# The mean depth of soil water uptake by two temperate grassland species over time subjected to mild soil water deficit and competitive association

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## Abstract

Little is known concerning the soil water use dynamics of white clover (WC) and ryegrass (RG) grown in mixtures. A greenhouse study, on a deep soil, was conducted to determine the mean depth of soil water uptake of WC and RG plants grown in a competitive association and subjected to a moderate soil water deficit. Plant growth period simulated that experienced by newly sown grassland in temperate regions. Three irrigation solutions, each containing a different hydrogen isotope (deuterium) concentration, expressed as delta notation ($\delta$), were provided at three different soil depths through specially constructed tubes and containers (0.50 m diameter, 1 m depth) in order to create a soil deuterium profile gradient. Young leaves and not the entire plant were harvested in order to preserve the competitive plant association over time. Patterns of leaf $\delta$D value were constant for both WC and RG. Lower leaf $\delta$D values in RG compared to WC was attributed to RG more efficient stomatal control. Increases in the mean depth of soil water uptake as soil water deficit increased was similar between plants. The mean depth of soil water uptake of WC was at all times greater than that of RG. After 3 months of competitive growth, WC roots obtained water from a soil depth 30% greater than that of RG. In our experimental conditions, the ability of WC to obtain water from substantially lower soil depths may give it a competitive advantage over RG during the period subsequent to pasture sowing if surface soil water deficits are experienced and deeper soil layers contain water.

## Introduction

The perennial ryegrass (*Lolium perenne* L.) – white clover (*Trifolium repens* L.) association is the mainstay of temperate pastoral agriculture in Europe. The success of the association is dependent on the competition between the two species for aerial (i.e. light) and soil resources (i.e. nutrients and water). Numerous studies have shown that the effects of root competition on plant growth are usually greater than those of shoot competition, at least during the initial months follow-

ing planting (Donald, 1958; Snaydon, 1971; Scott and Lowther, 1980; Wilson, 1988).

One of the most limiting factors affecting recently sown plants is water deficit. Moderate to severe drying of the upper soil layers occurs in many European grasslands in late spring and throughout summer. The dynamics of water availability and use are essential factors differentiating species survival in a pasture. Little, however, is known concerning the response of white clover and ryegrass to water deficit in a mixed pasture. Reduced white clover growth in pasture mixtures under drought has been attributed to its less extensive rooting system (Guobin and Kemp, 1992; Thomas, 1984). The ability of one species to extract

soil water compared to another species can be enhanced by greater root densities. Yet the capacity of white clover to extract water from deep soil reserves has also been reported (Guckert et al., 1993). In this case, a plant's ability to extract water from deep soil reserves is more advantageous to plant growth than to develop a larger root biomass or root density as root biomass may not always be directly indicative of a plants ability to acquire soil water.

Methods for the determination of the mean depth of soil water uptake for plants are not numerous and are difficult to put into practice. Excavation measurements provide a one-point-in-time snapshot of root distribution, but may provide very little insight into resource uptake rates or patterns of resource utilization. This is because root presence may not be a reliable indicator of either actual water or nutrient uptake dynamics in either time and space (Ehleringer and Dawson, 1992). Since water is the only source of hydrogen for plants and there is no fractionation of water during its root uptake by plants, the stable hydrogen isotope ratio (deuterium/hydrogen) of water contained within the xylem sap should reflect the water source that a particular plant uses (Dawson and Ehleringer, 1991; Wershaw et al., 1966; White et al., 1985; Zimmermann et al., 1966). Experiments using the analysis of deuterium concentration in water can allow the determination of the soil depth from which a plant obtains water (Dawson and Ehleringer, 1991; Flanagan et al., 1991; Flanagan and Ehleringer, 1991; White, 1988). This information may allow us to determine how differential utilization of water sources influences plant distribution, coexistence, and competition for water by plants in both time and space (Dawson and Ehleringer, 1991; Ehleringer et al., 1991).

A greenhouse study was conducted to (1) determine the effects of soil water deficit on the mean depth of soil water uptake for both white clover and ryegrass plants grown in a competitive association, and (2) establish and compare the kinetics of the soil depth of water extraction by roots during a 4–5 month period similar to that experienced during grassland establishment in Europe (seeds sown in April–May, and occurrence of drought in July–August).

## Materials and methods

### Experimental conditions

A completely randomized greenhouse experiment was performed in fall and winter 1996/97 at the University of Utah in Salt Lake City, USA. Two experimental designs were simultaneously carried out in containers and pots (described below). For both experiments, soil consisted of a homogeneous mixture of sand, peatmoss, vermiculite, woodmulch, perlite and top soil (mainly organic in nature and locally acquired from a field research site) in proportions of 1x2x3x4x4x4. Soil was allowed to dry by evaporation for several days in the open air in the greenhouse prior to pot and container filling. Seeds of ryegrass (RG) *'Preference'* and white clover (WC) *'Huia'* were first sown into small pots in order to obtain homogeneous seedlings for transplanting into experimental designs 45 days later. At the top of containers and pots, average daily air temperature (23+/-3 °C) and relative daily air humidity (30–45%) were maintained constant throughout the experiment by a cooling system and lights: Photosynthetic photon flux density developed by Hg and Na vapor lights was about $950+/-100$ $\mu$mol m$^{-2}$s$^{-1}$ depending on cloudy or sunny weather. Photoperiod was 14 h daylight, 10 h night. Climatic conditions during the night were as follows: Relative humidity, 60–90%; air temperature, 10–15 °C. Inoculation of WC with *rhizobium* was not performed and nodules on the roots of WC were not observed at harvest. The artificial soil and environmental conditions of Salt Lake City, Utah, where *rhizobium* are not commonly found did not favor natural *rhizobia* infection of WC roots.

### Experimental design 1

One and a half month old WC and RG seedlings, with roots uniformly cut to three centimeter length, were transplanted into eight PVC cylindrical containers (0.5 m diameter, 1 m depth). About 60 seedlings (30 WC and 30 RG) per container were transplanted in an alternative pattern at 50 mm spacing, to establish a competitive association (Lucero et al., 1999; Martin and Field, 1984). Soil was progressively filled and uniformly tamped for all containers. At time of soil container filling, three specially constructed plastic tubes (20 mm diameter) were placed in the containers at three different soil depths. These tubes were circular (0.25 m diameter), perforated on the lower side to allow trickle irrigation and placed in the middle of the container at 0.20, 0.55 and 0.90 m soil depth (Figure 1). Tubes were individually connected to the soil surface by additional tubes running vertically into the containers. These three tubes allowed three different concentrations of deuterium (discussed below) to
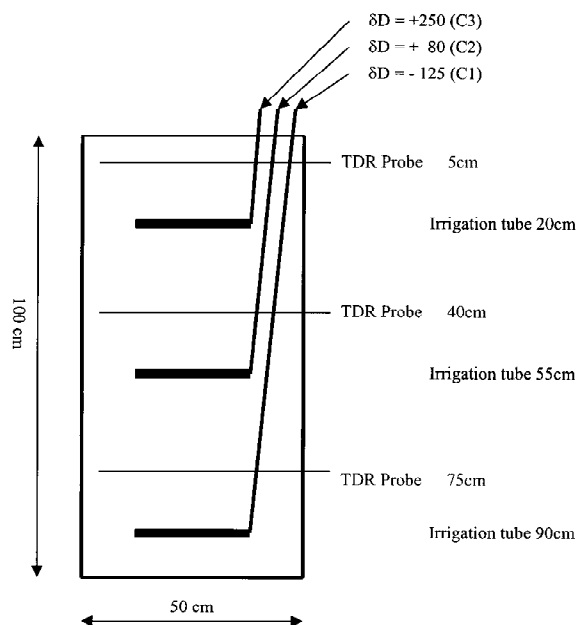
*Figure 1.* Diagram of containers in a greenhouse experiment indicating the placement by depth of TDR probes and irrigation tubes. Note how the three different deuterium concentration (C1, C2, C3) were applied at three different soil depths in order to obtain $\delta D$ values of, respectively, $-125$ ‰; $+80$ ‰; $+250$ ‰.

be provided at three soil depths in order to obtain a soil deuterium concentration gradient. Also at time of container soil filling, three TDR (time domain reflectometry) probes were horizontally placed at 0.05, 0.40 and 0.75 m soil depth to monitor and control soil water content. The TDR probes were constructed of stainless steel rods and modified to fit the container. To assure as homogeneous as possible greenhouse conditions, containers were placed on moveable carts and rotated daily.

*Experimental design 2*

Simultaneous to the set-up of '*Experimental design 1*', 45 day old seedlings of WC and RG were transplanted (one per pot) into 20 PVC pots (0.20 m diameter, 0.30 m depth): 10 pots with WC plants and 10 pots with RG plants. These plants were irrigated twice weekly as described below.

*Water deficit treatments*

*Experimental design 1*

Six of the eight containers were randomly separated into two groups of three containers (referred to as containers 1 to 6). Containers were superficially hand irrigated for 15 days after WC and RG plant transplant-

ing, to avoid dehydration of the soil surface. Then, in order to obtain two soil water content treatments, i.e. minimal (MIN) and moderate (MOD), containers 1, 2 and 3 were irrigated with twice as much water as containers 4, 5 and 6 during all the experiment. A complete nutrient solution (N, P, K at 20 mg $l^{-1}$ of water and microminerals) was applied daily. The two remaining containers were well-watered throughout the duration of the experiment and plants grown therein were designated as controls and used to determine reference (maximum) values of both net $CO_2$ assimilation rate (*A*) and pre-dawn leaf water potential ($\Psi_{pd}$) (Table 1).

At day 15, post-transplanting irrigation water was applied through the three specially constructed plastic tubes placed at the three soil depths. Nutrient solution continued to be surface applied once weekly. From 15 days post-transplanting onward, the amount of water needed for irrigation in order to maintain the two designated soil water content levels was determined by TDR readings taken weekly at the three soil depths. Tap water used for irrigation from day 15 post-transplanting onward was either unaltered or supplemented with two solutions of deuterium to create three deuterium water concentration solutions: Here in refered to as C1, C2, C3 (see Figure 1).

The soil deuterium gradient for root uptake was established by applying each different deuterium enriched water solution at a separate soil depth. This was done by using the specially constructed plastic tubes placed at the three different soil depths (Figure 1).

To measure effects of soil water deficit at the plant level, *A* and $\Psi_{pd}$ measurements were taken at day 49 and 99 post transplanting, respectively, on leaves of 6 RG and 6 WC plants per container. Net $CO_2$ assimilation rate, measured at the beginning of the afternoon, was expressed as a percentage of that measured on the plants grown under identical conditions in the two well-watered adjacent containers. The *A* measurements were performed with a portable IRGA system (Licor 6200, Li-cor, USA). The $\Psi_{pd}$ were measured with a pressure chamber (Scholander et al., 1965).

*Experimental design 2*

Three groups of six pots each of WC and RG plants were randomly selected. Two pots were eliminated. Each group was watered twice weekly with water having hydrogen isotope concentrations corresponding to that of the concentrations used in '*Experimental*

*Table 1.* The mean pre-dawn leaf water potential ($\Psi_{pd}$) and net $CO_2$ assimilation rate ($A$) as a % of maximum net $CO_2$ assimilation rate of controls of white clover (WC) and ryegrass (RG) plants grown in a competitive association in the greenhouse and subjected to a minimum (MIN) and a moderate (MOD) soil water deficit

| Treatment[a] | DAT[b] | Mean $\Psi_{pd}$ [c] | | Mean $A$[c] max | |
|---|---|---|---|---|---|
| | | WC | RG | WC | RG |
| | | ——— MPa ——— | | ——— % ——— | |
| Control Plants | 49 | −0.02 a | 0.03 a | 100 a | 100 a |
| | 99 | −0.02 a | 0.03 a | 100 a | 100 a |
| Minimum (MIN) soil water deficit | 40 | −0.05 a[d] | 0.09 a | 105 a | 97 a |
| | 49 | −0.09 a | 0.12 a | 108 a | 92 a |
| | 70 | −0.19 a | 0.19 a | 98 a | 89 a |
| | 99 | −0.15 a | 0.32 b | 95 a | 79 b |
| Moderate (MOD) soil water deficit | 40 | −0.24 a | 0.41 b | 92 a | 66 b |
| | 49 | −0.26 a | 0.50 b | 94 a | 60 b |
| | 70 | −0.31 a | 0.52 b | 89 a | 55 b |
| | 99 | −0.22 a | 0.32 b | 92 a | 72 b |
| | 99 | −0.02 a | 0.03 a | 100 a | 100 a |

[a] Treatments compared only by date for WC and RG plants (across lines).

[b] DAT = days after transplanting.

[c] $\Psi_{pd}$ = pre-dawn leaf water potential; $A$ max = maximum $CO_2$ assimilation rate.

[d] Means followed by different letters are significantly different ($P$=0.05).

Mean values derived from ten replicates.

design 1'. As in 'Experimental design 1', the same two soil water deficit treatments were established 15 days post-transplanting in three pots for each treatment. These two soil water deficit treatments were thereafter maintained by the frequent weighing of pots and the re-watering of water loss, as well as by pre-dawn leaf water potential measurements. At three dates (49, 70 and 99 days post-transplanting), samples of water, soil and leaves (in replicates of three) of both plant species were analyzed in order to determine their hydrogen isotope concentration values (Table 2).

*Leaf and soil water isotopic composition and sampling*

In 'Experimental design 1', soil and plant samples gathered to determine $\delta$D were taken at pre-dawn on days 49 and 99 post-transplanting. For each date, soil samples collected to establish soil $\delta$D profiles, were taken at 0.10, 0.20, 0.30, 0.40, 0.55, 0.70 and 0.80 m depth. Soil samples were collected approximately 0.15 m from the border of the container with three replicates per soil depth sample. Soil samples collected were immediately placed in an airtight container and stored at −18 °C until analyzed. To determine plant $\delta$D values, three young tillers of six RG and three young leaves of six WC plants per container were randomly harvested. Only tillers and leaves and not the whole plant were harvested in order to keep the plant canopy intact and to preserve the competitive association throughout the experiment. Plants used for harvest were selected from a zone that was at least 0.10 cm from the border and at least 0.10 cm from the center of the container. Harvested tissue was individually placed into glass vials, sealed-tight and stored at −18 °C until water could be extracted.

Water extraction was 20 min for leaf tissue and 1 h for soil. For extraction, a cryogenic vacuum distillation apparatus was used (Ehleringer and Osmond, 1989). Final extracts were sealed under a vacuum in Pyrex glass tubes. Water samples were then reduced to diatomic hydrogen using a reduction reaction with zinc according to Coleman et al. (1982). A 10 ml subsample and the zinc alloy were sealed in an evacuated

*Table 2.* The H$_2$O isotopic composition ($\delta$D) of water, of soil (irrigated with three different water isotopic concentration solutions), and of white clover (WC) and ryegrass (RG) leaves grown in a greenhouse experiment and subjected to a minimum (MIN) and a moderate (MOD) soil water deficit

| DAT[b] | Irrigation | Well-watered plants (MIN soil water deficit)[a] | | | Water-stressed plants (MOD soil water deficit)[a] | | |
|---|---|---|---|---|---|---|---|
| | | Soil | WC | RG | soil | WC | RG |
| | | | | $\delta$D values | | | |
| 49 | 251 a | 228 a | 279 c | 275 c | 231 a | 277 c | 268 b |
| 70 | 252 a | 246 a | 288 bc | 291 c | 243 a | 288 bc | 286 b |
| 99 | 299 a | 279 a | 324 bc | 325 c | 285 a | 327 bc | 321 b |
| | | | | | | | |
| 49 | 74 a | 93 a | 139 c | 141 c | 96 a | 139 c | 135 b |
| 70 | 75 a | 81 a | 126 bc | 128 c | 80 a | 125 b | 124 b |
| 99 | 86 a | 85 a | 133 c | 130 c | 87 a | 132 c | 122 b |
| | | | | | | | |
| 49 | −125 a | −125 a | −82 c | −80 c | −125 a | −80 c | −86 b |
| 70 | −125 a | −125 a | −79 c | −84 bc | −125 a | −82 bc | −88 b |
| 99 | −125 a | −125 a | −80 cd | −79 d | −125 a | −82 bc | −84 b |

[a]Treatment H$_2$O $\delta$D values compared only across lines by depth and day.
[b] DAT = days after transplanting.
Means followed by different letters are significantly different (*P*=0.05).
Mean values derived from three replicates.

Vycor glass tube and baked at 500 °C for 60 min. The diatomic hydrogen resulting from the combustion was analyzed for its isotopic composition on a gas-phase isotope rationing mass spectrometer. Isotopic composition is expressed in delta notation ( ‰) as:

$$\delta D = [[(D/H_{sample})/(D/H_{standard})] - 1] \times 1000$$

where D is the deuterium and H is the hydrogen, with SMOW as the standard (Standard of Mean Ocean Water; D/H$_{standard}$ = 0.00015576).

Numerous studies have shown that during water uptake by roots and during water transport between the root and the shoot, the isotopic composition of xylem water remains unaltered from that of soil water (Dawson and Ehleringer, 1991; White et al., 1985; Zimmermann et al., 1966). Water isotopic composition remains unaltered until it reaches tissues undergoing water loss where the hydrogen isotopic concentration of leaves or green tissue samples can show significant evaporative isotopic enrichment. For herbaceous species, the roots, stem/caudex or non-photosynthetic basal rosette are usually collected for determination of $\delta$D. However, in our experiment, the harvesting of these plant parts of WC or RG would have necessitated the elimination of the total plant, which would have destroyed the competitive interaction between plants and species. To avoid this, only leaves from selected plants and not the whole plant were harvested. '*Experimental design 2*' was thus set up to determine the

deuterium enrichment in the leaves under each treatment condition. The difference between $\delta$D of leaves harvested in '*Experimental design 2*' (Table 2) and $\delta$D of soil water in '*Experimental design 2*' ($\delta$D leaf – $\delta$D soil) were calculated for WC and RG plants for each water deficit treatment. These fractionation values were then used to estimate the $\delta$D values of the roots (**root estimated $\delta$D**) of WC and RG plants for each water deficit treatment in '*Experimental design 1*' (Table 3) as follows: Root estimated $\delta$D = fractionation value − leaf measured $\delta$D. The air temperature and humidities during both experiments 1 and 2 ('*Experimental design 1 and 2*') were similar, allowing one to believe that the degree of evaporative leaf water enrichment was similar between the two experimental conditions.

The mean depth of soil water uptake per plant by date was determined by drawing an intersect from the **root estimated $\delta$D** value of each harvested plant leaf to the corresponding line on the respective container's soil $\delta$D profile (Figure 2a,b)

## Results

*Plant and soil water status (experimental design 1)*

Two distinct soil water content profiles were observed in the MIN soil water deficit treatment (Figure 3a). For

*Table 3.* Estimated values of isotopic composition of the roots ($\delta$D root estimated values) calculated from 6 white clover (WC) and 6 ryegrass (RG) plants grown in a competitive association in either a minimal soil water deficit (MIN) or a moderate soil water deficit (MOD) in 6 different containers. Plant leaf tissue were harvested at 49 (D49) and 99 days (D99) post-transplanting

| Soil Water deficit | Container 1 | | | | Container 2 | | | | Container 3 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | D49 | | D99 | | D49 | | D99 | | D49 | | D99 | |
| | WC | RG | WC | RG | WC | RG | WC | RG | WC | RG | WC | RG |
| | $\delta$D values | | | | | | | | | | | |
| MIN | −28 | −101 | −50 | 65 | 13 | −80 | 25 | 115 | −20 | −110 | 20 | 110 |
| | −16 | −115 | −2 | 115 | 21 | −60 | 14 | 112 | −12 | −82 | 24 | 60 |
| | −19 | −91 | −12 | 61 | 12 | −75 | 58 | 78 | −5 | −79 | 5 | 95 |
| | −37 | −88 | −19 | 95 | 6 | −98 | 26 | 62 | −18 | −101 | −18 | 71 |
| | −28 | −87 | 21 | 107 | 13 | −58 | −5 | 78 | −25 | −96 | 45 | 108 |
| | −19 | −80 | −15 | 79 | 13 | −96 | 18 | 57 | −26 | −95 | 1 | 85 |

| Soil Water deficit | Container 4 | | | | Container 5 | | | | Container 6 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | D49 | | D99 | | D49 | | D99 | | D49 | | D99 | |
| | WC | RG | WC | RG | WC | RG | WC | RG | WC | RG | WC | RG |
| | $\delta$D values | | | | | | | | | | | |
| MOD | 37 | 9 | 40 | 105 | 36 | −21 | 32 | 130 | 25 | −42 | −8 | 181 |
| | 31 | 20 | 20 | 188 | 29 | −12 | 56 | 165 | 36 | −12 | 23 | 72 |
| | 38 | 28 | −8 | 61 | 46 | 3 | 5 | 135 | 12 | −1 | 32 | 132 |
| | 60 | 18 | 43 | 95 | 45 | 7 | 78 | 121 | 10 | −19 | 0 | 168 |
| | 62 | 10 | 75 | 112 | 50 | 6 | 48 | 150 | 32 | −32 | 11 | 103 |
| | 40 | 23 | 22 | 121 | 49 | −18 | 101 | 185 | 23 | −8 | 6 | 129 |

the MIN soil water deficit treatment, no changes in the soil water content profile at the 0.40 and 0.75 m soil depth and a slight decrease at the 0.05 m soil depth were observed during the first 40 days post-transplanting. From day 40 onward, the soil water content declined dramatically in the soil surface layer (0.5 m) and decreased only slightly at the 0.40 and 0.75 m soil depths. The soil water content profile for the MOD soil water deficit treatment was at all times lower than that observed in the MIN soil water deficit treatment, at each respective soil depth. For the MOD soil water deficit treatment, decreases in the surface (0.5 m) soil water content began very early after transplanting, whereas a steady and progressive decline in soil water content over time was observed at the 0.40 m soil depth (Figure 3b). A pronounced decline in the soil water content at the bottom was observed during the first 40 days post-transplanting. This decrease was then corrected in order to obtain a relatively unchanged soil water content at this depth.

At the plant level, for the first 49 days of the experiment the level of soil water content in the MIN soil water deficit treatment had very little effect on pre-dawn leaf water potentials of either WC or RG (Table 1). During this time period, *A* rates were similar for WC and RG grown in the MIN soil water deficit treatment, and A rates were as well the highest compared to other dates and the MOD water deficit treatment. But from day 49 onward, the effects of the MIN soil water deficit were observed in decreased pre-dawn leaf water potentials: RG had a much lower pre-dawn leaf water potential than WC at day 99 (−0.32 vs −0.15 MPa, respectively). The *A* rates of WC were less affected by increasing soil water deficit levels than were those of RG. For the MOD soil water deficit treatment RG had both lower pre-dawn leaf water potentials and A rates than WC. It could be additionally observed in the MOD soil water deficit treatment that WC had higher A rates than RG at a comparable pre-dawn leaf water potential (Figure 4).

*Leaf water and root $\delta$D values*

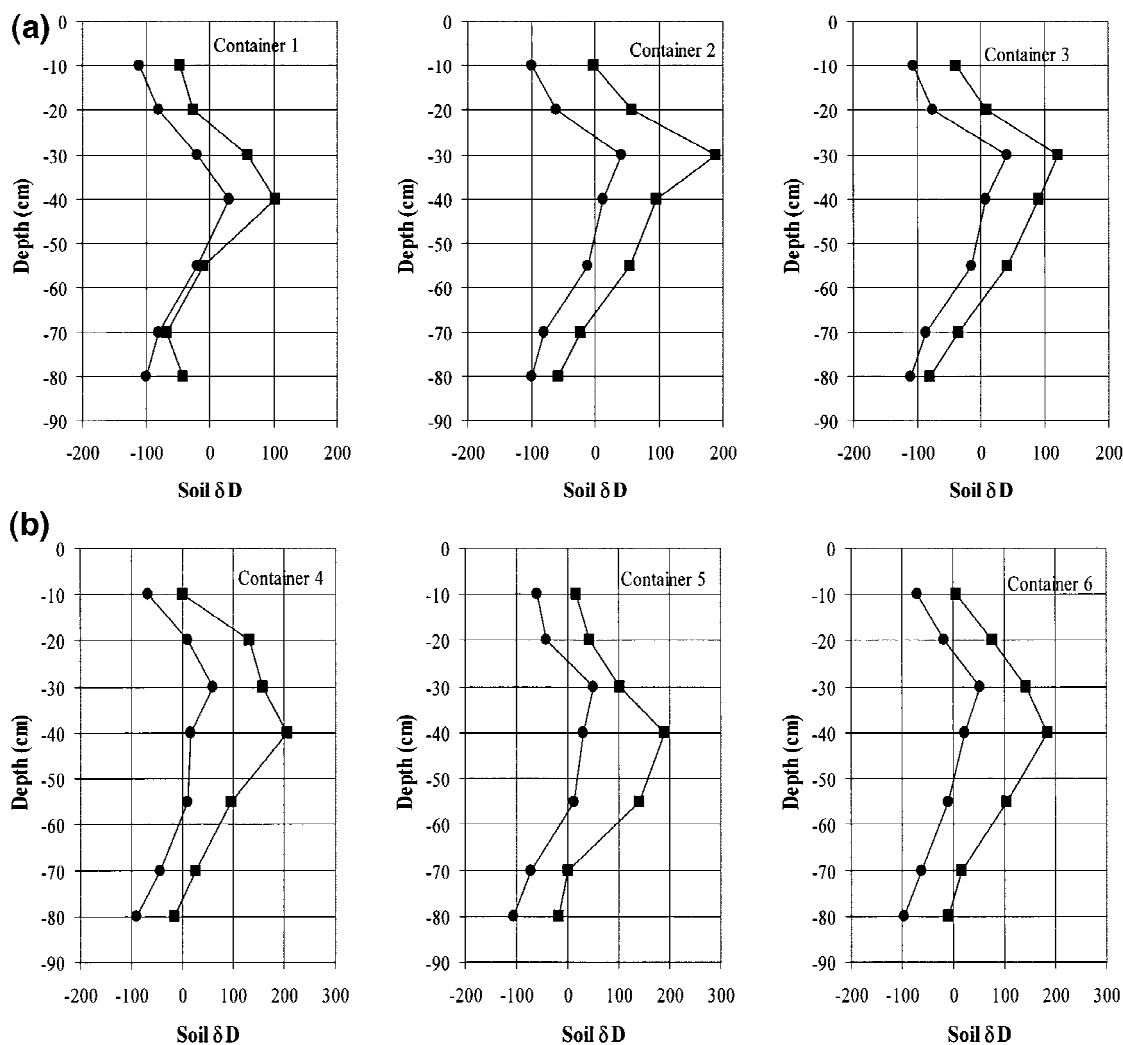The $\delta$D values measured in irrigation water and in soil

*Figure 2.* (a) Soil water isotopic composition ($\delta$D) profiles for containers 1, 2 and 3 from soil samples collected at days 49 (●) and 99 (■) post-transplanting. Containers 1, 2 and 3 were subjected to a minimal (MIN) soil water deficit treatment. (b) Soil water isotopic composition ($\delta$D) profiles for containers 4, 5 and 6 from soil samples collected at days 49 (●) and 99 (■) post-transplanting. Containers 4, 5 and 6 were subjected to a moderated (MOD) soil water deficit treatment.

water over time did not vary (Table 2). The expective $\delta$D evaporative enrichment of xylem water was observed at the leaf level. The leaf $\delta$D values of WC and RG in the MIN soil water deficit treatment and the $\delta$D values of WC in the MOD soil water deficit treatment were similar while the leaf $\delta$D values of RG in the MOD soil water deficit treatment were slightly lower. As previously explained, the leaf $\delta$D values measured in '*Experimental design 1*' were substracted from the fractionation values obtained in '*Experimental design 2*', which were then used to calculate **root estimated $\delta$D** values. The fractionation values calculated from '*Experimental design 2*' (not shown) were, re-

spectively, 45.7 ‰ for well-watered WC; 45.5 ‰ for well-watered RG; 44.1 ‰ for water-stressed WC, and 39.0 ‰ for water-stressed RG.

### $\delta$D values and the mean depth of soil water uptake

Soil $\delta$D profiles determined by depth at day 49 and 99 post transplanting were only slightly different for each container (Figure 2a,b). The soil $\delta$D profile slopes for both MIN and MOD soil water deficit treatments were similar with $\delta$D increasing from the soil surface to the 0.30–0.40 m depth and thereafter decreasing as soil depth increased. The upper soil surface area in fact did
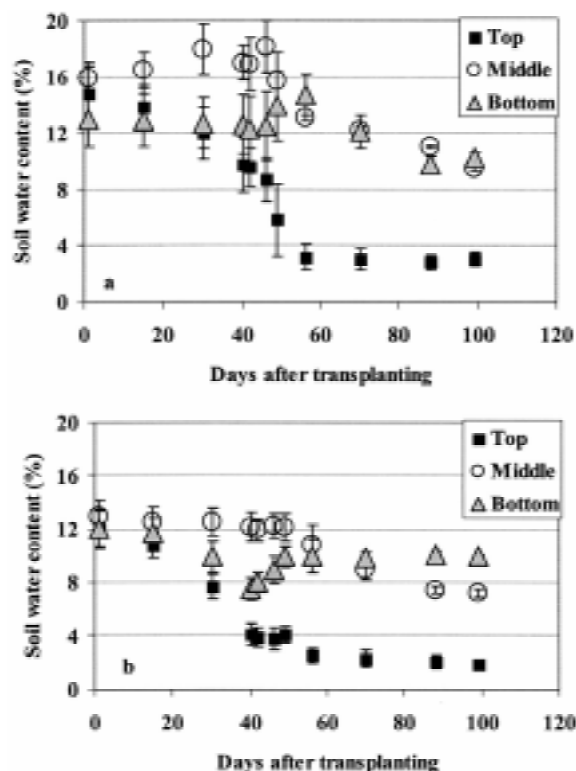
*Figure 3.* Soil water content profiles (%) over time of two soil water deficit treatments: a = Minimum; b = Moderate. Soil samples taken at 0.05, 0.40 and 0.75 m depth. Data are the means of three replicates. Bars indicate standard deviation.
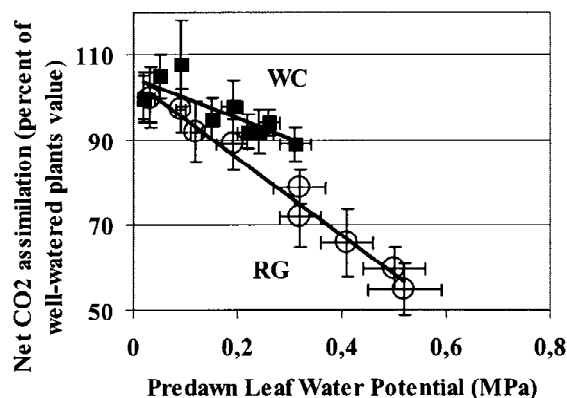


*Figure 4.* Relationship between pre-dawn leaf water potentials (MPa) and net $CO_2$ assimilation rate (*A*) of white clover (WC) and ryegrass (RG) plants grown in a competitive association in the greenhouse and subjected to either a minimal (MIN) or moderated (MOD) soil water deficit. Net $CO_2$ assimilation rate (*A*) is expressed as a% of maximum value of the well-watered plants. The maximum values were 13.98 $\mu$mol m$^{-2}$ s$^{-1}$ for WC and 9.35 $\mu$mol m$^{-2}$ s$^{-1}$ for RG. Data are the means of 10 replicates. Bars indicate standard deviation.

not receive deuterium-enriched water as the first subground irrigation tube providing deuterium-enriched water was placed at the 0.25 m depth. The soil water deficit profiles of the MOD soil water deficit treatment (Figure 2b) were more homogeneous between containers than that of the MIN soil water deficit treatment profiles (Figure 2a) probably because less tap water was provided to these containers during the post-transplanting period: The dilution of deuterium-enriched water was, therefore, less pronounced. The spatial variability of soil $\delta$D for each container was small and soil $\delta$D increased with time. White clover root estimated $\delta$D values were always greater than RG root estimated $\delta$D values at 49 days post-transplanting (Table 3). Raygrass root estimated $\delta$D values were always greater than WC root estimated $\delta$D values at 99 days post-transplanting.

As previously described, to determine the mean depth of soil water uptake per plant, an intersect was drawn from each final root estimated $\delta$D value (Table 3) to the corresponding container's soil $\delta$D profile line (Figure 2a,b). Where the root estimated $\delta$D value intersected the soil $\delta$D profile line twice (due to the bifurcated nature of the soil $\delta$D profile line) the two values of mean depth of soil water uptake were calculated and duly represented (Figure 5a). At 49 days post-transplanting, the use of the soil $\delta$D value corresponding to the lower soil region as the area from which plants obtained water was not considered, as plant roots had not yet grown into this lower soil level. But for the determination of the mean depth of soil water uptake at 99 days post transplanting, this assumption could not be made. Therefore, at 99 days post-transplanting, the soil depth where the root estimated $\delta$D value transected the soil $\delta$D profile line was considered more appropriate and therefore used to determine the mean depth of soil water uptake. At all times the estimated mean depth of soil water uptake for WC was greater than that of RG.

Decreased above ground dry matter growth was correlated with increased soil water deficit (Table 4). Ryegrass root dry matter yield was about twice that of WC. It was additionally observed that WC did not produce nodal roots. The top soil was too dry and the canopy density too great to allow stolon-soil contact. Therefore, water for WC plant use was acquired through the main tap root and not from roots initiating from stolons.

*Table 4.* Above and below ground dry matter yields and root/shoot ratios of white clover (WC) and ryegrass (RG) plants subjected to a minimal (MIN) or a moderate (MOD) soil water deficit and harvested at 99 days post-transplanting in a greenhouse experiment

| Soil water deficit Treatment | Dry Matter Yield | | | | | |
|---|---|---|---|---|---|---|
| | Above Ground[a] | | Below ground[b] | | Root/Shoot Ratios[b] | |
| | WC | RG | WC | RG | WC | RG |
| | ——— g/plant ——— | | | | | |
| MIN | 4.25 c | 6.25 a | 1.16 | 2.24 | 0.27 | 0.36 |
| MOD | 3.47 d | 5.03 b | 1.25 | 2.09 | 0.36 | 0.42 |

[a]Means followed by different letters are significantly different ($P$=0.05).
[b]Below ground dry matter yields were rough estimates and therefore statistical analysis were not done. Mean values derived from 12 replicates.

## Discussion

### Leaf fractionation

Our results indicated that for well-watered WC and RG plants, as well as for WC subjected to a moderate drought, the difference between soil and leaf $\delta$D values (i.e. water fractionation) remained constant for the three month time period following transplanting (Table 2). Our results showed a small but significant decrease of the leaf $\delta$D value of RG subjected to a moderate soil water deficit as compared with the leaf $\delta$D values of WC similarly grown and those of well-watered WC and RG plants. Water stress can potentially affect leaf water isotopic composition through reductions in plant stomatal conductance (Flanagan, 1993). The two possible causes of this effect of water stress on leaf isotopic water composition are, (1) an increase in leaf temperature which results in an increase of the leaf to air vapor pressure difference (this would increase $\delta$D values), or (2) a reduction in transpiration rate (this would decrease $\delta$D values).

In our study, we attribute the differences in measured $\delta$D leaf values between WC and RG subjected to a moderate soil water deficit to differences in plant stomatal control. For the same leaf pre-dawn water potential value, net $CO_2$ assimilation rate efficiency remained lower for RG than for WC (Figure 4). Several authors (Grieu et al., 1995; Guckert et al., 1993; Johns, 1978; Lucero et al., 1999; Thomas, 1984) have reported that in conditions of mild drought, WC has a less efficient control of stomatal closure than RG. Consequently, at the same leaf water potential, the transpiration rate is lower for RG than WC, and leaf water fractionation for $\delta$D would as well be lower for RG than WC, as was observed in our study.

### Mean depth of soil water uptake

White clover root estimated $\delta$D values were greater than RG root estimated $\delta$D values at day 49, but lower at day 99 post-transplanting. When these respective root estimated $\delta$D values are transected with the soil $\delta$D profile lines, it is clear that the mean depth of soil water uptake of WC was always greater than that of RG. Differences in the mean depth of soil water uptake by two species can be explained by one of three possibilities: (1) genetic differences that would affect root growth, (2) different root growth responses to a soil drying gradient, or (3) different root growth responses to root competition.

### Root genetic differences

The analysis of measured parameters, i.e. pre-dawn leaf water potentials, the proportions of maximum $A$, TDR measurements and the values of the mean depth of soil water uptake between 0 and 49 days after transplanting, indicated that: (1) the minimum soil water deficit had no effect on either WC or RG, and (2) the moderate soil water deficit slightly affected both WC and RG. It was noted that WC plants grown in a deep soil at a minimum soil water deficit grew roots deeper than RG, even though RG had twice as much root biomass as WC. Higher root biomass growth for RG compared to WC has been previously reported (Evans, 1977; Thomas, 1984; Whitehead, 1987). Evans (1977) reported that root hairs of *Lolium perenne* were longer and more frequent than in white clover – and that, therefore, the calculated surface of the root hair cylinder and the volume within the root hair cylinder were several times greater than those of clover. It was concluded that this could give ryegrass a strong
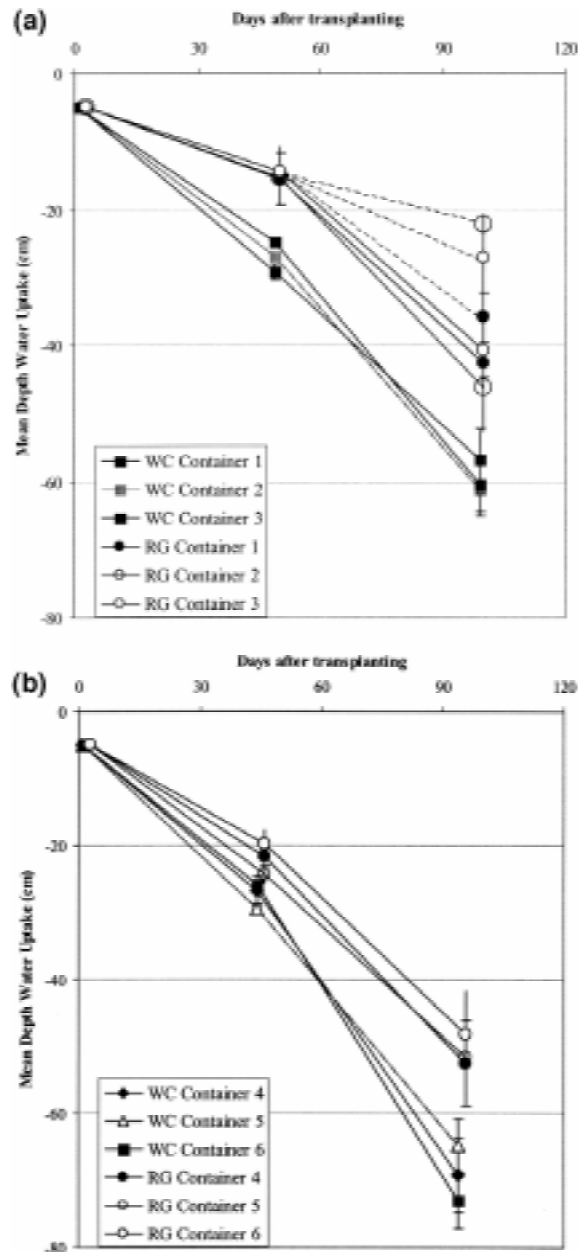
*Figure 5.* (a) Mean depth of soil water uptake over time of white clover (WC) and ryegrass (RG) plants grown in a competitive association in the greenhouse and subjected to a minimum (MIN) soil water deficit. Dotted lines (- - -) are estimates of mean depth of soil water uptake calculated with conservative soil water isotopic concentration values. Data are the means of three replicates. Bars indicate standard deviation. (b) Mean depth of soil water uptake over time of white clover (WC) and ryegrass (RG) plants grown in a competitive association in the greenhouse and subjected to a moderate (MOD) soil water deficit. Data are the means of three replicates. Bars indicate standard deviation.

competitive advantage over the clovers in nutrient and water uptake. Burch and Johns (1978) have equally reported that WC did not extract soil water from depths much below 0.35 m during 12 weeks of study. White clover has a thicker, shorter and less branched root system with shorter and fewer hairs than RG (Caradus, 1990; Haynes, 1980) and the majority of WC roots have been reported to remain in the upper 0.20 (Frame and Newbould, 1986) to 0.45 m of the soil profile (Blaikie and Mason, 1990). Yet root biomass is not always indicative of a plant ability to acquire soil resources and our observations of the capacity of WC to extract water from deeper soil reserves may give it a competitive advantage in deep soil subjected to drought following spring seeding. Our observations of the capacity of WC to obtain water from deeper soil reserves than RG has been previously reported (Guckert et al., 1993; Høgh-Jensen and Schjoerring, 1997).

*Root adaptation to drought*

The mean depth of soil water uptake by WC was always greater than that of RG regardless of soil water status. Deep root penetration (Grieu and Aussenac, 1988) and high root densities have long been recognized as important for water uptake (Ritchie and Jordan, 1972). In pasture, it has been observed that over three quarters of plant roots remain in the top 0.10 m (Jupp and Newman, 1987; Mehanni and Repsys, 1986). The near-surface soil is, therefore, subjected to intensive drying by both direct evaporative water loss, as well as by root water extraction. Therefore, as drought becomes established, upper soil water levels can reach the lower limits of availability for plant uptake.

Water still available deeper in the soil profile during times of drought may be available for plant growth if plant roots can access it. Klepper (1991) found root systems of young cotton plants deeper in the soil with gradual drying of a deep and initially moist soil, than when water was frequently applied to the soil surface. The propensity of plant roots to grow toward wetter areas has been recognized by numerous authors (Eissenstat, 1991; Gallardo et al., 1994; Pregitzer et al., 1993). However, the ability of plants to exploit areas of higher water content is not universal (Carmi et al., 1993; Plaut et al., 1995). This plastic growth response of plant roots to drought and soil water supposes a reorientation in the allocation of plant C that is not well understood. Greater carbon allocation to the roots has been observed in white clover subjected to moder-

ate soil water deficit (personal data), even though root biomass simultaneously decreased. That WC roots, in our experimental conditions, extracted water from a lower mean soil depth than RG may give WC a distinct advantage during the 3–4 month period subsequent to seeding, when soil surface drying can be a problem.

Though the mean depth of soil water uptake for RG was less than that of WC, our results clearly show a substantial increase in the depth of soil water uptake by RG roots as the soil water deficit increased. Root elongation in dry soil has been observed to decline. This decline has been attributed to various causes, such as a concomitant increase in soil mechanical resistance to root penetration, or a decline in the turgor pressure of plant roots (Sharp and Davies, 1989). Though drought is known to affect root growth more than shoot growth in *Lolium perenne* (Gales, 1979), the increase in the depth of soil water uptake by RG suggests a stimulation of root growth in response to a moderate soil water deficit for this species. Davidson (1969) saw reductions in root weight in white clover and ryegrass grown from seed when soil water content increased during the first 6 months of plant growth.

Roots of RG respond to severe drought by producing new lateral roots (Jupp and Newman, 1987). It has been shown that root tips are more resistant to desiccation than other parts of the root system (Jupp and Newman, 1987; Zhang and Davies, 1991). Düring and Dry (1995) have reported that a moderate soil dehydration led to osmoregulation in root tips, but not in other parts of the roots. The maintenance of a favorable water potential in the root tips favors root elongation (Gowing and Davies, 1989). Moreover, Takahashi and Scott (1993) demonstrated that the sensory site of moisture gradients resides in the region of the root cap.

*Root response to Competition*

The similarity of the mean depth of soil water uptake by WC and RG subjected to a moderate soil water deficit from 0 to, at least, 49 days after transplanting provides evidence of a strong competitive exploitation of the water resource by both species. Although numerous works have looked at the effects of competition on WC and RG (Lüscher et al., 1992; Martin and Field, 1984; Menchaca and Connolly, 1990), few studies have focused on the effect of both competition and drought (Guobin and Kemp, 1992; Lucero et al., 1999; Thomas, 1984).

Martin and Field (1984) determined RG to be more competitive than WC due to the combined effects of its more aggressive root and shoot system. They stated that the greater root competitive ability of RG may have been due to its ability to exploit nutrients in successive soil layers in advance of white clover with the aid of its faster growing root system (Evans, 1973, 1977). Previous studies have as well demonstrated the poor competitive ability of white clover in association with ryegrass for P (Jackman and Mouat, 1972a, b; Mouat and Walker, 1959a;), K (Mouat and Walker, 1959a) and S (Walker and Adams, 1958). This poor competitive ability of white clover for nutrients with low mobility was determined to be due to differences in root morphology (Evans, 1977) and/or root cation exchange capacity (Blaser and Brady, 1950; Mouat and Walker, 1959b). But the morphology of the root system may be less important with respect to nitrate and water acquisition, as our results give WC an advantage over RG in the recovery of deeper reserves of soil water.

Just as leaves are the principal organs of most aboveground competition for light between plants, the roots constitute the fronts of below ground interference between plants – fine roots of one plant may influence the fine root distribution of neighboring plants and this may, or may not, be mediated by local resource depletion – such as water (Caldwell et al., 1996). Collins et al. (1996) determined that severe competition exists between white clover and ryegrass at very early stages of growth. Thomas (1984) observed that droughting reduced the shoot growth of grass and clover monoculture equally, but affected clovers more than ryegrass in mixtures – possibly because clover had much smaller root systems. In a shallow soil, Lucero et al. (1999) have shown that, in conditions of moderate soil water deficit, competition between WC and RG plants reduced the root growth of ryegrass to a greater extent than that of white clover. Huber-Sannwald et al. (1998) observed that the morphological plasticity of the stolon of a perennial grass was influenced by the presence of the neighbouring species, mainly depending on their root density, much more than by the local nutrient enrichments. Other forms of below-ground interference may include species-specific recognition and avoidance of neighboring plant roots (Huber-Sannwald et al., 1996, 1997, 1998; Krannitz and Caldwell, 1995; Mahall and Callaway, 1991, 1996).

## Conclusion

Our results show that the soil water uptake of WC was always deeper than that of RG. Additionally, soil water deficit stimulated root growth regardless of the root morphological differences between the two species. After 3 months of competitive growth, WC roots obtained water from a soil depth 30% greater than that of RG, which is substantial. Results from this study indicate that WC may have a competitive advantage over RG when surface and near-surface soil experiences droughting during the early months subsequent to pasture sowing.

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