ grasses, possibly depends on its longer growing duration. In this study there is no evidence to support this contention, but the interpretation is complicated by the initially larger plants of Aristida which were used.

Nutrient Uptake and Accumulation

Data in the literature provides no clear account of element distribution in water stressed plants. Experiment 4.2.1 showed that the contents of all four elements in the shoots of Panicum and Aristida were only slightly reduced by water stress whilst their growth was substantially reduced. In contrast, total nutrient content in Avena was reduced more than its growth. This strongly supports the results of the experiments presented in Chapter Three, that nutrient uptake of these three species differs greatly depending on the severity of water stress imposed.

The results in experiment 4.2.2, in which the soil moisture content was returned to the required levels daily, showed that there were greater decreases in growth than in N uptake at both high and low N supply in water stressed plants of Avena. On the other hand, N uptake in Panicum was reduced more than its growth at high N but the effect was reversed in low N treatments. This indicates that the decreased growth of Avena and Panicum were primarily due to low water availability not low N availability.

Nutrient uptake was highly correlated with water use under water stress conditions (Fig 4.3a to 4.3d). The nutrient uptake/water use ratios in all the three species were fairly constant with time after four days of withholding water. Since the rates of water use declined substantially from four to eleven days, this indicates that the higher the transpiration rate, the greater was the the nutrient uptake. This is in general agreement with Verasan and Phillips (1978) who reported N, P and K uptake of corn to be linearly related to cumulative transpiration. However, it disagrees with O'Toole and Baldia (1982) who observed the stressed rice plants continued taking up nutrients but the uptake rates were not as closely related to cumulative transpiration as in the control plants. In the present experiment nutrient uptake of well-watered plants was not closely related to water use except for total cation uptake by Avena roots. Aristida and Panicum under well-watered conditions showed consistent deceases in the ratios of nutrient uptake to water use, suggesting that nutrient uptake under these conditions was influenced more by the capacity of the roots to absorb nutrients than by transpiration.

In the case of Aristida, the increased total root N, K and Ca with increasing water stress may suggest an adaptive mechanism. It also suggests that nutrient absorption by the roots was still effective even under severe water stress. This agrees with Tanguilig *et al.*(1987), who found that root N and K uptake of maize was increased with water stress. They then suggested that the ability of maize to grow better than rice under water stress conditions was due to the continued uptake of nutrients, especially K, allowing osmotic adjustment to occur. The decline of the total shoot K in stressed Aristida, on the other hand, may be due partly to the capacity of the decreased transpiration stream to transport nutrients from roots to shoots. The roots themselves were still able to absorb nutrients from the soil, as demonstrated by their increased nutrient content. Similarly, transport of N, Ca and Mg to the shoots occurred even at the reduced transpiration rates. The reduced shoot K levels must therefore have been caused by reduced transport. This suggests that the effect of water stress on transport to the shoots was independent of its effect on uptake into the roots.

Transpiration and nutrient uptake in Avena were closely correlated in both roots and shoots. This agrees with Greenway and Klepper (1969), for example, who found that the translocation of nutrients to the shoot was largely influenced by the transpiration rate. The result is, however, in contrast with those for Aristida discussed above.

The soil moisture level has a marked effect on the absorption of solutes by whole plants. Barber (1962) distinguised three mechanisms of ion movement to the root surface: mass flow, diffusion and root interception. He suggested that when nutrient concentration in the soil system was high in a well-watered soil, mass flow dominated the uptake processes and was closely related to cumulative transpiration. As the soil dries, highly soluble ions such as $Cl^-$ and $NO_3^-$ will increase in concentration, together with equivalent increase in the total concentration of cations such as $K^+$, $Na^+$, $Ca^{2+}$ and $Mg^{2+}$ (Pitman, 1981). In drier soils, on the other hand, Nye and Tinker (1977) showed that diffusion coefficients near roots decreased sharply due to the local decreases in water level. They presented evidence from experiments at varying soil moisture levels in which transpiration was minimised so that transport was solely by diffusion. They found that reduced soil moisture caused reduced uptake in spite of the increased concentration of ions in the soil solution. In the present experiment after four days, the stressed treatments of all the three species continued to take up nutrients and the total uptake/water use ratios were generally constant with time. This may indicate a shift in the dominant role of mass flow to that of diffusion in transport of nutrient to the root surface.

Other workers have reported that nutrient uptake by plants is decreased under water stress conditions mainly due to reduced transpiration (e.g. Greenway and Klepper, 1969; O'Toole and Baldia, 1982; Yambao and O'Toole, 1984) and impaired active transport and membrane permeability (Hsiao, 1973) which resulting in reduced root absorbing power. Water stress also caused substantial death of root cortex and root hairs in *Lolium* (Jupp and Newman, 1987). Furthermore, evidence of decreased ion absorption due to reduced root absorption power, as affected by water stress was also reported by Dunham and Nye (1976). This may suggest that the reduced nutrient uptake by the stressed plants for all the three species in the present study was closely linked to soil water status. A decline in soil moisture was associated with the decrease in diffusion rate of nutrients from the soil matrix to the absorbing root surface (Viets, 1972). It may also suggest that the greater decrease in nutrient uptake by Avena than Panicum and Aristida under water stress conditions was primarily due to its substantially reduced growth. Whilst the ability of Panicum and Aristida to grow better than Avena under water stress conditions may be partly due to their ability to maintain the roots' absorptive capacity to continue uptake of nutrients, especially K, so allowing osmotic adjustment to occur.

The present investigation has shown that nutrient uptake of Panicum, Avena and Aristida was highly correlated with water use under water stress conditions. The uptake of well-watered plants was not closely related to water use except for total cation uptake by Avena roots. It also suggests that the effect of water stress on transport to the shoots was independent of its effect on uptake into the roots. The transport of nutrients from the roots to the shoots occurred even at reduced transpiration rates.

These relationships warrant further investigations using other methods of inducing water stress. The direct approach of varying the availability of water in the soil, in all the experiments discussed earlier, has shown the need to consider movement of ions in the soil as well as properties of plants in assessing the effects of water flow on nutrient uptake. In the next Chapters (Chapter 5 and 6), experiments in which PEG was used to induce constant levels of water stress are described. These solutions of high osmotic potential were used in order to avoid the problems of nutrient transfer in dry soil and to show the effect of low water potentials in the solution on uptake by the roots, transport to the shoot and associated effects on growth.

# CHAPTER FIVE

## Studies of Polyethylene Glycol-induced Water Stress on Growth, Water Consumption and Nutrient Uptake of *A.funiculata*, *A.fatua* and *P.miliaceum*

# CHAPTER FIVE

## STUDIES OF POLYETHYLENE GLYCOL-INDUCED WATER STRESS EFFECTS ON GROWTH, WATER CONSUMPTION AND NUTRIENT UPTAKE OF *A.fatua* (Avena), *A.funiculata* (Aristida) AND *P.miliaceum* (Panicum).

### 5.1 Introduction

One of the factors which continuously interacts with the effects of soil water stress on nutrient uptake is the effect of total salt concentration. The use of PEG offers the possibility of separating water stress from salt effects. Although PEG-induced water stress is osmotic in nature and may not be exactly the same as that occurring in soils, it is a sensitive method which can create small degrees of water stress that are not easily induced in soils on a continuous basis (Lagerwerff *et al*, 1961). Short duration stresses at different levels may have significant effects on water use, growth and nutrition of $C_3$ and $C_4$ grass species of different origins. In order to investigate these effects, this study attempts to determine the effects of PEG-induced water stresses on water consumption and nutrient uptake of Panicum, Avena and Aristida at different levels of N and Ca nutritions, under varying levels of osmotic stress.

### 5.2 Experiments

#### Experiment 5.2.1 PEG induced water stress effects on water consumption and growth of Panicum, Avena and Aristida.

*Material and Methods*

The seeds of Avena and Panicum were sown in trays of vermiculite on 3/4/88, whilst plants of Aristida were derived by vegetative propagation from a single parent, starting about 10 days earlier. A week later the plants were transplanted into plastic tubular containers (of volume of 65 $cm^3$) filled with vermiculite, and were grown under well watered conditions and supplied with half strength Long Ashton solution. After 25 days, when the roots systems were well developed, they were transferred (together with the root-vermiculite mass) into tubes of water-

rinsed semipermeable membrane (dialysis tubing). The stems of the plant-membrane systems were inserted into slits cut into styrofoam caps which were fitted tightly on to the 250 $cm^3$ jars. To avoid problems with the open ends of the membranes, they were folded at the base and raised back to the tops of the containers. The plant-membrane systems were equilibrated for 3 hours in distilled water and then transfered to nutrient solutions containing 0, 7, 15, and 22% of PEG 4000 (% w/v). A randomised complete block design with three replication was used. The plants were grown in aerated solution culture jars of PEG for eight days. Water use by the plants was determined by weighing the jars daily and the water loss was calculated. Ambient air temperatures and humidities were recorded.

Plant transpiration rates were calculated from daily water loss data. Evaporation through the styrofoam caps was determined from jars with no plants. Evaporation losses were deducted from the total water losses before determining transpiration. Water lost was replaced daily to maintain 250 $cm^3$ water/jar. At the end of experiment, shoot fresh weights and water use were determined.

## *Results*

### Water Consumption

The daily water consumption of plants of all the three species (Fig 5.1a to 5.1c), followed the changes in air temperature and relative humidity with the exception of the first 3 days (Fig 5.2). During this adaptation period the rate of transpiration decreased in all treatments with greater responses shown by the controls, but very further little difference due to water stress severity. The adaptation period was followed by a 'response period' during which the patterns of water consumption for all three species were broadly similar, in that they were decreasing with increasing applied stress. Changes in water consumption in response to air temperature fluctuations became smaller as the concentration of PEG increased.

The daily water consumption of the controls of Panicum, Avena and Aristida (Fig 5.1a, 5.1b and 5.1c, respectively), were consistently higher than other treatments. All the three species responded proportionally to the levels of PEG-induced water stress. The water consumption decreased with increasing stress, although the 15% and 22% PEG stressed plants of Avena showed no differences between the treatments. The rates of water consumption by the three species, increased with time, with the exception of the adaptation period and the controls of Panicum and Avena.

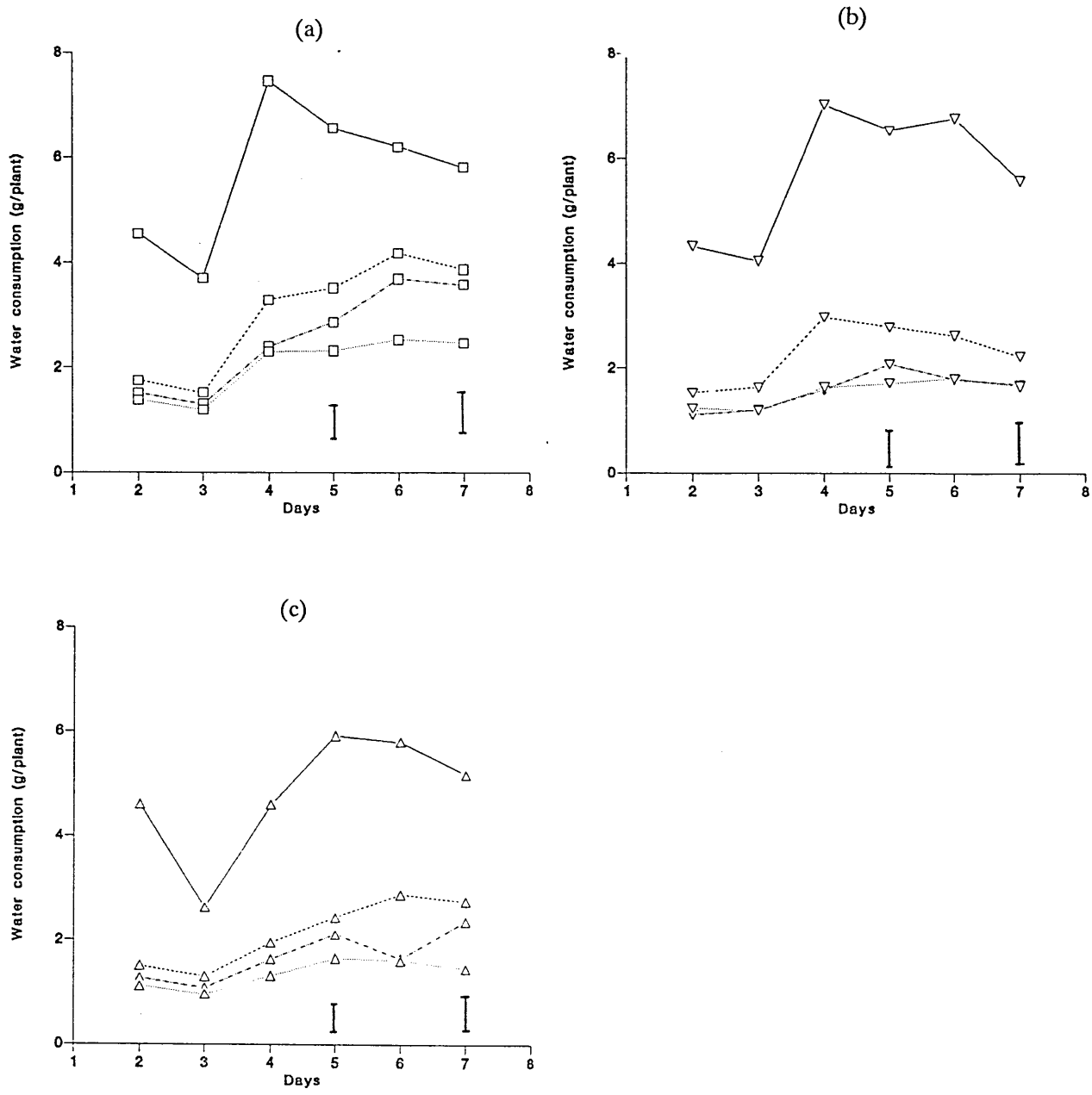



**Figure 5.1** Effects of osmotic stress level on the daily water consumption (g/plant) by: a) Panicum ( □ ) b) Avena ( ▽ ) and c) Aristida ( △ ).
Vertical bars are pooled SE's at the selected means.
Control (————), 7% PEG (- - - - - - -), 15% PEG (—·—·—·—) and 22% PEG (·····).

Plant Growth and Amount of Water Consumed

The effects of the four levels of osmotic stress on growth and total water consumption of the three species are presented in Table 5.1 and plotted vs the stresses in Fig 5.3a and 5.3b. The shoot fresh weight responses for all three species were nearly linear, but their directions differed. Panicum and Avena showed decreases, whilst Aristida showed an increase with increasing stress (Fig 5.3a). This was reflected in the analysis of variance where the main effects of stress and of the Species x Stress interaction were non-significant, but this changed to significance when linear regressions were fitted. Panicum was substantially heavier than the other two species, giving a highly significant species effect (Table 5.1).

Total water consumption from the PEG-stressed plants was significantly influenced by the level of stress imposed. The greater the imposed stress, the lower the amount of water consumed (Fig 5.3b). In all three species, vigorous new root growth occurred between the vermiculite and the membranes in the solution cultures, although there was less new root growth with 15 and 22% PEG.

Commentary

In this experiment, the shoot fresh weight of Aristida was increased with increasing levels of water stress, which suggests an adaptive feature of plants. The most probable explanation is that the PEG could have diffused across the semipermeable membrane into the root-vermiculite mass and absorbed by the plants. This may be possible since the semipermeable membrane (dialysis tubing) that was used in this particular experiment had a minimum molecular weight exclusion limit of 12,000 to 14,000. Based on this assumption, a higher molecular weight PEG such as PEG 20,000 was used in later experiments.

The 3-day adaptation period in this experiment was not unexpected, and should be taken into consideration in any experiments using PEG or other osmotic potential modifiers. The equilibration time is necessary for plants to adapt to the osmotic potential of the nutrient solution, and also to overcome any transplantation shock.

Table 5.1 Effects of varying levels of osmotic stress (PEG) on shoot fresh weight and total water use of Panicum (PM), Avena (AV) and Aristida (AR)

| Concentration of PEG 4000 (% w/v) | Shoot Fresh Weight (g/plant) | | | Total Water Consumption (g/plant) | | |
|---|---|---|---|---|---|---|
| | PM | AV | AR | PM | AV | AR |
| 0 (Control) | 0.4746 | 0.3100 | 0.1600 | 34.27 | 34.26 | 28.73 |
| 7 | 0.5133 | 0.2233 | 0.1467 | 18.12 | 13.74 | 12.84 |
| 15 | 0.4500 | 0.2300 | 0.2000 | 15.37 | 9.43 | 10.08 |
| 22 | 0.3700 | 0.2100 | 0.1800 | 12.19 | 9.21 | 8.08 |
| Significance level | | | | | | |
| Stress (OS) | | ns | | | *** | |
| Lin | | * | | | *** | |
| Quad | | ns | | | *** | |
| Dev | | ns | | | ** | |
| Species (SP) | | *** | | | *** | |
| OS x SP | | ns | | | ns | |
| Lin | | * | | | ns | |
| Quad | | ns | | | ns | |
| Dev | | ns | | | ns | |
| LSD (P<0.05) | | | | | | |
| Stress (OS) | | - | | | 2.507 | |
| Species (SP) | | 0.0455 | | | 2.171 | |
| OS x SP | | - | | | - | |

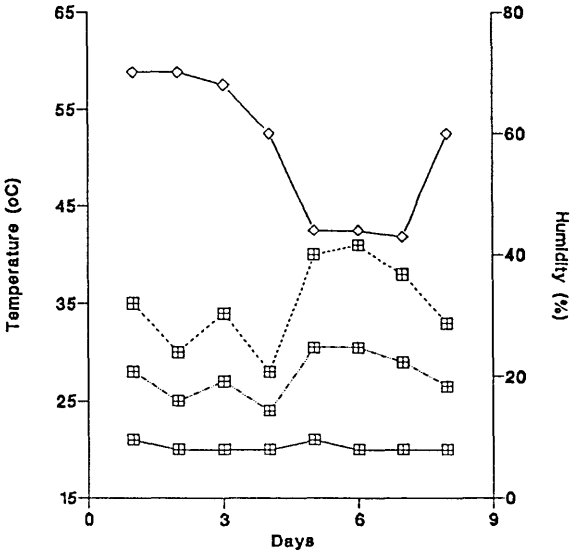


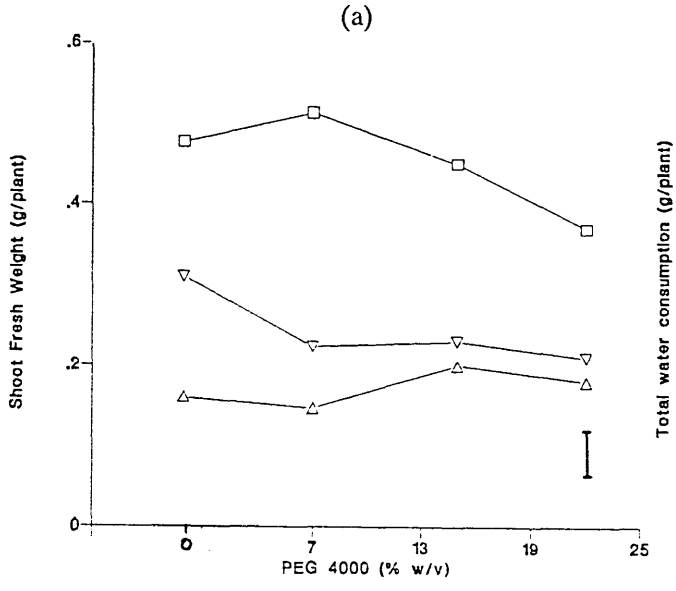

**Figure 5.2** Daily air temperature and humidity inside the cubicle of glasshouse.

Humidity : ◇——◇

Temperature: ⊞——⊞ Minimum

⊞·····⊞ Mean

⊞- - - - -⊞ Maximum



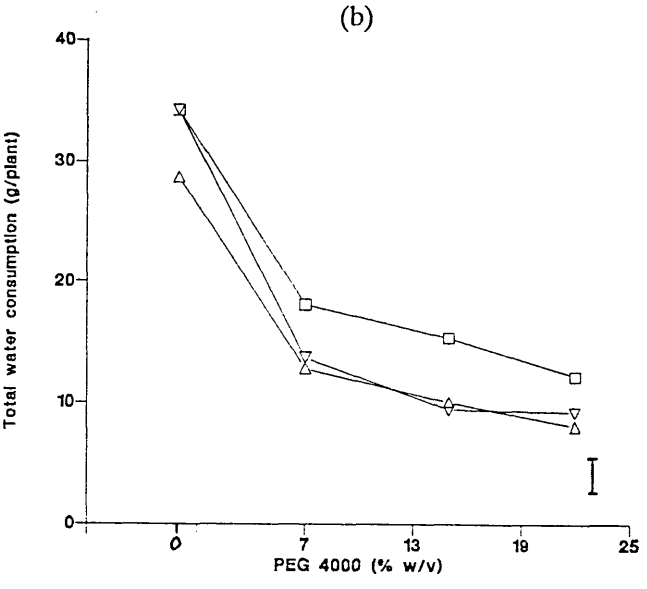




**Figure 5.3** Effects of osmotic stress level on: a) Shoot fresh weight (g/plant) b) Total water consumption (g/plant), by Panicum ( □ ), Avena ( ▽ ) and Aristida ( △ ). Vertical bars are pooled SE's

**Experiment 5.2.2 PEG induced water stress effects at three levels of Calcium nutrition on water consumption, growth and uptake of Panicum and Avena.**

*Material and Methods*

The seeds of Avena and Panicum were sown in trays of vermiculite on 24/4/88. A week later the plants were transplanted into plastic tubular containers filled with vermiculite, and were grown under well watered conditions and supplied with half strength Long Ashton solution. After three weeks, they were transferred into tubes of water-rinsed semipermeable membrane. The stems of the plant-membrane systems (root-vermiculite mass) were inserted into slits cut of styrofoam caps which were fitted tightly on to the 250 $cm^3$ jars. To avoid problems with the open ends of the membranes, they were folded at the base and raised back to the tops of the containers. The plant-membrane systems were equilibrated for 3 hours in distilled water and then transfered to nutrient solutions containing three levels of Ca: 0.449 mM (18.00 ppm), 1.322 mM (53.00 ppm) and 3.99mM (160.00 ppm), and concentrations of 0, 8, and 20 (%w/v) of PEG 20,000. A randomised complete block design with three replications was used. The plants were grown in aerated solution culture jars of PEG for eight days. Water use by the plants was determined by weighing the jars daily and replaced the transpired water. Evaporation through the styrofoam caps was determined from jars with no plants. Evaporation losses were deducted from the total water losses before determining transpiration. At the end of experiment, fresh and dry weights of shoot and root were determined, and root nutrient contents analysed.

*Results*

Plant Growth and Total Water Consumption

The changes in shoot fresh weight, total dry weight, root-shoot ratio and dry matter ratio of the two species to three levels of PEG-induced water stress and calcium nutrition are shown in Fig 5.4a to 5.4d. Analysis of variance of the four variables in Table 5.2 showed that the main effects of water stress and the interaction of Water Stress x Species (for shoot fresh weight and total dry weight) were significantly different, but the differences were confined to the linear term when regressions were fitted.

The shoot fresh weight of the control Panicum was considerably higher than other treatments. Weights of both species were greatly reduced by water stress, with Panicum showing a greater effect than Avena (Fig 5.4a). Generally, Ca supply showed little effect on shoot fresh weight of either species although both treatments of the stressed Avena and the control Panicum showed slight increases with increasing Ca supply. This difference between the species caused the interaction effects of Water Stress x Species to be highly significant (Table 5.2).

As expected, the total dry weights of the controls of both species were consistently higher, whilst the stressed treatments were decreased proportionally to the levels of stress imposed (Fig 5.4b). Total dry weights of both species again showed little effects due to Ca supply. Panicum was consistently much heavier than Avena. This confirmed the significant main effects of species and water stress.

Water stress caused large increases in the root-shoot ratios of both species (Fig 5.4c). Increasing Ca supply, however, showed no consistent effects in either species. Avena maintained consistently higher ratios than Panicum. These differences again caused the main effects of Water Stress and Species to be highly significant.

The dry matter ratios of both species were significantly increased with increasing water stress, but again showed little effects due to Ca supply (Fig 5.4d).

The total water consumption of the stressed plants of the two species were greatly influenced by the level of stress applied (Fig 5.4e). Both species again showed little effect due to Ca supply although the control Panicum showed slight increases with increasing Ca levels.

Nutrient content (root)

The total N contents in control Panicum was considerably higher than other treatments (Fig 5.5a). Root N contents of both species were greatly reduced as water stress increased, with Panicum showing a much larger response than Avena. The contents in the two species mostly increased with increasing Ca supply although there was a gradual decrease in control Avena.

The root K in all treatments of Panicum and 20% PEG stressed treatments of Avena, increased with increasing Ca supply, whilst both the control and 8% PEG stressed treatments of Avena showing small decreases (Fig 5.5b). The content of Panicum was consistently lowered as water stress increased, whilst Avena showed inconsistent responses.

Table 5.2 Analyses of variance of the effects of PEG-induced water stress at three levels of Ca on shoot fresh weight, total dry weight, dry matter ratio and root-shoot ratio of Panicum and Avena.

| Source of variation | df | Shoot fresh weight (g/plt) | Total dry weight (g/plt) | Root-shoot ratio | Dry Matter Ratio |
|---|---|---|---|---|---|
| | | Mean Square (MS) | | | |
| Water Stress(WS) | 2 | 0.909*** | 0.0199*** | 0.237*** | 0.0175*** |
| Lin | 1 | 1.680*** | 0.0364*** | 0.457*** | 0.0350*** |
| Quad | 1 | 0.139*** | 0.00337* | 0.0169ns | 0.000045ns |
| Calcium (Ca) | 2 | 0.00021ns | 0.000089ns | 0.00557ns | 0.000525ns |
| Species (SP) | 1 | 2.237*** | 0.0841*** | 0.851*** | 0.0000704ns |
| WS x Ca | 4 | 0.00088ns | 0.000159ns | 0.00504ns | 0.00047ns |
| Lin | 2 | 0.00165ns | 0.000282ns | 0.00888ns | 0.00073ns |
| Quad | 2 | 0.00011ns | 0.0000356ns | 0.00120ns | 0.00021ns |
| WS x SP | 2 | 0.172*** | 0.00179ns | 0.0060ns | 0.000287ns |
| Lin | 1 | 0.326*** | 0.00346* | 0.00546ns | 0.000107ns |
| Quad | 1 | 0.0189ns | 0.000115ns | 0.00654ns | 0.000467ns |
| Ca x SP | 2 | 0.00258ns | 0.000254ns | 0.00687ns | 0.000159ns |
| WS x Ca x SP | 4 | 0.00351ns | 0.000116ns | 0.00704ns | 0.000251ns |
| Lin | 2 | 0.00401ns | 0.000222ns | 0.00447ns | 0.000283ns |
| Quad | 2 | 0.00301ns | 0.0000105ns | 0.00961ns | 0.000219ns |
| Residual | 34 | 0.01045 | 0.0007537 | 0.0207 | 0.000507 |
| Total | 51 | 0.09371 | 0.003037 | 0.0414 | 0.00112 |

**Figure 5.4** Effects of calcium level on: a) Shoot fresh weight (g/plant) b) Total dry weight (g/plant) c) Root/shoot ratio d) Dry matter ratio (Total DW/Total FW) e) Total water consumption (g/plant), in Panicum ( □ ) and Avena ( ▽ ) at different levels of osmotic stress. Vertical bars are pooled SE's
Control (————), 8% PEG (--------) and 20% PEG (—··—··—··—).

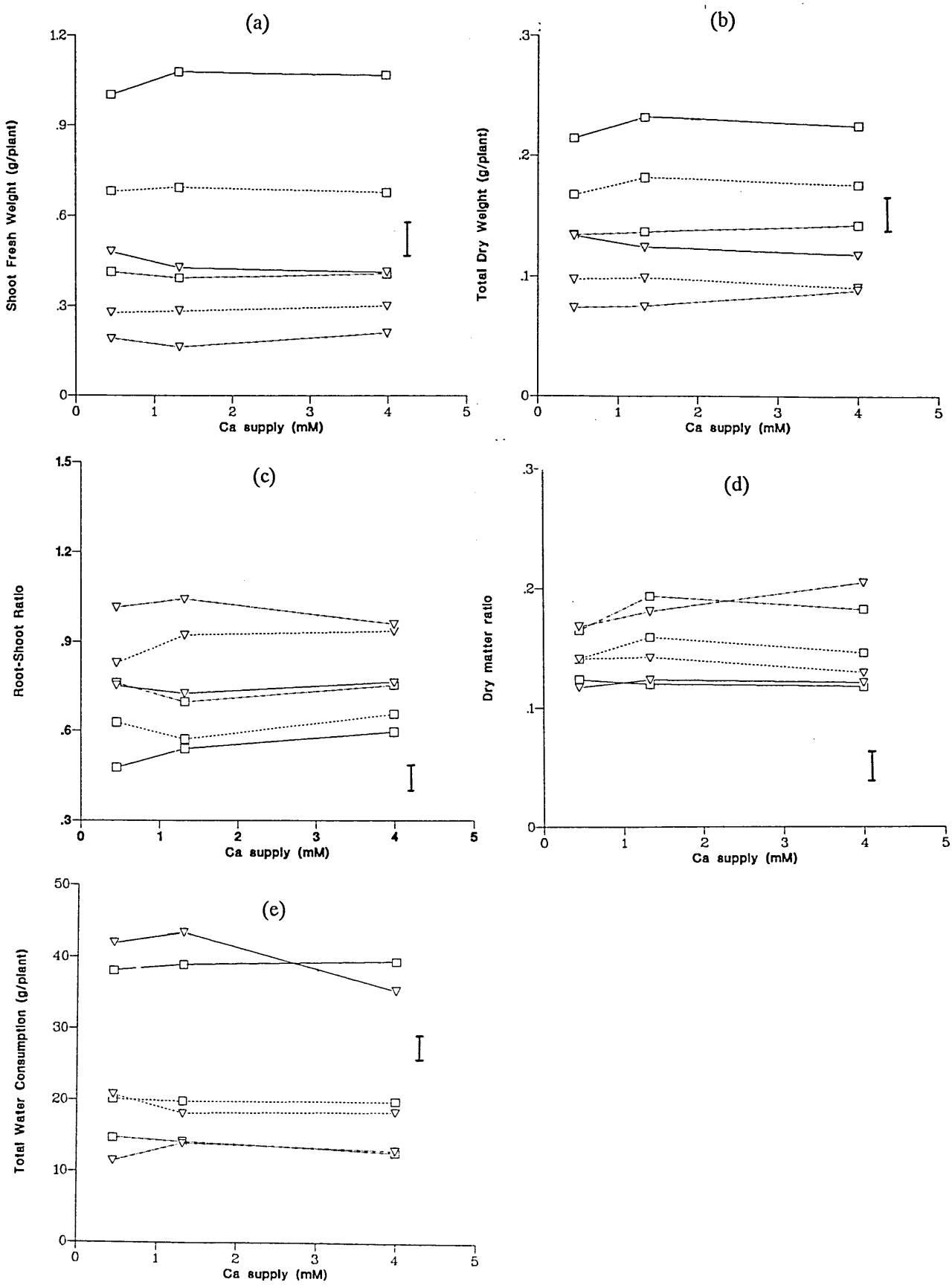
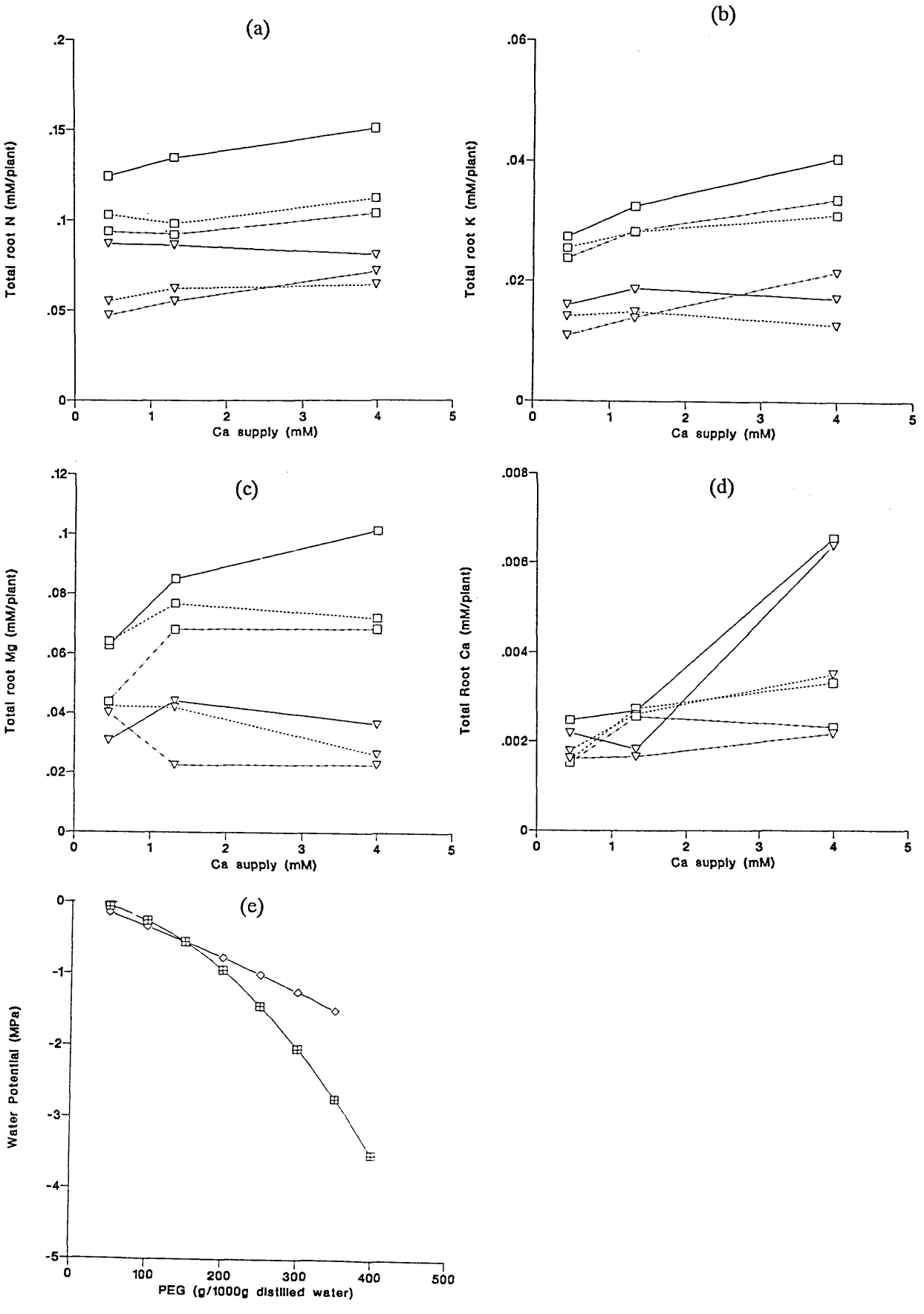

(a)
Shoot Fresh Weight (g/plant)
1.2
.9
.6
.3
0
0 1 2 3 4 5
Ca supply (mM)
(b)
Total Dry Weight (g/plant)
.3
.2
.1
0
0 1 2 3 4 5
Ca supply (mM)
(c)
Root-Shoot Ratio
1.5
1.2
.9
.6
.3
0 1 2 3 4 5
Ca supply (mM)
(d)
Dry matter ratio
.3
.2
.1
0
0 1 2 3 4 5
Ca supply (mM)
(e)
Total Water Consumption (g/plant)
50
40
30
20
10
0
0 1 2 3 4 5
Ca supply (mM)

**Figure 5.5** Effects of calcium supply on: a) Total root N b) Total root K c) Total root Mg d) Total root Ca (mMol/plant), at different levels of osmotic stress in Panicum ( □ ) and Avena ( ▽ )

Control (——————), 8% PEG (- - - - - - - -) and 20% PEG (—··—··—··—).

e) Relationship between PEG concentration (g/1000g distilled water) and water potential (MPa) of PEG 4000 ( ◇ ) and PEG 20 000 ( ⊞ ) by Steuter, 1981.


(a)
Total root N (mM/plant)
.2
.15
.1
.05
0
0 1 2 3 4 5
Ca supply (mM)
(b)
Total root K (mM/plant)
.06
.04
.02
0
0 1 2 3 4 5
Ca supply (mM)
(c)
Total root Mg (mM/plant)
.12
.1
.08
.06
.04
.02
0
0 1 2 3 4 5
Ca supply (mM)
(d)
Total Root Ca (mM/plant)
.008
.006
.004
.002
0
0 1 2 3 4 5
Ca supply (mM)
(e)
Water Potential (MPa)
0
-1
-2
-3
-4
-5
0 100 200 300 400 500
PEG (g/1000g distilled water)

The total Mg in roots of Panicum and the control of Avena were increased with increasing levels of Ca supply, whilst both the stressed treatments of Avena showed small decreases (Fig 5.5c). The content of Panicum was decreased with increasing stress, whereas Avena showed inconsistent responses.

The root Ca in both species increased with increasing levels of Ca supply, with the control treatments showing larger responses than the stressed (Fig5.5d). There were no consistent differences between the species.

Commentary

The shoot fresh weights and the total amounts of water consumed by the two species used in this experiment were more severely affected by water stress than those in experiment 5.2.1. This might be explained in part by the different range of water stresses applied through using PEG's of different molecular weights. Since, however, the relationship between PEG concentration and water potential is non-linear, as demonstrated by Steuter *et al* 1981, and shown in Fig 5.5e, the differences in water potential were not all that large. The highest imposed potential of experiment 5.2.1 would be -0.86 MPa compared with -0.94 MPa in this experiment. Other differences might be due to the effects of weather, possibly associated with the warm and bright conditions which occured during this particular experiment.

## Experiment 5.2.3 PEG induced water stress effects at two levels of nitrogen supply on water consumption, growth and uptake of Panicum, Avena and Aristida.

### *Material and Methods*

The seeds of Avena and Panicum were sown in trays of vermiculite on 26/5/88. A week later the plants were transplanted into plastic tubular containers filled with vermiculite, and were grown under well watered conditions and supplied with half strength Long Ashton solution. After three weeks, they were transferred into tubes of water-rinsed semipermeable membrane. The stems of the plant-membrane systems were inserted into slits cut of styrofoam caps which

were fitted tightly on to the 250 $cm^3$ jars. To avoid problems with the open ends of the membranes, they were folded at the base and raised back to the tops of the containers.

The plant-membrane systems were equilibrated for 3 hours in distilled water and then transfered to nutrient solutions containing two levels of N: 1.356 mM (19.00 ppm) and 12.134 mM (170.00 ppm) and concentrations of 0, 8, and 20 (%w/v) of PEG 20,000. A randomised complete block design with three replications was used. The plants were grown in aerated solution culture jars of PEG for eight days. Water use by the plants was determined by weighing the jars daily and replaced the transpired water. Evaporation through the styrofoam caps was determined from jars with no plants. Evaporation losses were deducted from the total water losses before determining transpiration. At the end of the experiment, fresh and dry weights of shoots and roots were determined, and their N contents analysed.

## *Results*

Plant Growth and Water Consumption

The effects of PEG-induced water stress at two levels of N supply on shoot fresh weight, total dry weight, root-shoot ratio and dry matter ratio of the three species are shown in Fig 5.6a to 5.6d. Analysis of variance in Table 5.3 showed that the main effects of water stress for all the four variables and the interactions of Water Stress x Species (for shoot fresh weight and dry matter ratio) were significant, and that this was confined to the linear term when regressions were fitted.

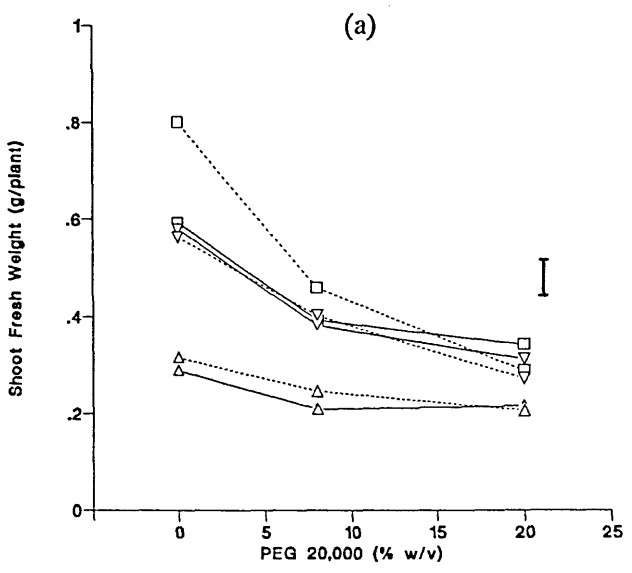
The shoot fresh weights of the three species decreased with increasing water stress, with Panicum and Avena showing much greater responses than Aristida (Fig 5.6a). Panicum was greatly reduced at the lower N supply under no water stress but the difference disappeared with increasing water stress. N levels showed little effect on shoot fresh weights of Aristida, whilst Avena showed inconsistent responses. These differences between the species caused the interaction effects of Water Stress x Species to be highly significant (Table 5.3).

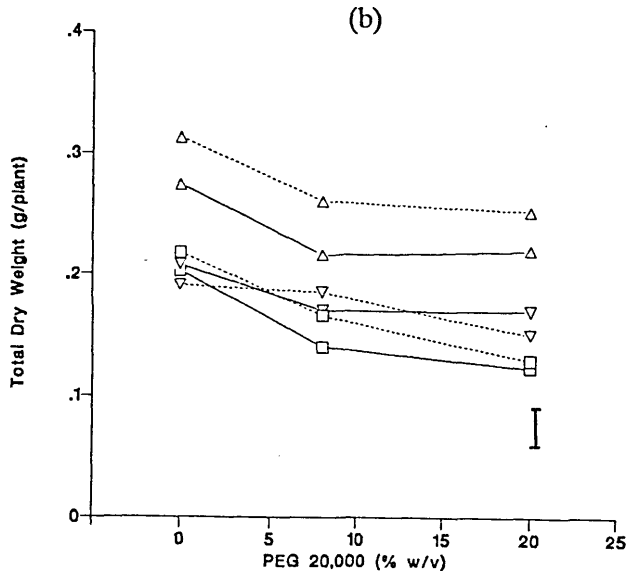
The total dry weight of Aristida was considerably higher than other species (Fig 5.6b). As expected, the total dry weights of Panicum and Aristida were consistently higher with high N supply, whilst Avena showed inconsistent responses. Weights of all three species were reduced with increasing water stress.

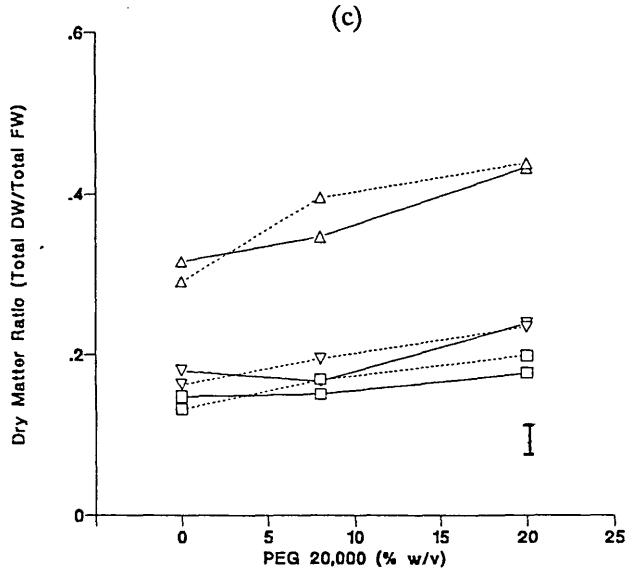
Table 5.3 Analyses of variance of the effects of PEG-induced water stress at two levels N nutrition on shoot fresh weight, total dry weight, dry matter ratio and root-shoot ratio of Panicum and Avena.

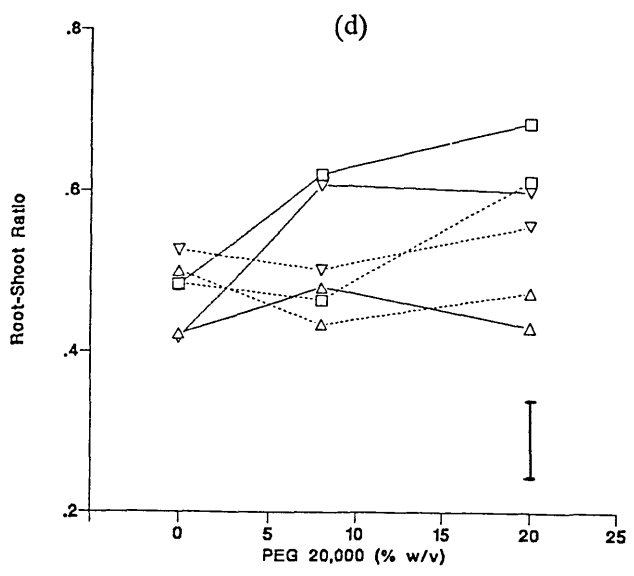
| Source of variation | df | Shoot fresh weight (g/plt) | Total dry weight (g/plt) | Root-shoot ratio | Dry Matter Ratio |
|---|---|---|---|---|---|
| | | Mean Square (MS) | | | |
| Water Stress(WS) | 2 | 0.296*** | 0.0176*** | 0.0350ns | 0.0305*** |
| Lin | 1 | 0.563*** | 0.0327*** | 0.0700* | 0.0602*** |
| Quad | 1 | 0.0293* | 0.00246ns | 0.00003ns | 0.000819ns |
| Nitrogen (N) | 1 | 0.00934ns | 0.00330* | 0.0117ns | 0.000573ns |
| Species (SP) | 2 | 0.261*** | 0.0441*** | 0.0489* | 0.223*** |
| WS x N | 2 | 0.0135ns | 0.000578ns | 0.0403* | 0.00292* |
| Lin | 1 | 0.0256* | 0.0000757ns | 0.0168ns | 0.00165ns |
| Quad | 1 | 0.00148ns | 0.00108ns | 0.0638* | 0.00419* |
| WS x SP | 4 | 0.0335*** | 0.00105ns | 0.0138ns | 0.00333* |
| Lin | 2 | 0.0642*** | 0.00151ns | 0.0236ns | 0.00599** |
| Quad | 2 | 0.00290ns | 0.000597 | 0.00402ns | 0.000673ns |
| N x SP | 2 | 0.00848ns | 0.00231ns | 0.0101ns | 0.0000798ns |
| WS x N x SP | 4 | 0.00707ns | 0.0000808ns | 0.00140ns | 0.000306ns |
| Lin | 2 | 0.0133ns | 0.0000063ns | 0.00266ns | 0.000115ns |
| Quad | 2 | 0.000879ns | 0.000155ns | 0.00013ns | 0.000496ns |
| Residual | 34 | 0.004503 | 0.0007845 | 0.01154 | 0.0008505 |
| Total | 51 | 0.02908 | 0.0032088 | 0.01438 | 0.01092 |

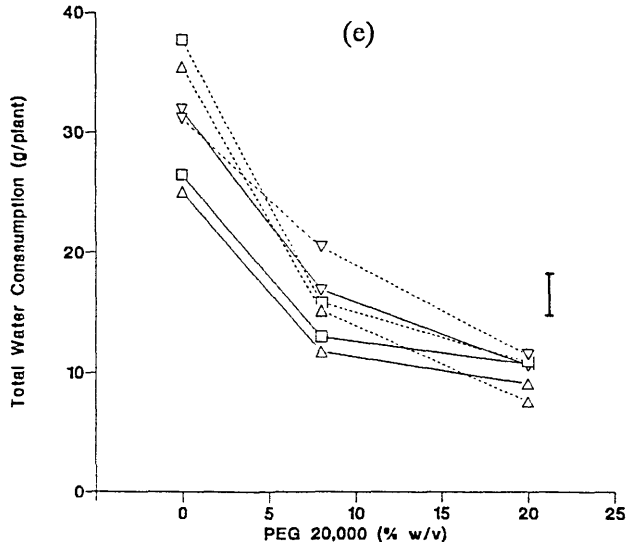
**Figure 5.6** Effects of PEG concentration on: a) Shoot fresh weight (g/plant)
b) Total dry weight (g/plant) c) Dry matter ratio (Total DW/Total FW)
d) Root/shoot ratio e) Total water consumption (g/plant),
in Panicum ( □ ), Avena ( ▽ ) and Aristida ( △ ).
Vertical bars are pooled S.E.'s
N level: 1.36 mM ( ——— ), 12.13 mM ( - - - - - - - ).


(a)
Shoot Fresh Weight (g/plant)
1
.8
.6
.4
.2
0
0
5
10
15
20
25
PEG 20,000 (% w/v)


(b)
Total Dry Weight (g/plant)
.4
.3
.2
.1
0
0
5
10
15
20
25
PEG 20,000 (% w/v)


(c)
Dry Matter Ratio (Total DW/Total FW)
.6
.4
.2
0
0
5
10
15
20
25
PEG 20,000 (% w/v)


(d)
Root-Shoot Ratio
.8
.6
.4
.2
0
5
10
15
20
25
PEG 20,000 (% w/v)


(e)
Total Water Consumption (g/plant)
40
30
20
10
0
0
5
10
15
20
25
PEG 20,000 (% w/v)

The dry matter ratios of the three species were markedly increased with increasing water stress, but showed little effects due to different levels of N supply (Fig 5.6c). Aristida maintained the highest ratios at all levels of water stress.

Water stress caused quite large increases in the root-shoot ratios of Avena and Panicum, with low N treatments showing much larger responses than high N (Fig 5.6d). Avena, on the other hand, showed no obvious changes to water stress. The ratios of Panicum and Avena, decreased with increasing N supply, although the ratio in Avena was slightly higher with high N under no water stress. Increasing N supply, however, showed no obvious effects in Aristida. These differences again caused the interaction of Water Stress x Nitrogen to be significant.

The total water consumption of the three species was reduced both by water stress and by reduced N supply, and these effects were cumulative (Fig 5.6e). Panicum showed much larger responses to N supply than the other two species, with the differences between the species being significant under no water stress, but not under stressed conditions.

Nitrogen Uptake

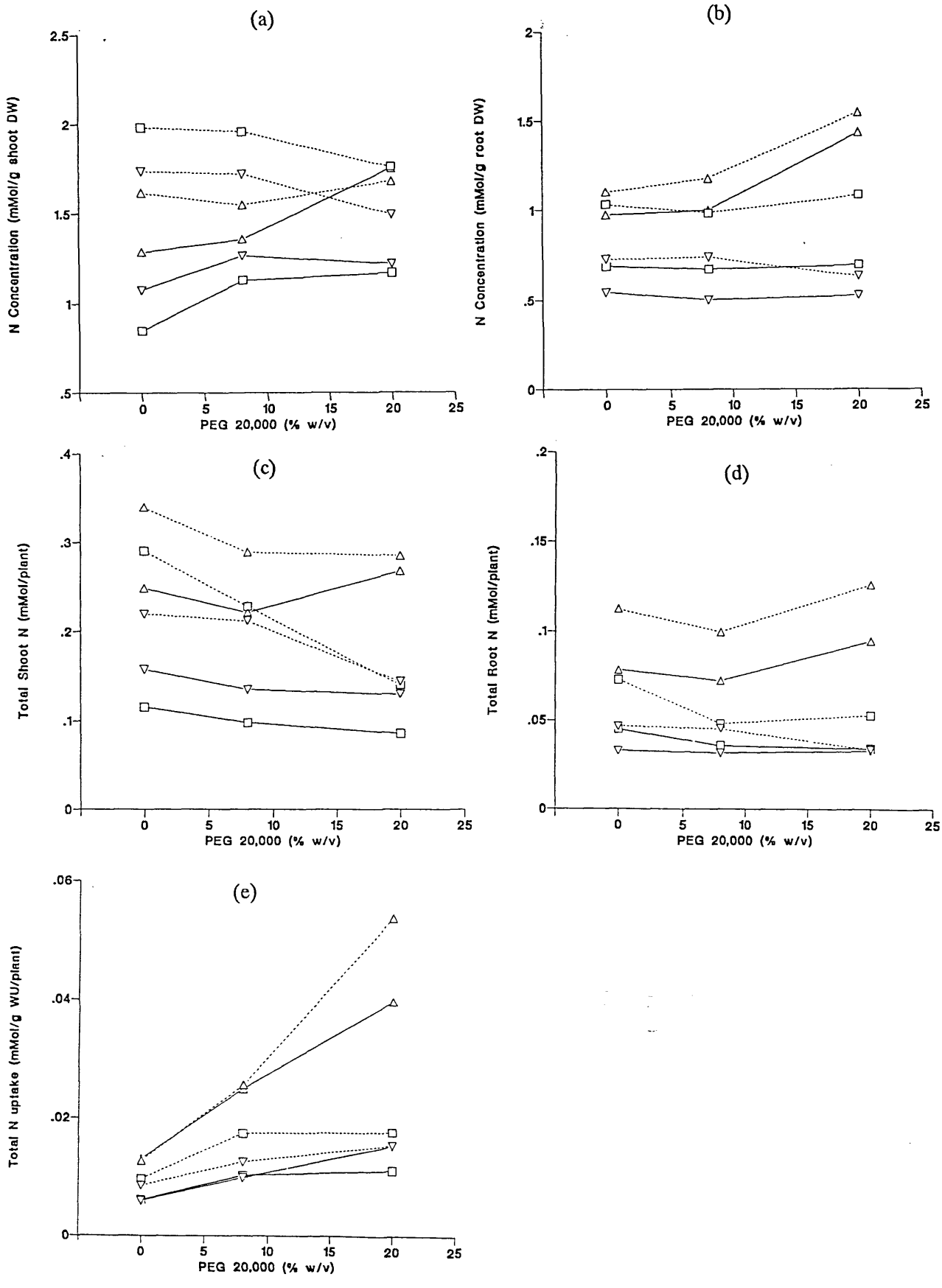
The concentration of N in the shoot of Panicum under high N supply was consistently higher than other treatments, and the concentrations of all the three species, as expected, were reduced with the lower N supply (Fig 5.7a). The concentrations in Panicum and Avena decreased with increasing water stress under high N, but showed opposite responses under low N. All treatments of Aristida, on the other hand increased with increasing water stress.

Root N concentration of Aristida was consistently higher than other treatments at all levels of water stress (Fig 5.7b). The concentration in Aristida increased with increasing water stress, whilst the concentrations in Panicum and Avena showed no obvious changes. The concentrations in all three species, as expected, were reduced with the lower N supply with Panicum showing the largest effects.

The total N contents in shoots of the three species were greatly reduced with the lower N supply. Aristida under high N was consistently higher than other treatments (Fig 5.7c). Values in all three species declined with increasing water stress, with high N plants showed greater responses, although the low N Aristida showed a slight increase with water stress.

**Figure 5.7** Effects of osmotic stress level on: a) Concentration of N in shoot (mMol/g DW) b) Concentration of N in root (mMol/g DW) c) Total shoot N (mMol/plant) d) Total root N (mMol/plant) e) Total N uptake (mMol/g water use/plant), in Panicum ( □ ), Avena ( ▽ ) and Aristida ( △ ).

N level: 1.36 mM ( ——— ), 12.13 mM (- - - - - - - ).


(a)
N Concentration (mMol/g shoot DW)
2.5
2
1.5
1
.5
0
5
10
15
20
25
PEG 20,000 (% w/v)
(b)
N Concentration (mMol/g root DW)
2
1.5
1
.5
0
5
10
15
20
25
PEG 20,000 (% w/v)
(c)
Total Shoot N (mMol/plant)
.4
.3
.2
.1
0
5
10
15
20
25
PEG 20,000 (% w/v)
(d)
Total Root N (mMol/plant)
.2
.15
.1
.05
0
5
10
15
20
25
PEG 20,000 (% w/v)
(e)
Total N uptake (mMol/g WU/plant)
.06
.04
.02
0
5
10
15
20
25
PEG 20,000 (% w/v)

The amounts of N in roots of the three species were again greatly reduced in the lower N level, with Aristida showing the largest differences (Fig 5.7d). Aristida under both levels of N, increased with increasing water stress, whilst Panicum and Avena generally showed little changes due to water stress.

Commentary

The increased root-shoot ratios in the stressed Avena and Panicum in this experiment, indicates a major repartitioning of assimilates into the roots under reduced water availability, which confirmed the observations of several earlier experiments. However, the root-shoot ratios of Aristida at the three different levels of water stress, which were more or less constant, may indicate one of the sources of its tolerance to water stress. The constant uptake of N in shoots and the increased content of roots associated with water stress in this species can similarly be explained. On the other hand, the decreased uptake of N in shoots of Panicum and Avena and the constant content of N with water stress in their roots, might be explained by impaired transport of nutrients from roots to shoots.

## 5.3 Discussion

Plant Growth and Water Consumption

Although PEG has been widely used to induce water stress in plants, the presence of PEG in nutrient solutions has been reported by several workers to cause undesirable effects. For instance, Jacomini, *et al.* (1988) found an amount of PEG of up to 15.4% of the total dry weight in roots of tomato after 12 days of 25% PEG 6000 treatment. Maize and pepper plants absorbed up to 14% of the PEG in solution and transported it unmetabolised through the plant (Jane, 1974; Lawlor, 1970). PEG contents of 1 to 3 mg/g fresh weight for roots of maize (Resnik, 1970) and 0.5 to 2 mg/ml of plant sap have also been reported (Janes, 1974). In the present investigation, semipermeable membrane (dialysis tubing) was used to separate the PEG but allow water and nutrients to diffuse to the root system (see Chapter One). Short-term (8-day periods) treatments of osmotic stress were imposed in order to avoid bacterial degradation of the membranes. The semipermeable membrane system described in the present experiments is similar to that used by Tingey and Stockwell (1977) and Yambao and O'Toole (1984). For example, Tingey and Stockwell (1977) grew beans (*Phaseolus vulgaris* L.) in a semipermeable membrane system which encased the root-vermiculite mass, and was then placed in nutrient solutions to which various amounts of PEG 20,000 were added. They also demonstrated that visual degradation of the membrane occurred only after 2 to 3 weeks in solution.

The results from experiment 5.2.1 suggest that PEG 4000 incorporated in the nutrient solution might have diffused across the semipermeable membrane into the root-vermiculite mass. This was possible since the membrane used in this experiment had a minimum molecular weight exclusion limit of 12,000 to 14,000. This is in agreement with Tingey and Stockwell (1977), who found that the PEG which did diffuse across the semipermeable membrane into the root-vermiculite mass comprised the low molecular weight fractions. The increased shoot fresh weight of Aristida with increased osmotic stress may have been caused by uptake of that PEG which had passed through the membrane. This would in turn suggest that Aristida was more capable of such uptake than Avena or Panicum. Similar observations in osmotically stressed maize seedlings with PEG 4000 were reported by Izzo *et al.* (1989). However, this problem did not appear in experiments 5.2.2 and 5.2.3, in which PEG 20,000 was used, which further supports the use of high molecular weight PEG's.

Several workers have reported that when PEG is used in hydroponic solutions, the aeration of the viscous solutions can cause foaming, which can seriously lower the $O_2$ content of the solution (e.g. Mexal *et al.* 1975). In the present investigation, the availability of $O_2$ would not be a problem since the requirement of the roots in the membrane system could be met via $O_2$ diffusion through the vermiculite rather than from the solution. Tingey and Stockwell (1977), using a similar membrane system to that used here, have shown that no net plant consumption of $O_2$ could be detected from the PEG solution.

The daily water consumption in all the three species in experiment 5.2.1, followed the changes in air temperature and relative humidity with the exception of the first three days (Fig 5.1a to 5.1c), whilst the plants equilibrated with the nutrient solutions by adapting to the different osmotic potentials of the stress treatments. This again agrees with Tingey and Stockwell (1977), who observed similar equilibration within one to four days, followed by normal diurnal patterns of water relations. Gergely, *et al.* (1980a) also observed similar adaptation responses with apple seedlings.

Total water consumption in stressed plants of all three species, was markedly decreased with increasing levels of stress. The amount of water comsumed by the stressed plants was closely related to the amount of PEG present in the nutrient solutions but this correlation differed between the species. There was no apparent difference in the total water consumption of high and low nitrogen plants under severe water stress (20% PEG treatment) conditions. For instance, Aristida despite having the highest total dry weight at both levels of osmotic stress, also showed the lowest amount of water consumed (Fig 5.6e). This partly reflects the greater ability of this species to resist water stress by increasing its efficiency of water use.

The reduced water use by plants growing in solutions of decreased osmotic potential is in agreement with Yambao and O'Toole (1984), who used a similar semipermeable membrane technique and observed that the transpiration rate of PEG-stressed rice declined sharply with decreases in leaf water potential. They suggested that during the light period leaf water potential decreased to levels which may have caused stomatal closure. In another study, leaf rolling was found to be an effective means of reducing transpiration rate in rice plants under water stress (O'Toole *et al.*, 1979).

Although decreases in the osmotic potential of the nutrient solutions markedly reduced total dry weights, the root/shoot ratios of Avena and Panicum were increased (Fig 5.6b and 5.6d). This indicates that root growth of these two species proceeded in preference to shoot growth in plants under water stress. The preferential development of roots over shoots has frequently

been observed in response to water stress (Brouwer, 1983). It has been cited as an adaptive mechanism to water stress that allows plants to exploit a larger volume of soil for water (Blum *et al.* 1983). The increase is caused by a shift in the partitioning of dry matter between roots and shoots but the underlying mechanisms are unclear (Lambers, 1983). Brouwer (1983) suggested that carbohydrate supply limited growth rate, but many workers disagree. For example, Schildwacht (1988) observed that differences in turgor pressure between roots and leaves could cause differences in their enlargement rates and be influenced by water stress. This in turn could lead to the observed shift in the dry matter root/shoot ratio.

In contrast to these adaptive responses, the constant root/shoot ratios of Aristida reflect the overall tolerance of this species. This is in agreement with Izzo *et al.* (1989), who observed that the constant root/shoot ratio at -0.44 and -0.88 MPa osmotic potential in the nutrient solutions and suggested this response as an indication of one of the sources of water stress tolerance in maize.

The results in experiment 5.2.3 also revealed that PEG-stressed plants of Avena and Panicum under low N produced more root dry matter than in high N. Increasing water stress and N supply, however, showed no effects in Aristida. This then caused significant interactive effects of nitrogen with water stress in that the greatest amount of root growth occurred in nitrogen deficient stressed plants. This is in agreement with many reports showing that nitrogen deficiency sharply reduced top growth whilst root growth was either unaffected or moderately reduced (e.g. Davidson, 1969; Drew, 1975). The increased root/shoot ratios of nitrogen deficient PEG-stressed plants have also been observed in rice by Yambao and O'Toole (1984).

Nutrient Uptake and Accumulation

The results in experiment 5.2.3 showed that the total N contents in shoots of the three species were reduced both by the lower N supply and by water stress, although the low N plants of Aristida showed a slight increase. However, the plants supplied with high N showed greater responses than low N treatments. On the other hand, the content of N in roots of Aristida increased with increasing water stress, but generally showed little change in Panicum or Avena. The amount of N in roots of the three species were also reduced with the lower N supply. This suggests that the amounts of N absorbed by the roots in the three species depended more on the amounts supplied than on effects due to water stress, and the effect was shown most obviously in Aristida. For example, plants grown in 12.13 mM N had greater total N uptake,

associated both with greater dry matter and higher N concentrations, than those grown in 1.36 mM N.

The concentrations of N in the shoots of Panicum and Avena decreased with increasing water stress under high N, but showed the opposite responses under low N. The concentrations of N in the roots of these two species, on the other hand, showed no obvious changes with water stress. In Aristida, the concentrations of N in all treatments and in both shoots and roots increased with water stress. Since N concentrations in the shoots of low N plants increased whilst their dry weights decreased in response to water stress, it can be inferred that the uptake of N in the shoots of low N Panicum and Avena was less affected by water stress than their growth, whilst the reverse responses were seen in the high N treatments. This suggests that the increased root/shoot ratios of low-N plants of Panicum and Avena under water stress might have enhanced both their water use efficiencies, and their nutrient uptake. On the other hand, the N uptake in Aristida at both low and high N was generally unaffected by water stress, presumably due at least partly to its constant root/shoot ratio.

Many workers have presented evidence that the internal water relations of plants subjected to water stress are altered depending on the level of N. For example, Radin and Parker (1979), reported that stomatal closure in N-deficient plants of cotton began at a much higher leaf water potential than in N-sufficient plants. In another study, they also demonstrated that abscisic acid (ABA) concentrations and stomatal sensitivity to ABA increased in low-N plants (Radin *et al.*, 1982). Yambao and O'Toole (1984) observed that low N rice plants maintained higher leaf water potentials throughout the stress period. This is supported by Tanguilig *et al.* (1987), who suggested that the ability of maize and soybean to grow better than rice under water stress conditions was due to their ability to maintain turgor as a result of their slower decline in leaf water potential brought about by lower transpiration rates and the continued uptake of nutrients which allowed osmotic adjustment to occur.

The results in experiment 5.2.3 also demonstrated that in general, the total shoot N decreased due to the reduced demand for nutrients, whilst the roots' capability to absorb nutrients was unimpaired since their N contents remained relatively constant. This suggests an impaired transport of nutrients from the the roots to the shoots. Similar responses were also found in PEG-stressed maize (Izzo *et al.*, 1989). This therefore supports the results of the earlier experiments in soil which suggested that the effect of water stress on transport to the shoots was independent of its effect on uptake by the roots.

Many workers have reported that the decreased N uptake by plants under water stress conditions was primarily due to reduced water uptake. For example, Yambao and O'Toole (1984) found that the total N content in PEG-stressed rice plants increased linearly with cumulative transpiration but plants supplied with high N contained more N than those in low N. They concluded that since stressed plants transpired less, they therefore accumulated less N. Frota and Tucker (1978), who studied red kidney bean plants also observed a high positive correlation between N and water uptake under both water and salt stress. Greenway and Klepper (1969) concluded that water flow accelerated anion transport to the shoot after the ions had been taken up by the roots. They found total P uptake to decrease with nutrient solution potential as a result of reduced P transport to the shoot. These reports show similar trends to those found for Panicum and Avena in the present investigation, in that the ratios of N uptake to water use were nearly constant (Fig 5.8e) so that the decreased N uptake of stressed plants could be due to their reduced water consumption (Fig 5.6e).

In constrast, N uptake in Aristida was not found to be related to water use, since the ratio of uptake to water use increased substantially with increasing stress (Fig. 5.7e). This is in agreement with Erlandsson (1975), who found, firstly, that the uptake of K, P and water by the roots of intact wheat seedlings decreased when the water potential of the root medium was decreased by the addition of PEG. However, increasing the humidity around the shoots from 60 to about 100% RH, so as to decrease water loss, actually increased the uptake of ions. He concluded that the decreased uptake of ions induced by the PEG treatment did not depend on low water uptake or on any plugging effect by PEG. In another study, he further suggested that low water potentials affected both membrane permeability and active uptake of ions (Erlandsson, 1979). He also found that the effect of low water potential on the membranes and the uptake mechanism was due not only to increase of ABA in the plants but also involved other factors. Thus in the present study, it is suggest that water stress had little effect on the uptake of N in Aristida since its uptake was not related to the uptake of water.

The important points arising from the present investigation are that the total dry weights of the three species were reduced by water stress but high N plants suffered greater growth reduction than low N. Plant water stress reduced total N uptake in shoots of both N treatments, but the uptake in low N Panicum and Avena was less affected by water stress than their growth, partly due to the greater root growth than in high N. In contrast, the uptake at both N levels in Aristida and its root/shoot ratio was unaffected by water stress.

Decreased plant growth caused by water stress, low N supply or both led to decreases in total water transpired. Decreased transpiration reduced the nitrogen contents in Panicum and Avena and this probably affected growth during water stress. However, the uptake of N in Aristida was not affected by reduced water uptake, so that any change in growth was due to water stress *per se* and not to interacting effects of N nutrition. These relationships were further investigated in the next chapter, the final set of experiments which involved growing plants in nutrient film channels using split-root systems. This technique allowed nutrient uptake to be distinguished from overall water uptake, and gave a better indication of species differences in their response to water stress.

# CHAPTER SIX

## Studies on the Efficiency of Roots *A.fatua*, *A.funiculata* and *P.miliaceum* Using a Split-Root System

# CHAPTER SIX

## STUDIES ON THE EFFICIENCY OF ROOTS OF *A.fatua* (Avena), *A.funiculata* (Aristida) and *P.miliaceum* (Panicum) USING A SPLIT-ROOT SYSTEM.

### 6.1 Introduction

Split-root systems have also been used by many workers to study the effects of differing root environments (e.g. Blackman and Davies 1985 and Coutts 1982). They studied the effects of stressed and unstressed root environments on the corresponding portions of the shoots of maize and Sitka spruce seedlings by dividing the roots of individual plants between containers which differed in water regime. The aims of the experiments in this chapter are to investigate and compare the capabilities of Aristida, Avena and Panicum roots to retranslocate N from the N-fed halves of split-root systems, supplied with various levels of N, ranging from very high to deficient, to the N-free halves. The efficiencies under water stress conditons were also investigated by adding PEG.

### 6.2 EXPERIMENTS

**Experiment 6.2.1** The growth and translocation of N in Avena, Aristida and Panicum using the split-root technique with four levels of N supply.

#### *Material and Methods*

The seeds of Avena and Panicum were sown in trays of vermiculite on 1/5/89, whilst plants of Aristida were always derived by vegetative propogation from a single parent, starting about 8 days earlier. A week later the seedlings of Avena and Panicum were
pruned to two seminal roots by removing the primary roots, whilst two equal-sized adventitious roots were selected for Aristida plants. The plants were transferred to a nutrient film culture

installation made up of pairs 1.2 m long PVC channels (see Plate 2), where each root was grown in a separate channel containing nutrient solutions with different levels of N. Nutrient solutions from 7 litre reservoirs, were circulated through the channels at a flow rate of 1 litre per minute.

(electrical conductivity)
The nutrient solutions were changed at regular intervals, whilst their cF/and pH levels were maintained at 2000-2500 micromhos and 6.0-6.2 respectively following daily checks. The development of other adventitious roots was prevented by keeping the root-bearing nodes dry and above the nutrient solutions. The stems of plants were inserted through slits cut into polystyrene board which supported the plants. The channels were then covered with black (below) and white (above) polythene sheet. The experimental treatments were arranged in a split-plot design with three replications. The nutrient solutions were made up as shown in the table below.

| Treatment | mM N in nutrient solution supplied to | |
|---|---|---|
| | Root A | Root B |
| N0 N1 | 0 | 3.569 (50 ppm N) |
| N0 N2 | 0 | 10.707 (150 ppm N) |
| N0 N3 | 0 | 17.844 (250 ppm N) |
| N1 N1 | 3.569 | 3.569 |

The N-free nutrient solution contained $CaCl_2$ (to replace $Ca(NO_3)_2$), and KCl (to replace $KNO_3$) plus other components as in the complete nutrient solution of Cooper (1979)

After two weeks, fresh and dry weights of shoot and root were determined, and their nutrient contents analysed.



Plate 2. PVC channels where each root was grown in a separate channel containing nutrient solutions with different levels of N.

## *Results*

### Plant Growth

The effects of the four levels of N on shoot and root growth of the three species are presented in Fig 6.1a to 6.1f. Analyses of variance showed that there were significant differences between species for all the variables, but the effect of nitrogen and its interaction with species were not significant (Table 6.1).

The shoot fresh weights of Panicum were considerably higher than the other two species (Fig 6.1a). Differences in N supply to the N-fed root halves produced no significant responses, although there was a gradual increase up to the highest levels of N. Avena and Aristida showed no obvious changes with N supply. The control treatment (N1N1) of Panicum was slightly higher than the low N (N0N1), whilst the controls of Avena and Aristida showed no differences from the other treatments.

The root-shoot ratios of Avena were consistently higher than the other species, whilst Panicum were the lowest (Fig 6.1c). Increasing N supply to one of the root halves showed no consistent effects with Aristida or Panicum but generally caused an increase in the ratios of Avena.

The changes in root fresh weights of Panicum, Avena and Aristida are shown in Fig 6.1d, 6.1e and 6.1f respectively. The fresh weights of roots receiving N were always greater than those of the N-free roots for all the three species. The weights were also greater than the roots of control plants, both of which received the same level of N (N1N1), except for Aristida which had more or less the same weights as the control. The weights of N-free roots of Panicum and Avena generally increased when the roots of the other halves received a higher N supply but Aristida showed no obvious changes.

### Shoot Nutrient Concentrations

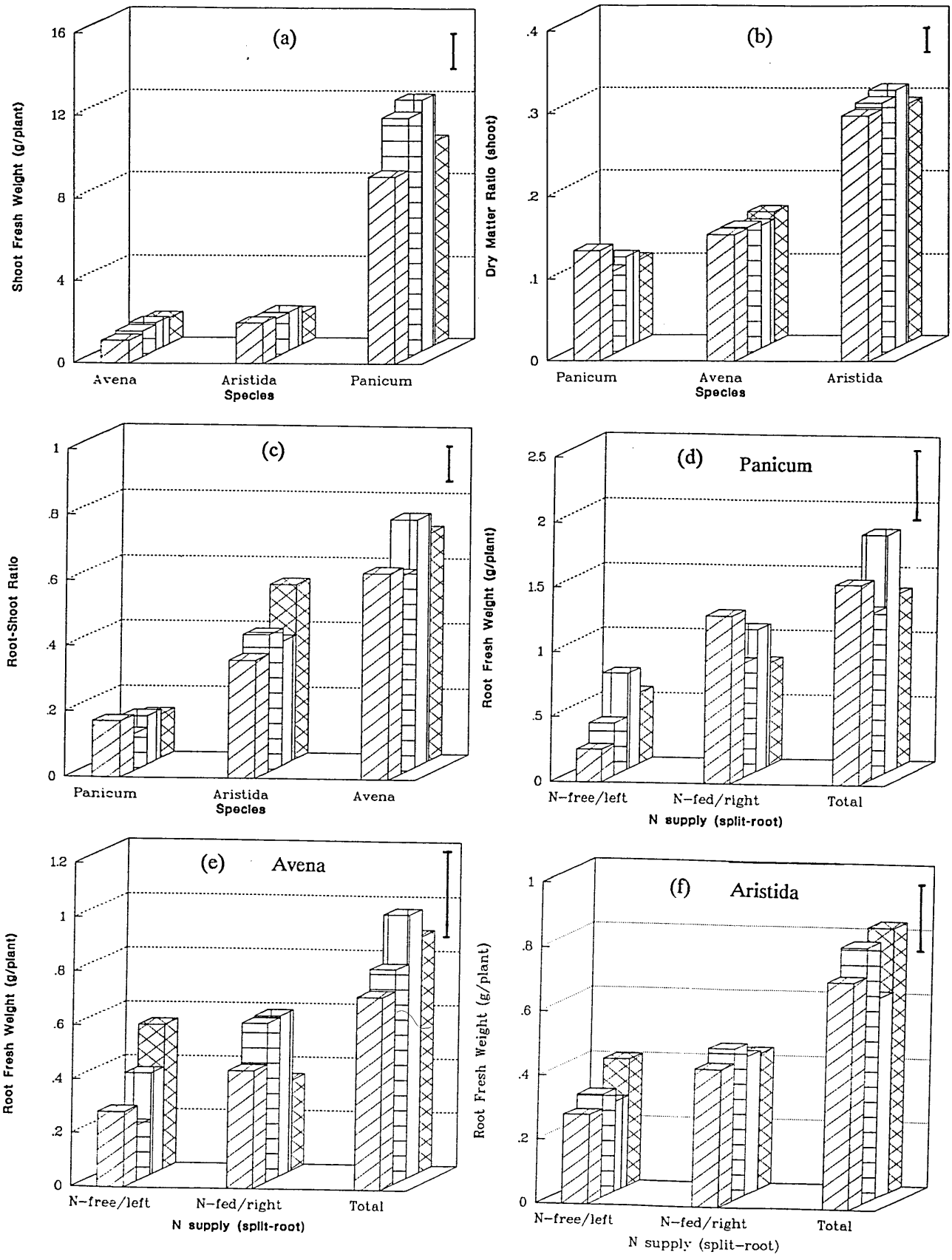
The shoot N concentrations of the three species gradually increased with increasing N supply to the N-fed roots, although Aristida levelled off at N0N2 (Fig 6.2a). The concentrations in the control treatments of Panicum and Aristida were slightly higher than other treatments, whilst the control of Avena was slightly lower than N0N1 treatments but higher than the other treatments.

Table 6.1 Analyses of variance of the effects of four levels of N supply on shoot fresh weight, shoot dry weight, shoot dry matter ratio and root-shoot ratio of Panicum, Avena and Aristida grown with split-root system.

| Source of variation | df | Shoot fresh weight (g/plt) | Shoot dry weight (g/plt) | Root-shoot ratio | Shoot Dry Matter Ratio |
|---|---|---|---|---|---|
| | | | Mean Squares (MS) | | |
| Nitrogen (N) | 3 | 2.427ns | 0.0471ns | 0.0114ns | 0.000287ns |
| Species (SP) | 2 | 336.981*** | 3.116*** | 0.846*** | 0.119*** |
| N x SP | 6 | 2.023ns | 0.0213ns | 0.0113ns | 0.000426ns |
| Residual | 22 | 2.397 | 0.0632 | 0.00963 | 0.000448 |
| Total | 33 | 22.610 | 0.239 | 0.0608 | 0.00761 |
| Grand total | 35 | | | | |

**Figure 6.1** Effects of N level on: a) Shoot fresh weight (g/plant) b) Dry matter ratio (Total DW/Total FW) c) Root/shoot ratio, in Panicum, Avena and Aristida d) Root fresh weight (g/plant) in Panicum e) Root fresh weight (g/plant) in Avena f) Root fresh weight (g/plant) in Aristida. All plants grown in NFT with split-root. Vertical bars are pooled S.E.'s

N0N1 (  ), N0N2 (  ), N0N3 (  ) and N1N1 (  ).


(a)
Shoot Fresh Weight (g/plant)
16
12
8
4
0
Avena
Aristida
Panicum
Species
(b)
Dry Matter Ratio (shoot)
.4
.3
.2
.1
0
Panicum
Avena
Aristida
Species
(c)
Root-Shoot Ratio
1
.8
.6
.4
.2
0
Panicum
Aristida
Avena
Species
(d) Panicum
Root Fresh Weight (g/plant)
2.5
2
1.5
1
.5
0
N-free/left
N-fed/right
Total
N supply (split-root)
(e) Avena
Root Fresh Weight (g/plant)
1.2
1
.8
.6
.4
.2
0
N-free/left
N-fed/right
Total
N supply (split-root)
(f) Aristida
Root Fresh Weight (g/plant)
1
.8
.6
.4
.2
0
N-free/left
N-fed/right
Total
N supply (split-root)

Shoot K concentrations in Panicum were consistently higher than the other species, whilst Aristida was the lowest (Fig 6.2b). Different levels of N supply showed little effect on K concentrations in the three species, although the low N and the control treatment gave the lowest concentrations in Panicum and Avena respectively.

Shoot Mg concentrations in Panicum gradually decreased with increasing N supply, whilst the control gave the highest (Fig 6.2c). The low N plants gave the highest concentrations in Avena, whilst the controls gave the highest values in Aristida.

The shoot Ca concentrations in Avena were consistently higher than other species, and Panicum the lowest (Fig 6.2d). Different N levels produced inconsistent responses for shoot Ca, but the concentrations in Panicum and Avena generally decreased towards the higher N levels, whilst Aristida showed no changes.

Root Nutrient Concentrations

The N concentrations in N-fed roots of all the three species were higher than in N-free roots, with Panicum and Avena showing much larger responses than Aristida (Fig 6.3a). The control plants in which both roots received the standard N application (N1N1), had the same concentrations in both sides of the roots for all the three species.

The K concentrations in both roots (N-fed and N-free) of the three species were not affected by the different levels of N supply (Fig 6.3b). Regardless of N supply, both roots had the same concentrations in all the species, with arisitida showing the lowest values.

The concentration of Mg in both roots of Panicum were not affected by N supply, whilst the N-fed roots of Avena and Aristida were generally higher than the N-free roots (Fig 6.3c).

The root Ca in all three species showed inconsistent responses although the low N and the control treatments of Avena showed the highest concentrations (Fig 6.3d).

Commentary

The plants of Panicum were substantially larger than Aristida and Avena, even after a preliminary trial to study timing, aimed at producing uniform plant sizes for all species at the

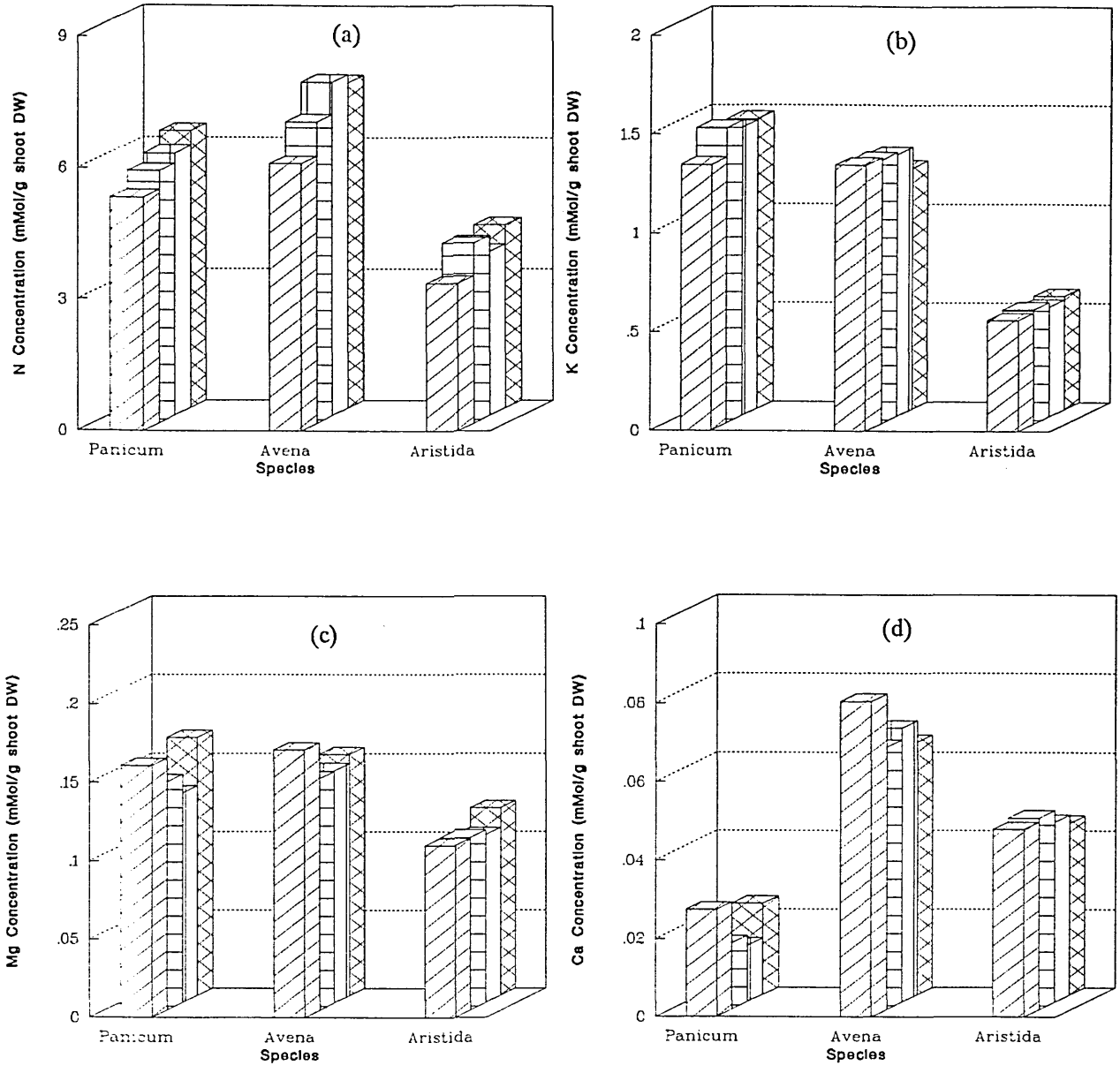



**Figure 6.2** Effects of N level on the concentration of: a) N b) K c) Mg d) Ca (mMol/g DW), in shoot of Panicum, Avena and Aristida grown in NFT with split-root.

N0N1 ( ), N0N2 ( ), N0N3 ( ) and N1N1 ( ).

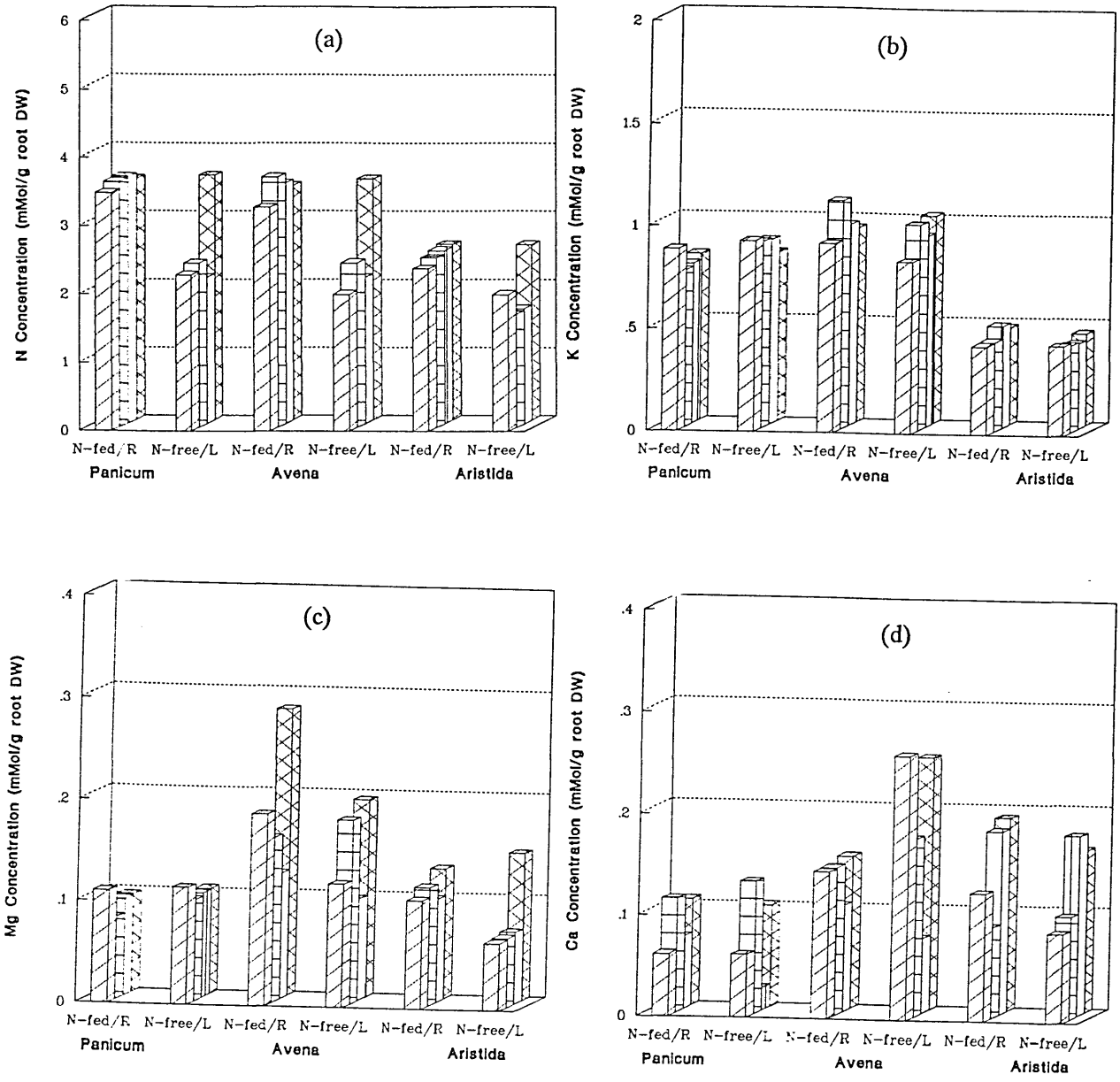



**Figure 6.3** Effects of N level on the concentration of: a) N b) K c) Mg d) Ca (mMol/g DW), in root of Panicum, Avena and Aristida grown in NFT with split-root.

N0N1 ( ), N0N2 ( ), N0N3 ( ) and N1N1 ( ).

L = left side, R = right side

beginning of the experiment. This arose because Panicum produced short, highly branched seminal root systems which took longer to reach a total length suitable for use with this experimental technique. Because of this difficulty, Panicum was not used in later split-root experiments.

In this experiment, for all three species, the higher N concentrations (N0N2 AND N0N3) supplied to one side of the root systems did not show higher concentrations of N in tissues than the control treatments (N1N1) which received the same low N concentrations to both parts. The most likely explanation is that the roots from the N-fed parts were not efficient enough to fully utilize the high N in the solutions within the short period of this experiment. Since one of the main reasons for performing this experiment was to follow the re-circulation of nutrients, following the technique of Lambers *et al.* (1982), and since this is best done under conditions of N stress, the low N level (N0N1) was used in the next experiment to compare the roots efficiencies under water stress conditions.

### Experiment 6.2.2 The growth and translocation of N in Avena and Aristida under water stress, using the split-root technique.

#### *Material and Methods*

The plants of Aristida were derived by vegetative propagation from a single parent on 3/6/89. Two weeks later the plants were pruned to two equal-sized adventitious roots. In a subsequent experiment, seeds of Avena was sown in trays of vermiculite on 31/7/89 and a week later the seedlings were pruned to two seminal roots. The plants were transferred to a nutrient film culture installation made up of pairs 1.2 m long PVC channels, where each root was grown in a separate channel containing nutrient solutions with different levels of N.

Nutrient solutions from 7 litre reservoirs, were circulated through the channels at flow rates of 1 litre per minute. Every day, the nutrient solutions were checked and corrected for changes of water level, pH and conductivity. The development of other adventitious roots was prevented by keeping the root-bearing nodes dry and above the nutrient solutions. The stems of plants were inserted through slits cut into polystyrene board which supported the plants. Evaporation and algal growth was prevented by covering the channels with black and white polythene sheet.

After having grown for one week, when the roots were well developed, the nutrient solutions were changed. In the stressed treatments, the water potential was gradually lowered by adding polyethylene glycol (PEG 20,000) at an average rate of 3% (w/v) per day. After 7 days a total of 22% of PEG 20,000 was reached. The experimental treatments were arranged in a split-plot design with two replications. The nutrient solutions were made up as shown in the table.

| Treatment | mM N in nutrient solution supplied to | |
|---|---|---|
| | Root A | Root B |
| N0 N1 | 0 | 3.569 (50 ppm ) |
| N0S N1S (PEG stress) | 0 | 3.569 (50 ppm ) |
| N1 N1 | 3.569 (50 ppm) | 3.569 (50 ppm ) |
| N1S N1S (PEG stress) | 3.569 (50 ppm) | 3.569 (50 ppm) |

The N-free nutrient solution again contained $CaCl_2$ (to replace $Ca(NO_3)_2$), and KCl (to replace $KNO_3$) plus other components as in the complete nutrient solution of Cooper, 1988.

After 15 days, fresh and dry weights of shoots and roots were determined, root volumes and root lengths were measured and their nutrient contents analysed. Since the roots were clumped together inside the channels, they had to be bulked within each main plot treatment for all determinations. The data on Aristida and Avena from the two consecutive experiments were analysed separately but are presented together in order to compare the pattern of responses between the two species.

## *Results*

### Plant Growth

The effects of water stress on shoot and root growth of the two species are presented in Fig 6.4a to 6.4f. Analyses of variance showed that both species responded significantly to water stress treatments, whilst the effects of nitrogen and its interactions with water stress were only significant in Aristida except for nitrogen in shoot fresh weight (Table 6.2).

Table 6.2 Analyses of variance of the effects of water stress on shoot fresh weight and dry matter ratio of Aristida and Avena grown with split-root system.

| Source of variation | df | Aristida | | Avena | |
|---|---|---|---|---|---|
| | | Shoot fresh weight (g/plt) | Shoot dry Matter Ratio | Shoot fresh weight (g/plt) | Shoot Dry Matter Ratio |
| | | Mean Squares (MS) | | | |
| Nitrogen (N) | 1 | 0.422ns | 0.00830* | 0.726ns | 0.000458ns |
| Water (WS) | 1 | 9.791*** | 0.0318*** | 36.577*** | 0.00694*** |
| N x WS | 1 | 2.668* | 0.00617* | 1.0791ns | 0.0000848ns |
| Residual | 35 | 0.519 | 0.001305 | 0.5657 | 0.0001272 |
| Total | 38 | 0.817 | 0.00242 | 1.531 | 0.000314 |
| Grand Total | 39 | | | | |

The shoot fresh weights of Avena were considerably higher than those of Aristida (Fig 6.4a). The weights consistently decreased in the stressed treatments of both species, with Avena showing the greater response, although the differences were not quite significant, probably due to the rather low degree of replication in the experiment. The non-stressed plants of the control treatments (N1N1) of both species were again consistently higher than other treatments. The stressed plants in which both sides of the roots received N (N1SN1S) were slightly lower than the N0SN1S treatment in Aristida, whilst Avena showed no differences across treatments.

In contrast to the shoot fresh weights, the total dry weights of Aristida were consistently higher than Avena (Fig 6.4b). The controls (N1N1) of both species were again higher than other treatments, and were reduced by water stress. The increments in weight of N0SN1S plants of both species were more or less the same as N1SN1S plants.

The change in root-shoot ratios of Avena were much higher than Aristida (Fig 6.4c). Water stress caused large increases in the ratios of both species, with Avena showing much larger effects. The ratios of both species showed little effect of N supply although the low N (N0N1) showed slightly higher values than the controls.

The dry matter ratio of Aristida was substantially higher than Avena (Fig 6.4d). N1SN1S Aristida showed the highest ratio, whilst the stressed Avena generally showed slightly higher ratios than the non-stressed treatment.

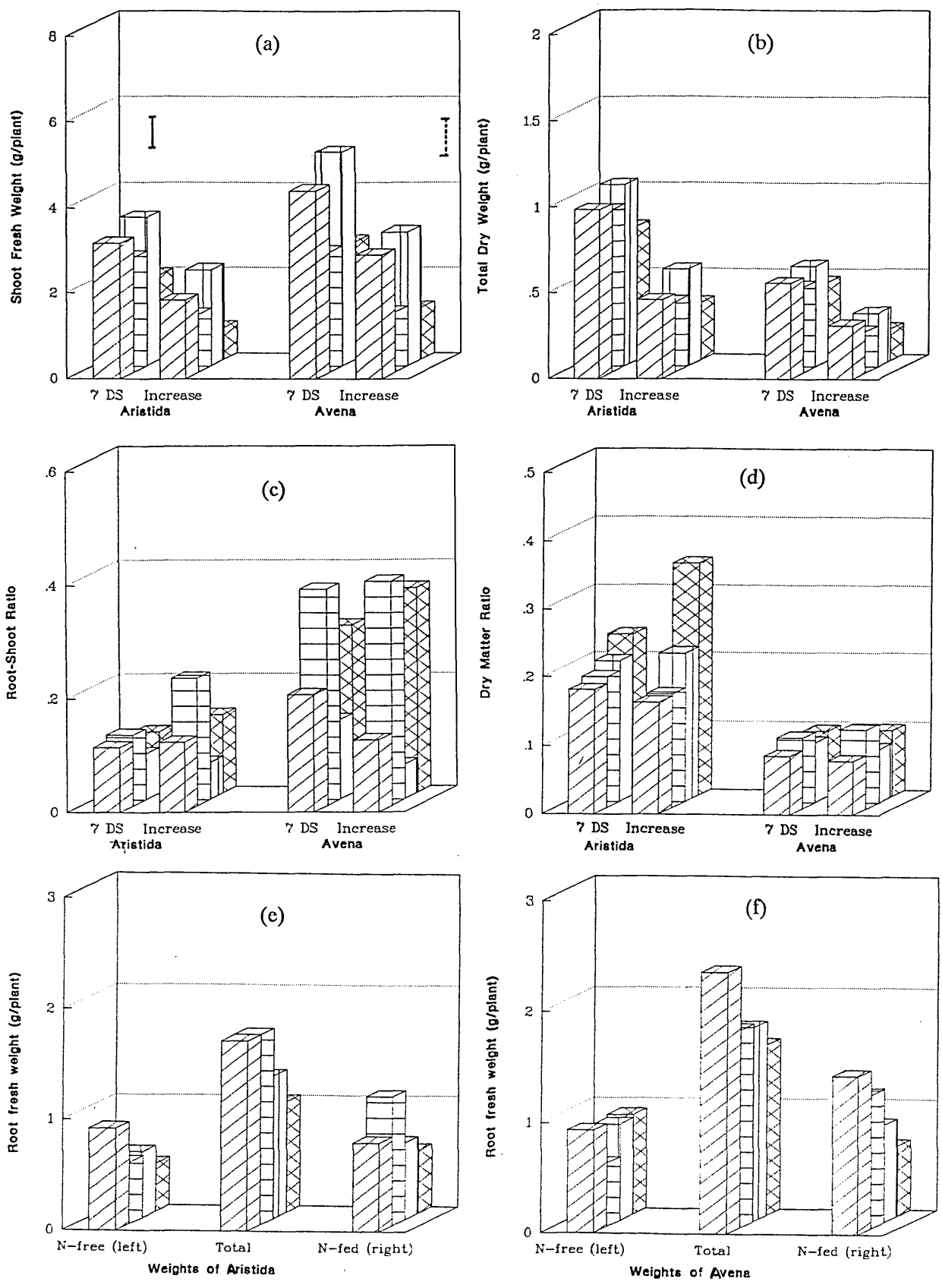
The changes in root fresh weights of Aristida and Avena are shown in Fig 6.4e and 6.4f respectively. The weights of the N-fed roots of N0N1 and N0SN1S plants of both species were greater than those of the N-free roots (see Plate 3), except for N0N1 Aristida which had slightly lower weights than the N-free roots. The N-fed roots of N0N1 and N0SN1S Avena were also greater than the N1N1 and N1SN1S plants, whilst the controls of Aristida showed dissimilar weights on both sides. The total weights of NON1 and N0SN1S Aristida were greater than the controls, whilst the N0N1 of Avena was higher than other treatments.

The root volumes ($cm^3$) of both species generally followed a similar pattern to root fresh weights, in which the volumes of the N-fed roots were higher than the controls (N1N1), except for Aristida where the volumes on both sides were dissimilar (Fig 6.4g and 6.4h).

The N-fed roots of the stressed treatments (N0SN1S) of both species had much longer roots than other treatments (Fig 6.4i and 6.4j). Avena N0N1 and N0SN1S roots were also longer than

**Figure 6.4 (a-f)** Effects of osmotic stress on: a) Shoot fresh weight (g/plant) b) Total dry weight (g/plant) c) Root/shoot ratio d) Dry matter ratio (Total DW/ Total FW), in both Avena and Aristida e) Root fresh weight (g/plant) in Aristida f) Root fresh weight (g/plant) in Avena. All plants grown in NFT with split-root.
Vertical bars are pooled S.E.'s
N0N1 (  ), N0SN1S (  ), N1N1 (  ) and N1SN1S (  ).

DS = days after stress
Increase = increment after 7 DS


(a)
Shoot Fresh Weight (g/plant)
8
6
4
2
0
7 DS
Increase
Aristida
7 DS
Increase
Avena
(b)
Total Dry Weight (g/plant)
2
1.5
1
.5
0
7 DS
Increase
Aristida
7 DS
Increase
Avena
(c)
Root-Shoot Ratio
.6
.4
.2
0
7 DS
Increase
Aristida
7 DS
Increase
Avena
(d)
Dry Matter Ratio
.5
.4
.3
.2
.1
0
7 DS
Increase
Aristida
7 DS
Increase
Avena
(e)
Root fresh weight (g/plant)
3
2
1
0
N-free (left)
Total
N-fed (right)
Weights of Aristida
(f)
Root fresh weight (g/plant)
3
2
1
0
N-free (left)
Total
N-fed (right)
Weights of Avena

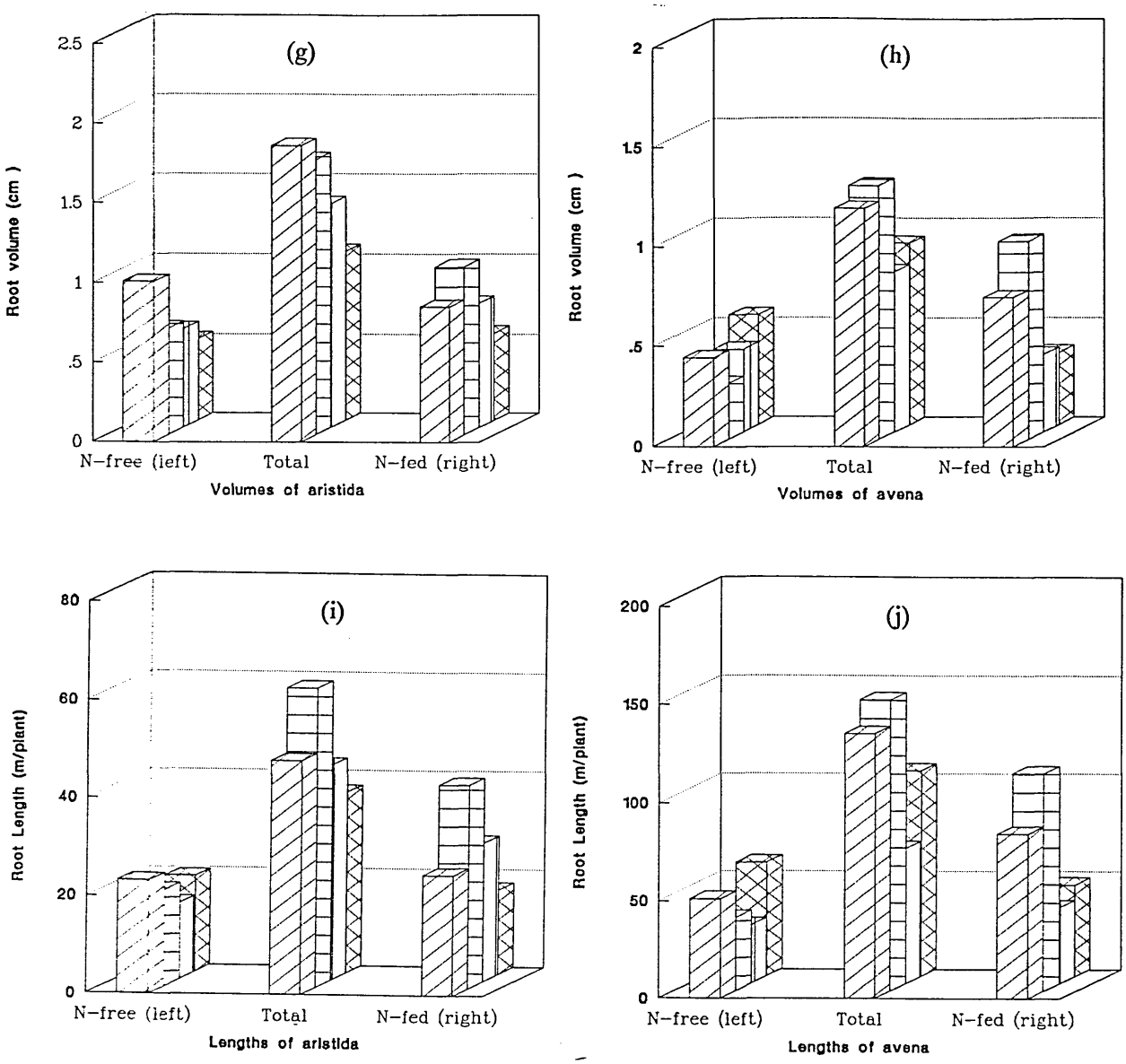



**Figure 6.4 (g-j)** Effects of osmotic stress on: g) Root volume ($cm^3$) of Aristida h) Root volume ($m^3$) of Avena i) Root length (m) of Aristida j) Root length (m) of Avena. All plants grown in NFT with split-root.

N0N1 ( ), N0SN1S ( ), N1N1 ( ) and N1SN1S ( ).

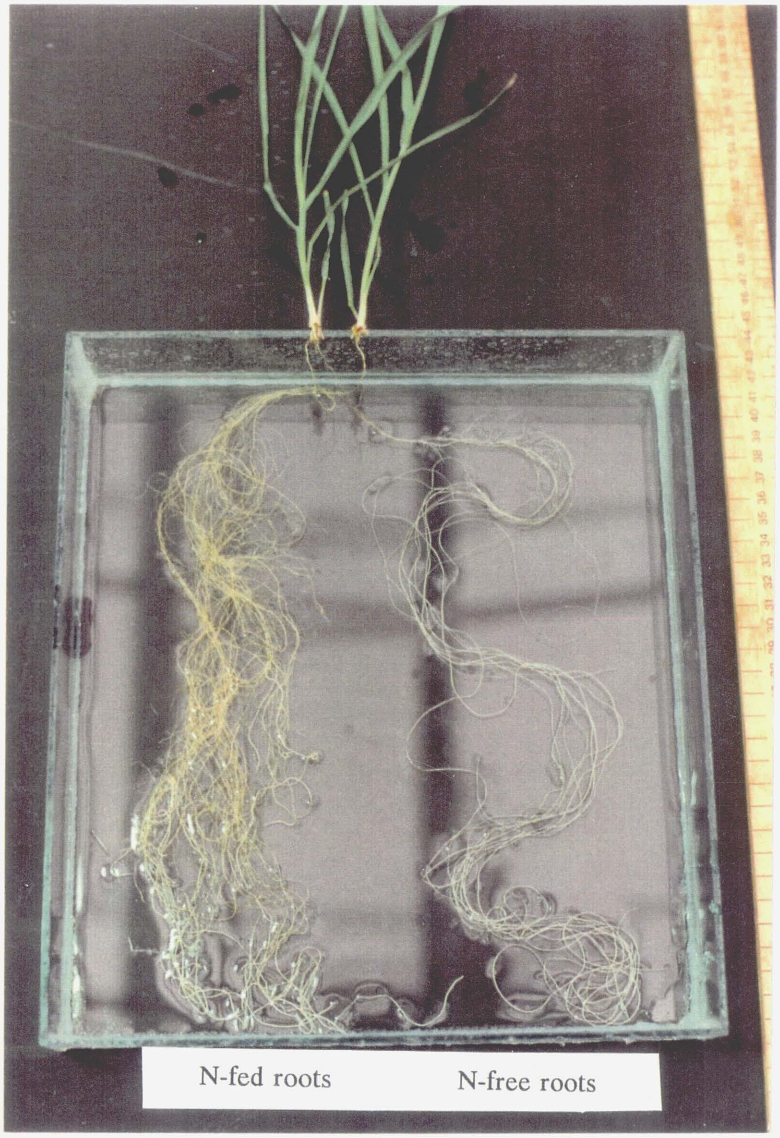



Plate 3. The morphology of the split root system. N0N1 treatment of *Avena fatua plants*.

N1N1 and N1SN1S, whilst the N-free and N-fed roots of N0N1 Aristida had more or less the equal lengths.

Shoot Nutrient Concentrations

The concentrations of N, P, K, Mg and Ca in the shoot dry weights of the two species are illustrated in Fig 6.5a to 6.5e.

The shoot N concentrations in Avena were consistently higher than Aristida (Fig 6.5a). In general, the concentrations of all treatments in both species were not affected by water stress, although the N1SN1S plants were slightly higher than other treatments in Avena.

The concentrations of shoot P, K, Mg and Ca in both species similarly showed no obvious changes due to water stress in any of the treatments. The concentrations of K and Ca were consistently higher in Avena and Aristida respectively, whilst the shoot P and Mg were generally the same in both species (Fig 6.5b to 6.5e). Stressed Avena also showed slightly higher concentrations of Ca than non-stressed treatments.

Root nutrient concentrations

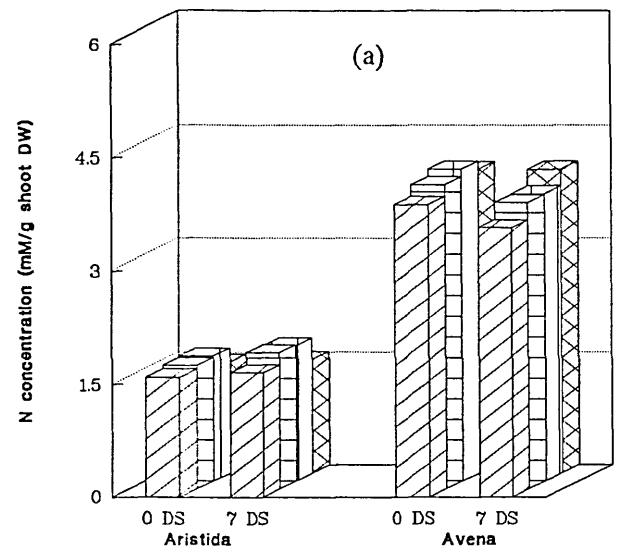
The root N concentrations in Avena were much higher than Aristida, whilst the concentrations in N-fed roots of N0N1 and N0SN1S treatments of both species were higher than in N-free roots, with Avena showing the larger responses to N supply (Fig 6.6a and 6.6b). The concentrations at 7 days on each side of the roots in stressed (N01N1S and N1SN1S) Aristida were slightly lower than the non-stressed treatments (N0N1 and N1N1), whilst the responses of Avena to water stress on both sides of the roots were inconsistent. The concentrations of N on the same N-fed or N-free roots of both species declined throughout the experimental period, although the concentrations in N-free roots of N0N1 and N1N1 Aristida showed no obvious changes.

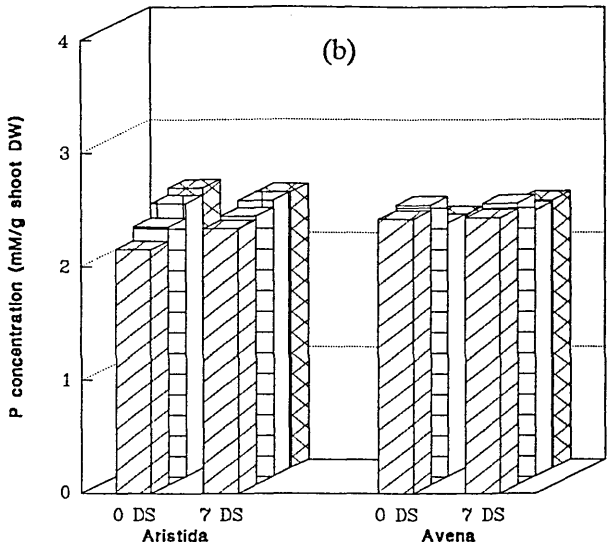
The concentrations of K in both sides of the roots of N0N1 Aristida were much higher than other treatments, whilst the concentrations at 7 days in Avena showed no obvious differences between any of the treatments (Fig 6.6c and 6.6d). The concentrations in both species were unaffected by water stress although the N0SN1S Aristida was greatly reduced compared with the non-stressed (N0N1).

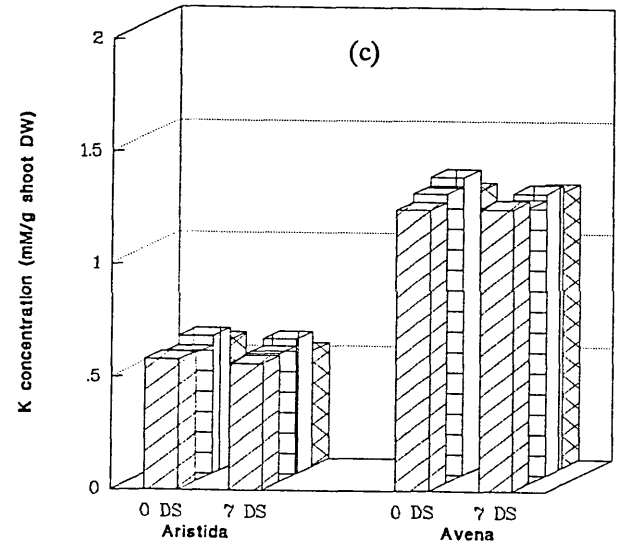
**Figure 6.5** Effects of osmotic stress on the concentration of: a) N b) P c) K d) Mg e) Ca (mMol/g DW), in shoot of Avena and Aristida. All plants grown in NFT with split-root.

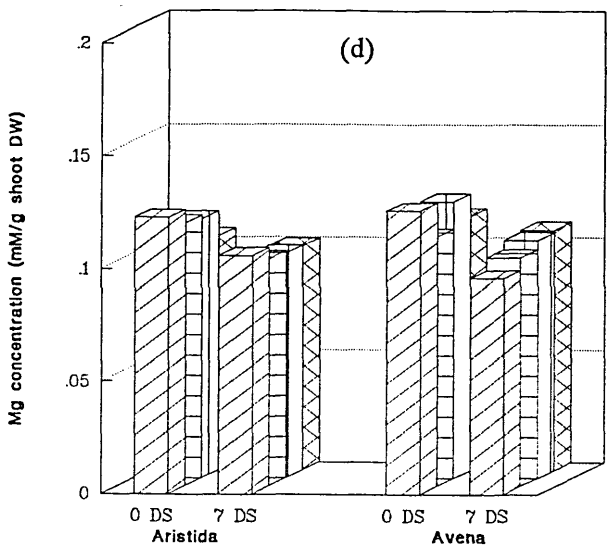
N0N1 (  ), N0SN1S (  ), N1N1 (  ) and N1SN1S (  ).

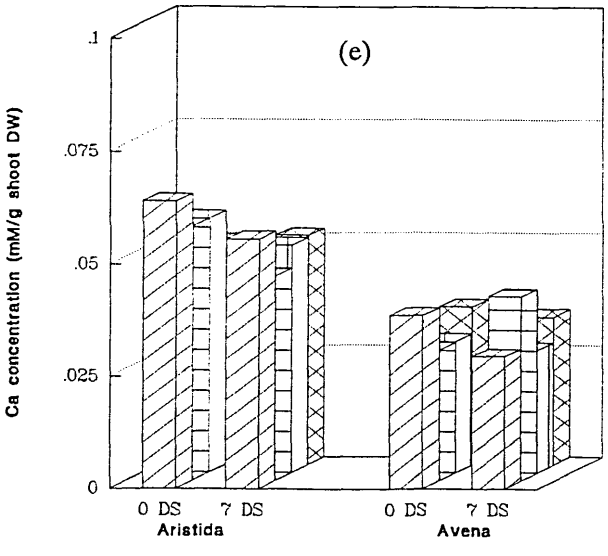
DS = Days after stress


(a)
N concentration (mM/g shoot DW)
6
4.5
3
1.5
0
0 DS
7 DS
Aristida
0 DS
7 DS
Avena


(b)
P concentration (mM/g shoot DW)
4
3
2
1
0
0 DS
7 DS
Aristida
0 DS
7 DS
Avena


(c)
K concentration (mM/g shoot DW)
2
1.5
1
.5
0
0 DS
7 DS
Aristida
0 DS
7 DS
Avena


(d)
Mg concentration (mM/g shoot DW)
.2
.15
.1
.05
0
0 DS
7 DS
Aristida
0 DS
7 DS
Avena


(e)
Ca concentration (mM/g shoot DW)
.1
.075
.05
.025
0
0 DS
7 DS
Aristida
0 DS
7 DS
Avena

The concentrations of Mg in roots of N0N1 Aristida at 7 days was much higher than other treatments at the same time. In Avena, the concentrations in N1N1, N1SN1S and in N-fed roots of N0N1 Avena had higher values than other treatments (Fig 6.6e and 6.6f). Stressed Aristida (N1SN1S) showed a slightly higher concentration than the non-stressed treatment (N1N1), whilst the non-stressed and stressed treatments of the Avena controls showed more or less the same concentrations.

The final concentrations of Ca in the stressed roots (N0SN1S and N1SN1S) of the two species were about 6 times greater than in roots of other treatments, except for N-free roots of the stressed N0SN1S Aristida where the concentration was greatly reduced (Fig 6.6g and 6.6h).

The final concentrations of P in roots of both species were inconsistent in response to water stress and N supply, although the concentrations in N-fed roots of N0N1 and in roots on both sides of the controls Aristida were slightly higher than in any other treatment (Fig 6.6i and 6.6j).

Shoot nutrient content

The shoot N content of N, P, K, Mg and Ca of the two species are illustrated in Fig 6.7a to 6.7e. The patterns of change of shoot nutrient contents of both species were again broadly similar, and generally followed the same patterns as shoot weights, in which the stressed treatments of both species showed slightly higher contents than the non-stressed.

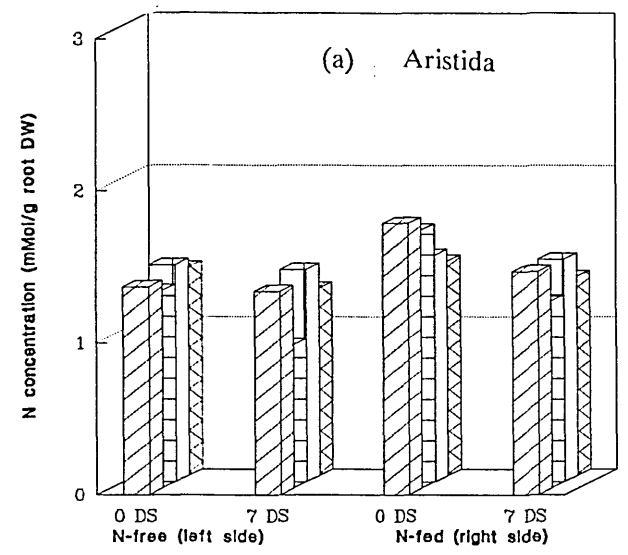
The shoot N contents in both species were generally reduced with water stress, although the N0N1S plants of Aristida were similar to the non-stressed treatment (Fig 6.7a). The N1N1 plants, as expected, consistently possessed the highest contents in both species.

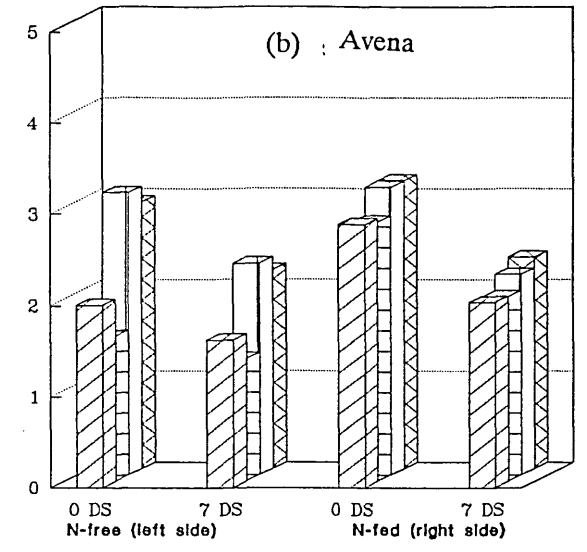
The contents of shoot P, K, Mg and Ca of both species followed similarly patterns to N, in that they were reduced with water stress. The contents of all nutrients were consistently higher in Aristida, except for K which more or less the same in both species (Fig 6.7b to 6.7e). Non-stressed plants of the controls in both species always showed slightly higher contents.

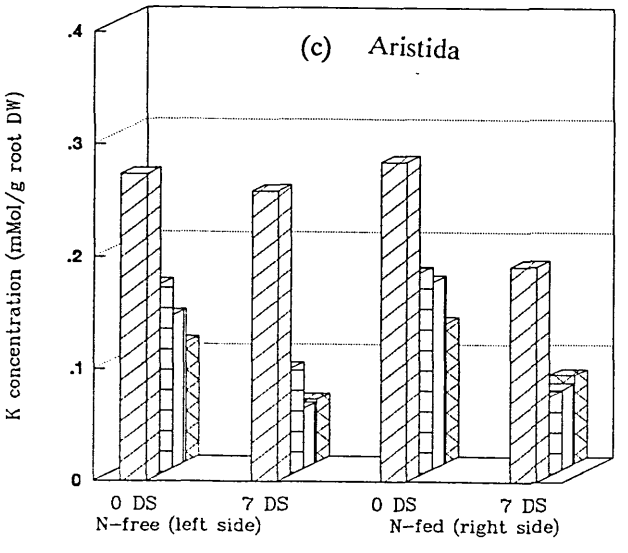
**Figure 6.6 (a-f)** Effects of osmotic stress on the concentration of: a) N in root of Aristida b) N in root of Avena c) K in root of Aristida d) K in root of Avena e) Mg in root of Aristida f) Mg in root of Avena (mMol/g DW). All plants grown in NFT with split-root.

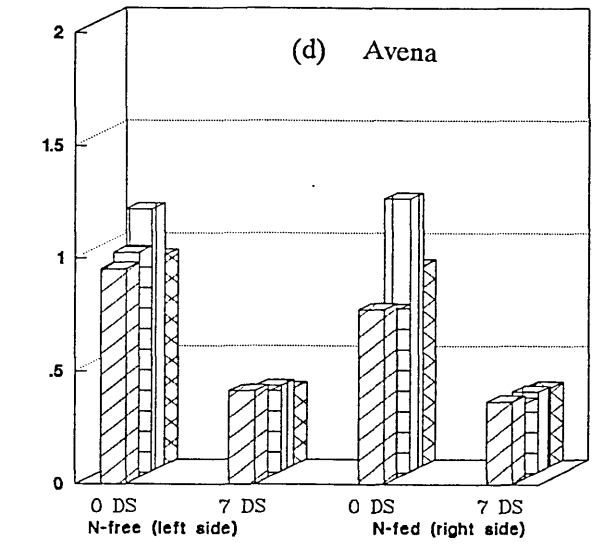
N0N1 (  ), N0SN1S (  ), N1N1 (  ) and N1SN1S (  ).

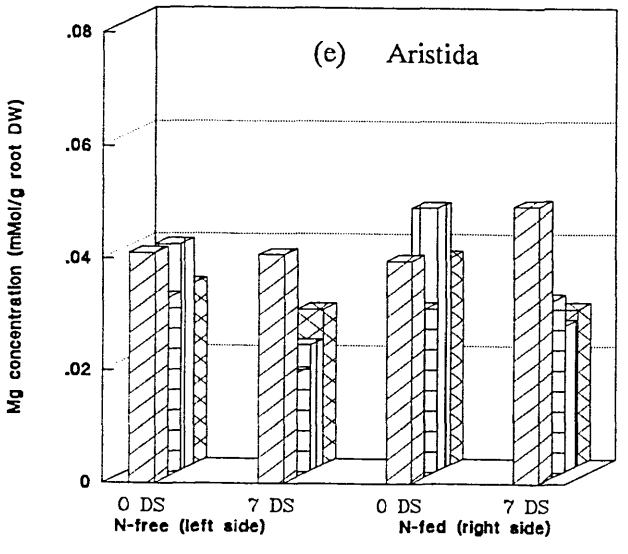
DS = Days after stress

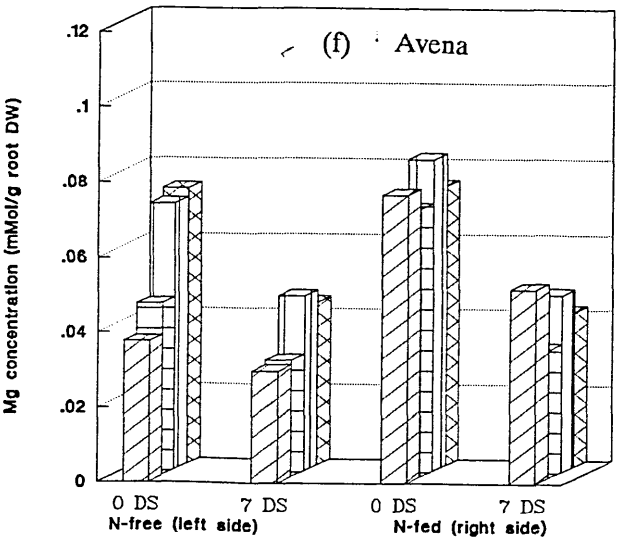

(a) Aristida
N concentration (mMol/g root DW)
3
2
1
0
0 DS
7 DS
0 DS
7 DS
N-free (left side)
N-fed (right side)


(b) Avena
N concentration (mMol/g root DW)
5
4
3
2
1
0
0 DS
7 DS
0 DS
7 DS
N-free (left side)
N-fed (right side)


(c) Aristida
K concentration (mMol/g root DW)
.4
.3
.2
.1
0
0 DS
7 DS
0 DS
7 DS
N-free (left side)
N-fed (right side)


(d) Avena
K concentration (mMol/g root DW)
2
1.5
1
.5
0
0 DS
7 DS
0 DS
7 DS
N-free (left side)
N-fed (right side)


(e) Aristida
Mg concentration (mMol/g root DW)
.08
.06
.04
.02
0
0 DS
7 DS
0 DS
7 DS
N-free (left side)
N-fed (right side)


(f) Avena
Mg concentration (mMol/g root DW)
.12
.1
.08
.06
.04
.02
0
0 DS
7 DS
0 DS
7 DS
N-free (left side)
N-fed (right side)

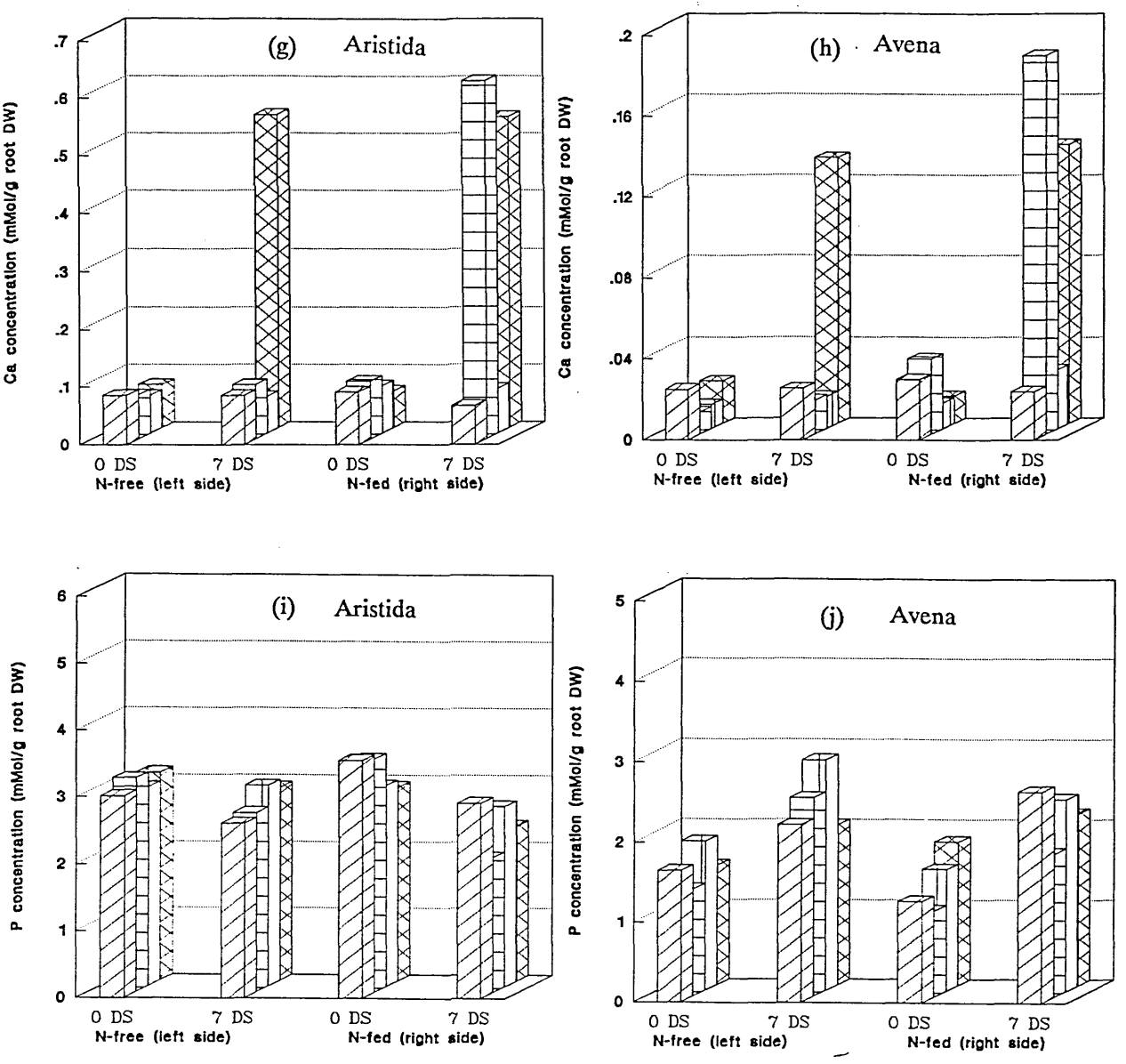


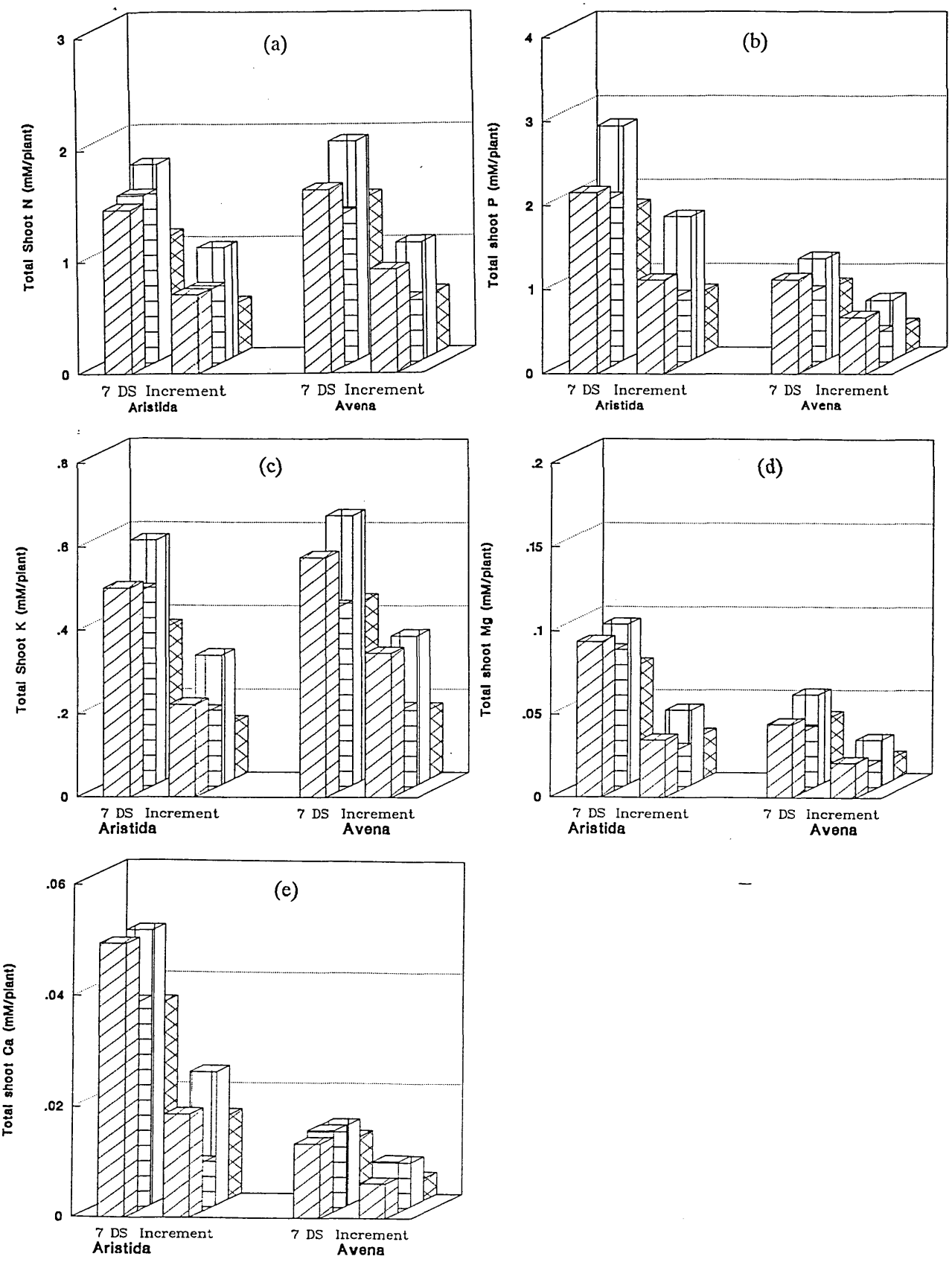

**Figure 6.6 (g-j)** Effects of osmotic stress on the concentration of: g) Ca in root of Aristida h) Ca in root of Avena i) P in root of Aristida j) P in root of Avena (mMol/g DW). All plants grown in NFT with split-root.
N0N1 ( ), N0SN1S ( ), N1N1 ( ) and N1SN1S ( ).

DS = Days after stress

**Figure 6.7** Effects of osmotic stress on the total shoot: a) N b) P c) K d) Mg e) Ca (mMol/plant), in both Avena and Aristida grown in NFT with split-root.

N0N1 (  ), N0SN1S (  ), N1N1 (  ) and N1SN1S (  ).

DS = Days after stress


(a)
Total Shoot N (mM/plant)
3
2
1
0
7 DS Increment
Aristida
7 DS Increment
Avena
(b)
Total shoot P (mM/plant)
4
3
2
1
0
7 DS Increment
Aristida
7 DS Increment
Avena
(c)
Total Shoot K (mM/plant)
.8
.6
.4
.2
0
7 DS Increment
Aristida
7 DS Increment
Avena
(d)
Total shoot Mg (mM/plant)
.2
.15
.1
.05
0
7 DS Increment
Aristida
7 DS Increment
Avena
(e)
Total shoot Ca (mM/plant)
.06
.04
.02
0
7 DS Increment
Aristida
7 DS Increment
Avena

Root nutrient content and N-increment

The root N contents of Aristida generally followed the same patterns as root fresh weights, in which the N-fed roots of N0SN1S always showed a slightly higher contents than other treatments at any sides of the roots system (Fig 6.8a). In contrast to Aristida, the total N content of the stressed plants of Avena were much higher than non-stressed treatments, and similarly the N-fed roots of N0SN1S plants posessed the highest content on both sides of the roots in all treatments (Fig 6.8b).

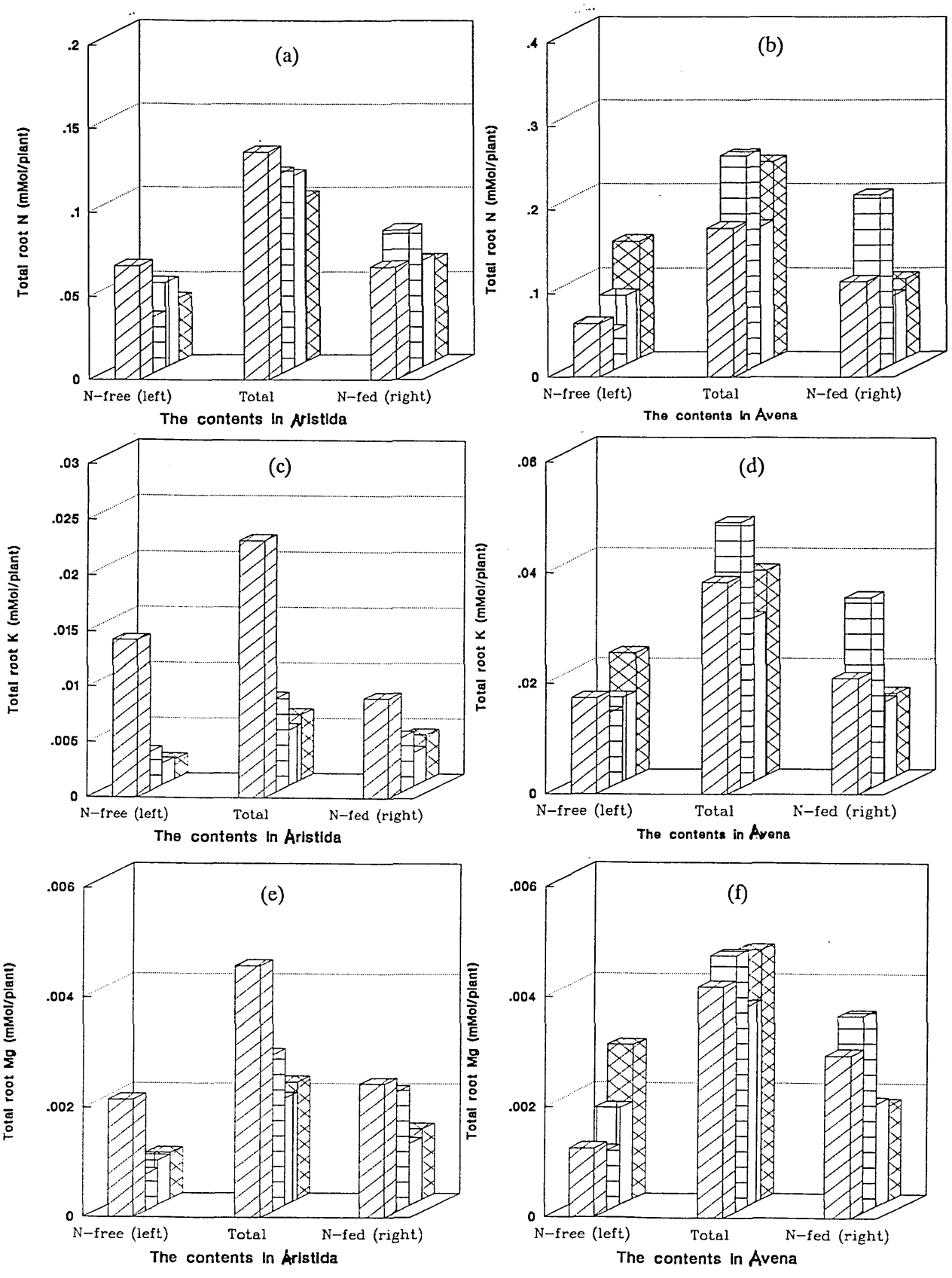
The contents of root P, K, Mg and Ca of both species generally followed the same patterns of responses as roots concentration, in that the total content of all nutrients in Avena and the content of Ca in Aristida were consistently increased with water stress (Fig 6.8c to 6.8j). The total contents of all nutrients were more or less the same in both species, except for Ca and K which consistently higher in Aristida and Avena respectively. Non-stressed plants of N0N1 Aristida always showed a higher contents of K and Mg on both sides of the roots compare to any other treatments, whilst the stressed plants (N0SN1S and N1SN1S) of Avena similarly showed the higher contents of roots K and Mg.

The increment and translocation of N at 7 days of stress in both roots and shoots is presented in Table 6.3. N-increment (net uptake) was calculated from data of total shoot or root N content during that period. Based upon assumption that all N was translocated by mass flow, the amount of N translocated in N-fed roots (Item 4 in Table 6.3) was estimated.
The N-increment in N-fed roots of N0SN1S treatments of both species were higher than in roots at either sides of the stressed control (N1SN1S). The uptake of N-fed roots in N0N1 treatment of the two species were lower than in N0SN1S roots.

Commentary

In this experiment, the water potentials of the stressed solutions were gradually lowered by adding PEG 20,000 over a period of 7 days, in the hope of avoiding stress shock and allowing time for adaptation to the final stress level. Injury to the roots could have resulted in an increased uptake of PEG and nutrients by opening the route of ion entry to a mass flow system to the root xylem. However, the substantial increase in concentrations of Ca in the stressed roots (N1SN1S) of both species probably revealed an impaired transport of nutrients from the roots to the shoots. The lower concentration of Ca in N-free roots of N0SN1S plants might similarly be explained if the stressed N-fed roots failed to retranslocate Ca to the other side of the root system.

**Figure 6.8 (a-f)** Effects of osmotic stress on the: a) Total root N in Aristida b) Total root N in Avena c) Total root K in Aristida d) Total root K in Avena e) Total root Mg in Aristida f) Total root Mg in Avena (mMol/g plant).
All plants were grown in NFT with split-root.
N0N1 (  ), N0SN1S (  ), N1N1 (  ) and N1SN1S (  ).


(a)
Total root N (mMol/plant)
.2
.15
.1
.05
0
N-free (left)
Total
N-fed (right)
The contents in Aristida
(b)
Total root N (mMol/plant)
.4
.3
.2
.1
0
N-free (left)
Total
N-fed (right)
The contents in Avena
(c)
Total root K (mMol/plant)
.03
.025
.02
.015
.01
.005
0
N-free (left)
Total
N-fed (right)
The contents in Aristida
(d)
Total root K (mMol/plant)
.06
.04
.02
0
N-free (left)
Total
N-fed (right)
The contents in Avena
(e)
Total root Mg (mMol/plant)
.006
.004
.002
0
N-free (left)
Total
N-fed (right)
The contents in Aristida
(f)
Total root Mg (mMol/plant)
.006
.004
.002
0
N-free (left)
Total
N-fed (right)
The contents in Avena

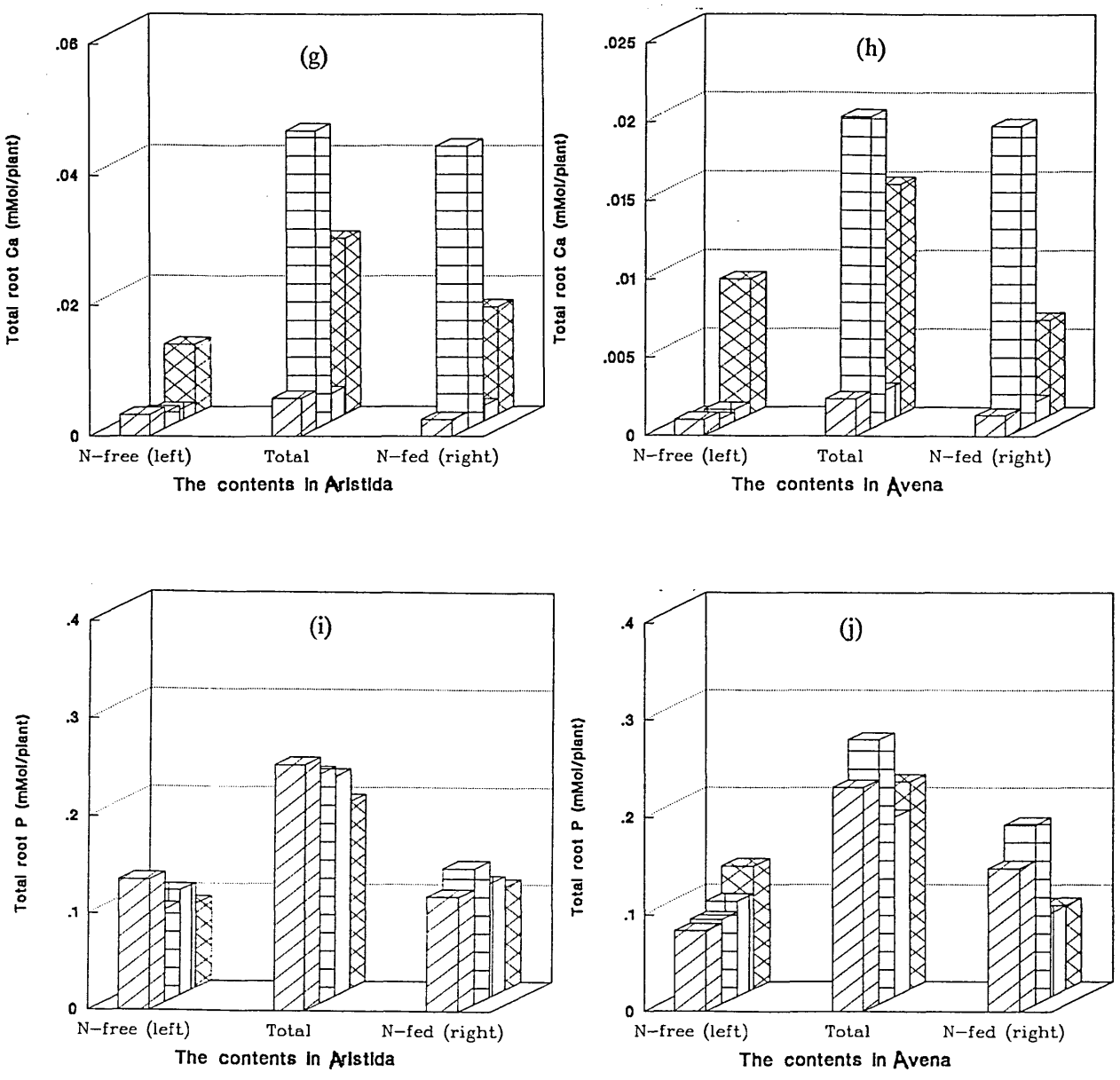



**Figure 6.8** (g-j) Effects of osmotic stress on the: g) Total root Ca in Aristida h) Total root Ca in Avena i) Total root P in Aristida j) Total root P in Avena (mMol/g plant). All plants were grown in NFT with split-root. N0N1 ( ), N0SN1S ( ), N1N1 ( ) and N1SN1S ( ).

## 6.3 Discussion

Development of plants with split-root systems

PEGs of various molecular weight grades (200-20,000), have been widely used as osmotic agents in solution culture to subject plant tissue to known levels of plant water stress (Kawasaki, *et al.*, 1983a, 1983b). Several reseachers (e.g. Lagerwerff *et al.*, 1961; Lawlor, 1970) have reported that PEG may enter and/or damage plant tissues, influencing the plant's response to changes in external potential at the root. Furthermore, PEG induces water stress very rapidly or instantaneously, which is uncharacteristic of stresses in the field (Lagerwerff *et al.*, 1961). This rapid development of stress can affect the mechanisms, including the amount of osmotic adjustment by which plants tolerate drought conditions, such that results obtained using PEG may not reflect plant performance in the field (Chapman and Fisher, 1988). In the present investigation, the water potentials of the nutrient solutions were gradually lowered by adding PEG 20,000 at an average rate of 3% (w/v) per day over a period of 7 days. This allowed time for osmotic adjustment and avoided the stress shock which could result in root injury.

Lawlor (1970) showed that when cotton plants had healthy roots, PEG 1000, 4000 and 20,000 were not absorbed in significant quantities. When the roots were injured there was a rapid uptake of all molecular sizes. PEG 200 and mannitol, however, could be absorbed by healthy roots. This is broadly in agreement with Janes (1974) who suggested that the PEG molecules entered the roots in a random manner and that the number of molecules entering the roots was related to the sizes of the pores. There was a appreciable number of pores of a size to permit passage of PEG 400 but fewer pores large enough for PEG 4000 to enter the roots of pepper plant. These ideas were supported by the present experiments, in that there were no visible symptoms shown by plants of any of the three species which could have been due to the presence of PEG in excessive amounts.

Another possibility is that plant injury might result from a substantial reduction of $O_2$ availability due to the addition of PEG (Mexal *et al.*, 1975). Barret-Lennard *et al.* (1988) demonstrated that hypoxia (low $O_2$ concentrations) in nutrient solutions caused serious damage to seminal root apices of wheat. In the present investigation, plants were grown in a nutrient film with only a thin layer (about 1 cm) of flowing solution so that $O_2$ would be readily available. A similar technique was succesfully used by Mougou *et al.* (1984) to study the effect of PEG-induced water stress on the photosynthetic parameters of four tomato species.

The present results demonstrated that the N-fed roots in the N0N1 and N0SN1S treatments of both species showed compensatory growth (Fig 6.4e and 6.4f). The weights of these roots were higher than those of each of the roots of the control (N1N1 and N1SN1S) plants except for N0N1 in Aristida. This is in agreement with by Lambers *et al.* (1982) who observed a similar pattern of response in $NO_3$-fed roots of wheat plants. They suggested that the increased dry weight of $NO_3$-fed roots was due to increased growth of laterals. In the present experiments, the increases in root volume and length in N-fed roots might similarly be explained.

The root/shoot ratios of Aristida and Avena were increased both with water stress and low N (Fig 6.4c), so that the stressed plants (N1SN1S) had similar weights to those in low N plants (N0N1). This confirmed the earlier results in Avena but disagreed with Aristida which had previously shown nearly constant ratios. These increases in root growth again agree with observations by several other workers (e.g. Rego *et al.* 1986; Hsiao and Acevedo, 1974). For example, Jarvis and Jarvis (1963) using PEG 1540 and 4000 to induce water stress effects observed a small but signifiant stimulation of root growth of *Lupinus albus* seedlings. Similarly, Malik *et al.* (1979) and Sharp and Davies (1979) demonstrated increases in length and dry weight of roots of cotton and maize seedlings as the soil became drier. They suggested that the root tips of maize seedlings were capable of osmotic adjustment which resulted in the maintenance of root turgor and growth (Sharp and Davies, 1979).

Translocation of N in the split-root system

The idea that plants may redistribute nutrients is not new. William (1955) observed the mobilisation of N and P, and S and P (Biddulph *et al.,* 1958) in bean plants, previously incorporated in leaves. Simultaneous translocation of minerals in and out of plant organs has been demonstrated by several workers. For example, cycling of K has been postulated to be associated with $NO_3^-$ uptake (Ben Zioni *et al.*, 1971). This also occurs in *Hordeum vulgare* (Greenway and Pitman, 1965), *Lycopersicum esculentum* (Armstrong and Kirby, 1979) and *Ricinus communis* (Kirby and Armstrong, 1980). Cycling of N has been determined in *Lupinus albus* (Pate *et al.*, 1979; Layzell *et al.*, 1981) and in *Triticum aestivum* (Simpson *et al.*, 1982; Lambers *et al.*, 1982).

In the present investigation it is clear that a large proportion of N may be recycled in both Aristida and Avena. The increments (net uptake) of N in root and shoot of both species, and the total translocation of N to the roots determined during the 7 day stress period are summarised in Table 6.3. The amount of N translocated to the N-fed roots (Item 6 in Table 6.3) was calculated by a method similar to that of Lambers *et al.* (1982).

$$N_{(\text{N-fed root})} = C_{(\text{N-fed root})} \times [N_{(\text{N-free root})} / C_{(\text{N-free root})}]$$

where: N = N translocated to the root

C = C (carbon) translocated to root

$N_{(\text{N-free root})}$ = increment of nitrogen in N-free root

In the present experiment, since the C/N ratio of the phloem sap feeding the roots was not determined, the calculation was based upon the assumptions that a fixed proportion of C transported to the roots contributed to increments in dry weight; that no N translocated to the N-free roots was recycled to the shoot; and that the C/N ratio in phloem sap was constant.

The total amount of N translocated to the N-fed roots of both species increased under water stress but Avena showed a greater response than Aristida (Item 6 in Table 6.3). On the other hand, the amount of N translocated to the total root system in Aristida decreased with water stress but showed an increase in the roots of Avena (Item 7 in Table 6.3). The results from this experiment also showed that the translocation of N to the N-fed roots of Avena was exceeded by its utilization in the N0N1 treatments (Item 8 in Table 6.3). This may suggest that the N-fed roots (N0N1 treatment) of Avena were completely dependant on the supply of N via the phloem instead of using recently absorbed N from the soil. This agrees with Pate *et al.*, (1979) who found that the roots of legumes continued to be fed by the N from the phloem even though it was fully dependent on symbiotic $N_2$-fixation as its source of N.

There is substantial evidence that specific mechanisms are involved in this circulation for mineral nutrients. For example, Simpson *et al.* (1982), using wheat plants grown with a limited supply of N, found that 79% of the N translocated in the xylem to the shoots was retranslocated in the phloem as reduced N back to the roots. Only 21% was actually incorporated into the root tissue and the remainder translocated back in the xylem to the shoots. The proportion of this dynamic component of N could be as high as 100% of the plants' daily uptake of N (Simpson *et al.*, 1982).

Table 6.3 Translocation and utilization of N (mMol/shoot or root) by Aristida (AR) and Avena (AV) plants during the 7 days water stress period

| Items | Treatments | | | |
|---|---|---|---|---|
| | N0N1 (non-stress) | | N0SN1S (stress) | |
| | AR | AV | AR | AV |
| 1. N-increment in shoots | 0.704 | 0.931 | 0.701 | 0.605 |
| 2. N-increment in N-fed roots | 0.0319 | 0.0134 | 0.0689 | 0.082 |
| 3. N-increment in N-free roots | 0.0274 | 0.0136 | 0.0080 | 0.00735 |
| 4. Dry weight increment in N-fed roots | 0.0230 | 0.0210 | 0.0595 | 0.0581 |
| 5. Dry weight increment in N-free roots | 0.0271 | 0.0141 | 0.0158 | 0.00999 |
| 6. N-translocation in N-fed roots (Item 4 x (Item 3/5)) | 0.0233 | 0.0203 | 0.0301 | 0.0427 |
| 7. N-translocation to total root system (Item 3 + 6) | 0.0507 | 0.0339 | 0.0381 | 0.0501 |
| 8. N-translocation to N-fed roots/N-increment of N-fed roots (Item 6/2 x 100) | 73.0408 | 151.493 | 43.687 | 52.0732 |
| 9. Proportion of N retranslocated to roots or may re-cycled (Item 7/(Item 1+2+3) x 100) | 6.642 | 3.539 | 4.898 | 7.208 |

In the present investigation, the total translocation of N to the roots was expressed as a proportion of the total uptake over 7 days (Item 9 in Table 6.3). In the N0N1 treatments this value was about 3.5% in Aristida and 6.6% in Avena, whilst the stressed treatments (N0SN1S) gave 4.9% in Aristida and 7.2% in Avena. Lambers *et al.* (1982), calculated a value of 11% for wheat seedlings in which one of the roots was supplied with different levels of N and the other deprived of N. They also showed that this value depended on the N supply. With a low supply of N, a smaller proportion of N translocated to the tops (81%) was incorporated than under conditions where the supply of N was greater (89%). They then suggested the level of N in the root environment not only affected the rate of N uptake and translocation but also the rate of N incorporation by the shoot.

The greater amount of N that was retranslocated to roots of Avena in plants grown under osmotic stress, as compared with plants grown without stress, may partly be an adaptation to stress. This is in agreement with Lambers *et al.* (1982), who observed a similar response in wheat plants grown under limited supply of N. In contrast, the pattern of N distribution between the shoots and roots in Aristida plants was less affected by water stress. The most likely explanation was that neither the growth nor the N increment in the shoot (Item 1 in Table 6.3) was changed by water stress. This shows that Aristida could tolerate the gradual increases of osmotic stress in the nutrient solutions over the 7-day period of this particular experiment. It contrasts with the results of previous experiments in which the growth of Aristida was markedly reduced with rapidly increasing osmotic stresses.

The results in the present experiments, suggest that the amount of N that is cycled in a plant is the consequence of a balance between its capacities to absorb and incorporate N. Thus Avena plants showed a large reduction in shoot growth due to water stress, which was associated with an increase in the total proportion of N that was cycled. This increase, however, was less than the increase in total root growth so that the concentration of N in water stressed roots actually declined (Fig. 6.6b). In contrast, Aristida plants which grew more normally at the low N level used (50 ppm) exhibited smaller changes in the patterns of N distribution between the shoots and roots. This suggests that the ability of Avena to cycle N must give it the potential to continue to develop its root system even when the roots encounter regions of soil poor in N. Roots growing through areas lacking nutrients would be entirely dependent on N translocated from the shoot via the phloem (Simpson *et al.* 1982).

# CHAPTER SEVEN

## General Conclusion and Suggestion For Further Study

# CHAPTER SEVEN

## 7.0 GENERAL CONCLUSION AND SUGGESTIONS FOR FURTHER STUDY

The results presented in this thesis have advanced the available information on some of the aspects of nutrient uptake in *Panicum miliaceum* (proso millet), *Avena fatua* (wild oat) and *Aristida funiculata* (a wild arid-zone weed), three grass species of different origins and habitats, in relation to water stress. Most of the effects have not previously been reported in the literature, whilst others provide confirmation of the results given only brief consideration in the past. Many interesting observations have been made during the course of this study. Some have been confirmed with reasonable certainty, other merit further study.

The investigation started with the hypothesis that wild arid-zone weeds, by nature of their evolutionary background, may be expected to show greater efficiencies of nutrient uptake and/or utilization than species which favour moister habitats, particularly under conditions of limited nutrient and/or water supplies. The main objective of the work was therefore to look for evidence of differences and similarities between the three species under soil moisture or osmotic stress and with varying levels of nutrients.

One of the most important features of the project was that two methods were used to induce water stress; withholding water from the soil and using an osmotic agent (PEG). It was established that using gradually increasing concentrations of PEG either in conjunction with a semi-permeable membrane, or in a circulating nutrient solution, had the advantage of inducing precise levels of osmotic stress whilst maintaining adequate root aeration. This system therefore resembled the stresses created by withholding water, but did so in a manner allowing more precise comparison between species. Similar inter-species differences were obtained by both methods, confirming that these methods of applying stress using PEG are valid.

Data have been presented confirming that there are differences in both uptake and utilization efficiencies in the three species in relation to N and/or K supply, and soil moisture regimes. It was quite conclusively shown that water stress caused the largest effects on the total dry weighs of Avena, followed by Panicum, and with no obvious changes in weights of Aristida. Indeed, it was clearly shown that Aristida developed the ability to produce maximum growth at very low levels of nutrients compared with Panicum and Avena. The agronomic or ecological implication is that if the three species were allowed to compete at low soil fertility and limited water supply, the growth of Aristida would be more nearly normal than Panicum followed by

Avena. All these results confirm the original hypothesis that the weedy species are more efficient than the crop species under stress conditions.

Literature concerning the effect of water stress on nutrient uptake is very scarce, and the little that there is comes to varying conclusions. This study produced fairly consistent trends in that, although water stress had varied effects on nutrient concentrations and contents, dry matter yields generally decreased faster than nutrient uptake in Panicum and Aristida. In contrast, total nutrient content in Avena was reduced more than its growth. The data presented also showed that the accumulation of divalent cations in both Panicum and Aristida was reduced under water stress, whilst that of Avena was unaffected (or possibly even increased under severe stress). The uptake of monovalent ions was consistently proportional to growth, except for the enhanced uptake of K in Aristida, which might well be an adaptive feature of this species. It would be worthwhile carrying out further experiments under both glasshouse and field conditions to examine both the nature and extent of these differences, since they could have important implications for adaptation to water stress in other crop species.

Another important conclusion to be drawn from this series of experiments is that nutrient uptake of Panicum, Avena and Aristida was highly correlated with water use under soil moisture stress conditions. The uptake of well-watered plants was not closely related to water use except for total cation uptake by Avena roots. However, the uptake of N in osmotically stressed plants of Aristida was not affected by reduced water uptake, so that any change in growth was due to water stress *per se* and not to interacting effects with N nutrition. Evidence was also provided that the effect of water stress on transport to the shoots was independent of its effect on uptake into the roots. Transport of nutrients from the roots to the shoots occurred even at reduced transpiration rates. More information on these effects of water stress on nutrient uptake is needed. It would be interesting to know whether the effects of water stress on root morphology and root death differ between these species and to relate such responses to the efficiency of nutrient uptake. It would also be useful to know whether root cells of Aristida are more resistant to water stress than those of the other species, or whether a degree of adaptation may occur during water stress enabling the remaining live cells to become more resistant.

In the split-root experiments, the method used, which involved growing plants in nutrient film channels, was found to be very useful in permitting half of each root system to be supplied with nutrients whilst the other half was kept in a deficient solution. Water stress which was imposed by incorporating PEG into the flowing solutions, allowed nutrient uptake to be distinguished from overall water uptake, and gave a better indication of species differences in their responses to water stress. The results provided evidence of species differences in the amounts of N that was cycled, and as a consequence differences in the balance between their capacities to absorb and

incorporate N. Avena plants showed a large reduction in shoot growth due to water stress, which was associated with an increase in the total proportion of N that was cycled. In contrast, Aristida plants which grew more normally at the low N level used (50 ppm) exhibited smaller changes in the patterns of N distribution between the shoots and roots. This would have considerable practical implications in that the ability of plants to cycle N must give them the potential to continue to develop their root system even when the roots encounter regions of soil poor in N.

The nutrient translocation study using this method of split-root was preliminary and needs to be developed by further investigations, possibly using some of the commonly available radioactive tracers (e.g. $^{15}N$, $^{86}Rb$ or $^{32}P$) and by extending the work to other species, particularly to drought-adapted crop and drought-tolerant arid-zone weed species of contrasting characteristics. It would be very interesting to perform experiments with these species and to interpret their responses in true ecological terms. It would be useful also to know whether the pattern of nutrient distribution between root and shoot was changed at different levels of osmotic stress or at different periods. This could be done by extending the experimental period.

The experiments in this study have involved growing plants in 10 cm pots and in water culture. The limitations of this and the caution which should be used in extrapolating the results to the field are fully appreciated. There were, however, advantages in using pot-grown and water cultured plants, particularly the fact that growing conditions and soil moisture/osmotic stress levels could be controlled reasonably accurately, thus allowing limited comparisons between experiments. It must be remembered, however, that in all the experiments which involved growing plants in small pots, the rate of soil drying was extremely rapid, so that plants would not neccessarily have behaved in the exactly the same way as in the field. The results do show, however, that many of the observed responses to water stress merit considerable further investigation, both in controlled environments and on a larger scale in the field.

There are other important aspects of this work which need to be pursued. The root distribution of plants on a field scale under both well-watered and water stressed situations requires investigation using radioactive tracers. The relationship between such a study and the efficiency of nutrients uptake by these species would also prove useful.

From an ecological point of view, the study of the nature of a plant's response on rewetting droughted soil is probably more important than what actually happens during the drought, and so particular emphasis should be given to the efficiency of recovery to normal responses after rewetting. The recovery of nutrient uptake efficiency would be particularly worthy of investigation.

There is now increasing evidence in the literature that plants may respond to 'signals' moving from roots to shoots as soil dries. Sharp and Davies (1985) suggested that abscisic acid which is produced in large quantities in water-stressed plants can stimulate the growth of root and also increase the partitioning of carbohydrates to roots. A study of this mechanism in relation to the efficiency of nutrient uptake would also be very useful in comparing these species.

In conclusion, this project has clearly shown that there are differences in both uptake and utilization efficiencies of nutrients by the three species, particularly under conditions of limited nutrient and/or water supplies. These differences were related to the nature of their evolutionary background and therefore support the hypothesis which initiated the work at the start of this thesis.

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APPENDIX I

## BOTANICAL FEATURES AND AGRONOMIC IMPORTANCE

### 1. *Panicum miliaceum* (common or proso millet)

*Panicum miliaceum*, is one of the earliest cereals known to have been domesticated. It is still grown widely in warm, arid areas such as India, the Middle East, and parts of Russia, China and Central Europe.

#### Botanical features

It is an annual warm-season $C_4$ grass, usually 30-100 cm tall, but frequently taller as a weed in tall crops (e.g. corn). The root system is shallow and adventitious. The glabrous or hairy stems are usually erect. Leaf sheaths are densely hairy and have overlapping margins. The ligule consists of a line of dense hairs and there are no auricles.

The much-branched panicles are at the top of the stem. The spikelets are borne singly and consist of a fertile and a sterile floret enclosed by two outer glumes (Bough *et al.*, 1986).

#### Agronomic importance

In parts of the United States and Canada it is grown as a minor crop for livestock feed or birdseed but it also recognised as a serious weed threat to other crops.

Its advantage as a crop is its ability to produce a greater yield than other cereals under hot, dry conditions. It has the lowest water requirement of any grain crop and grows quickly, requiring a growing seasons of only 60 days.

# APPENDIX II

2. *Avena fatua* (wild oat)

It originated from South West Asia and has rapidly spread to Europe, North America and Australia (Elliott, *et al.*, 1979). At present it is the characteristic wild oat of Great Britain, North West Europe, North America, North Africa, Central Asia and other regions with a similar climate.

## Botanical fatures

It is an annual $C_3$ grass, 30-150 cm. Culms tufted, erect at the base, smooth. Leaves green; sheath rounded on the back, the basal usually loosely hairy, the rest smooth; ligules blunt; blades rough and gradually pointed.

Infloresence; an open, nodding panicle, the branches mostly clustered, slender and rough, unequal. Spikelets loosely scatered, with all lemmas awned, breaking up at maturity beneath each lemma. Glumes finely pointed, as long as the spikelet, becoming papery except for thinner margins (Hubbard, 1984).

## Agronomic importance

A common weed of arable land and waste places, often abundant among wheat, barley and oats, particularly during winter crops. Introduced long ago, probably with seeds of cereals, now naturalized and widely distributed in the cultivated regions throught the world. Flowering: June to September.

## APPENDIX III

3. *Aristida funiculata* (wire or needle grass)

It occurs in south and south-east of Tibesti (Sahara) to a height of 1,500 m. Distributed in Arabia and other arid-zone regions in central Africa.

### Botanical features

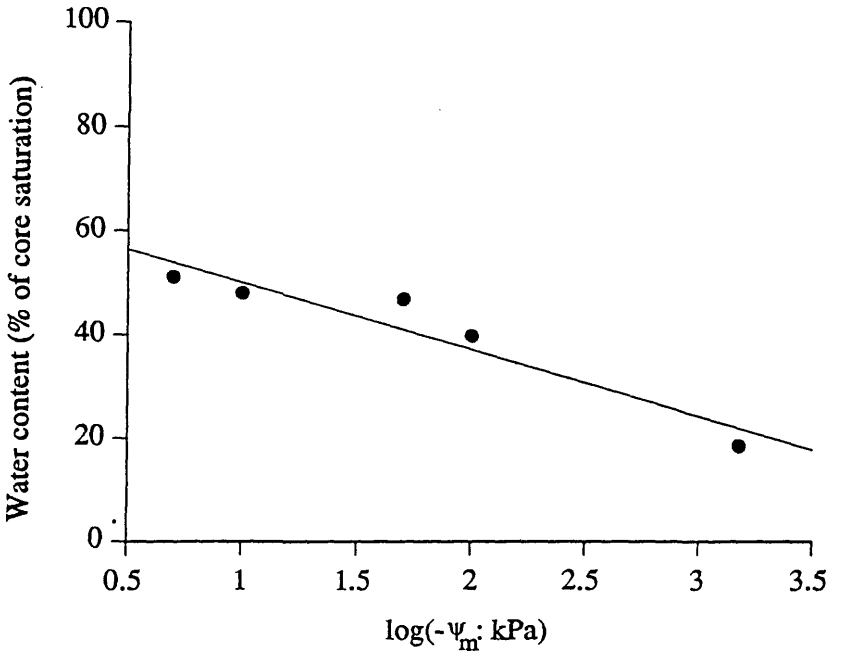
It is a strongly tufted and wiry perennial $C_4$ grass. All species are xerophytic and relatively deep rooted. Culms erect and hard wiry, smooth and hairless, sometimes branched at the lower nodes. Leaves are narrow, very hard, sparse, mostly on the culms; without auricles. Leaf sheaths generally longer than the internodes, loose, smooth and hairless. Leaf blades flat only at the very base, narrow, and becoming rolled inwards.

Inflorescence; an open panicle, spikelets few, the pedicel fine, flattened and rough along the margins. Spikelets, pale yellowish, at maturity breaking up with the floret falling free from the glumes, the glumes usually falling separately. Lemmas with rough awns.

### Agronomic importance

Aristida is a genus containing many noxious weed species, whose awns often injure livestock. The name is derived from the Latin *arista,* mean's awn. A native pasture species, it is generally of poor value but various species are of minor grazing use, particularly in dry regions.

## Appendix IV




The soil moisture-matric potential curve for the soil used in the experiments of Chapters 3 & 4.

Interpolated values for the limiting water potentials of the imposed treatments:

| | |
|---|---|
| 50% soil moisture content (Field Capacity): | -0.01Mpa |
| 37.5% soil moisture (3/4 Field Capacity): | -0.09MPa |
| 25% soil moisture (1/2 Field Capacity): | -0.86MPa |