COMPARATIVE WATER RELATIONS AND DROUGHT TOLERANCE AMONG ALFALFA CULTIVARS

By

Donald M. Bonner

A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfillment of the Requirements
for the Degree of

Master of Science

Department of Plant Science
University of Manitoba
Winnipeg, Manitoba

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A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree

of

MASTER OF SCIENCE

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Abstract

Comparative water relations and drought tolerance among alfalfa cultivars.

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Major Professor, Dr. Martin H. Entz.

A greater understanding of water relations in alfalfa and some of the traits involved in drought tolerance could be beneficial to future plant breeding work for arid and semi-arid regions. This study examined several divergent alfalfa cultivars over a wide range of soil moisture conditions in controlled watering and natural (field) environments with the objective of characterizing potential differences in plant water relations, productivity and water usage. In addition, the effect of pre-stress conditioning on water relations during a subsequent drought was explored in terms of general impact and possible cultivar differences. A third objective was to evaluate relationships between parameters and yield a better understanding of general water relations in alfalfa.

Several water relations parameters including relative water content, and total water, osmotic and turgor potentials were measured or calculated using both field-grown and container-grown alfalfa plants in 1991 and 1992. Aerial dry matter and root production, water usage and water use efficiency were also determined. Water application was controlled in the container-grown experiments to provide well-watered and droughted plant material for comparative measurements.

Differences in water relations, productivity and water use between the alfalfa cultivars were revealed. The relationships between these parameters are complex as most of the cultivars had at least one trait, such as a larger root system, better osmotic adjustment, lower detached leaf dehydration or lower plant water use, which could allow them to tolerate or avoid droughts. The combination and interaction of these traits will likely produce distinct results in different environments, which could make it difficult to identify and incorporate a specific trait into a breeding program and achieve predictable results. Overall, there appeared to be a tradeoff between productivity and drought tolerance.

Benefits of pre-stress conditioning were realized. However, these benefits did not occur to the same extent or were lost more quickly in some cultivars. Osmotic adjustment was determined to be a benefit of pre-stress conditioning, but this benefit was short-lived. Because superior water relations were maintained after the benefits of osmotic adjustment were lost, the contribution of other unidentified factors was implied.

Several general observations for alfalfa were also noted in this study. Although root mass differed in the controlled water study, the effective depth of water extraction in the field was generally similar between cultivars at 80 to 120 cm by the time of first cut and about 140 to 180 cm at the end of the season in 1991 and 1992, respectively. Osmotic adjustment of over 0.4 MPa was shown to occur in droughted versus well-watered alfalfa and is likely an important drought tolerance characteristic. Alfalfa cell walls are elastic relative to some other species, which may limit its drought tolerance potential, although the ability of cell walls to become more rigid when droughted, such as observed in 'Rangelander', may form the basis of drought tolerance in some alfalfa cultivars. Relationships between water relations variables indicated that turgor pressure was generally lost below a leaf relative water content of 72% and a stem water potential of -1.76 MPa in moderately to severely drought stressed alfalfa.

The relative importance of each drought tolerance trait likely varies depending on the specific environmental conditions and likely contributed to some of the inconsistencies observed in this study. These inconsistencies would make selection for drought tolerant traits from a group of cultivars with similar overall drought tolerance, using parameters measured in the current study, extremely difficult. Traditional methods of selection for forage yield over a number of site-years are simpler and likely render equal results.

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Acknowledgments

Upon completion of this thesis, there are a number of people that I wish to acknowledge for their contribution or support.

Thanks to my advisor, Dr. Martin Entz, for giving me the challenge of this project. His knowledge and background in this area taught me a great deal about plant water relations, while his enthusiasm strengthened my interest in agricultural research. His patience, as I struggled to complete this manuscript while working and raising a family, was appreciated.

Thanks also to my committee members: Dr. R. Sri Ranjan, Dr. S.R. Smith and Dr. W. Woodbury for their interest and input into this study.

Without technical and physical assistance the research could not have been completed. For this I thank: Keith Bamford, Terry Buss and Pam Ominski. Your hard work and dedication were very much appreciated.

The Canadian Wheat Board, and especially Dr. Paul Bullock, deserve thanks for accommodating me while I completed this thesis. Another former supervisor, Dr. G.H. Gubbels, deserves recognition for allowing me to take an active role in his research and for his persuasion to return to University. Jeff Babb, from the Canadian Grain Commission, is gratefully acknowledged for his advice and assistance with some of the statistical analysis.

Thanks to my parents, Jim and Pat, and mother-in-law, Virginia, for their support and also for their help with baby-sitting. I also owe special thanks to my parents for fostering my love, in general, for agriculture, and for teaching me to take pride in my work -- a quality that I have found especially beneficial in research.

Lastly, I would like to thank my wife Lorna, for her support and encouragement, and my daughter Jennifer, who, with a sparkle in her eye, provided me with the final drive to complete this project. To you Jennifer, I dedicate this thesis.

List of Symbols and Abbreviations

Ψw, WP - Water potential

π, OP - Osmotic potential

π_{100}, AOP - Leaf osmotic potential adjusted to full turgor

π_0 - Leaf osmotic potential at zero turgor

π_{r100} - Root osmotic potential at full turgor

OA - Osmotic adjustment

P - Turgor potential

RWC - Leaf relative water content

FW - Fresh weight

DW - Dry weight

TW - Turgid weight

TW:DW - Ratio of leaf turgid weight to dry weight

T_l - Leaf temperature

T_a - Ambient air temperature

T_c - Canopy temperature

g_l - Conductance

C.V. - Coefficient of variation

Ppt. - Precipitation

Rad. - Radiation

d - Day

h - Hour

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1.0 Introduction

Alfalfa (*Medicago sativa* L.) crops grown in Western Canada and other parts of the world are often exposed to periods of drought, due to seasonal or annual rainfall variability. Using models, Ash et al. (1992) estimated that the water deficit in alfalfa crops at the time of second cut averaged from 100 to 225 mm in the Eastern Canadian Prairies (southern Manitoba and south-eastern quarter of Saskatchewan). This huge deficiency has a significant impact on alfalfa productivity and survival. Without augmenting the available moisture supply via irrigation, the only other alternative to increase productivity under these conditions is to identify cultivars that have drought resistance characteristics. The ability of alfalfa to utilize water efficiently may be related to several morphological or physiological characteristics. For example, a deeper or more prolific root structure would allow a plant to avoid drought by increasing the available water to the plant. Alternatively, a greater level of osmotic adjustment would increase turgor potential maintenance and allow growth at lower plant water potentials.

Comparing alfalfa cultivars, which have been identified as having drought resistance capabilities, with other less drought resistant cultivars, may increase the knowledge of physiological characteristics or other mechanisms for drought resistance in alfalfa. One cultivar, 'Wilson', has shown greater productivity as compared with 'Mesilla' when grown under drought conditions in New Mexico (Anonymous, 1987b, Melton et al., 1989). 'Rangelander' alfalfa was selected for long-term persistence (Heinrichs et al., 1979), which may be partly linked to superior drought resistance. Current knowledge of drought resistance characteristics, especially the potential for osmotic adjustment as a mechanism for increased drought adaptation in alfalfa, is limited (Sheaffer et al., 1988).

Several techniques for measuring plant water relations have been developed and utilized in the past (O'Toole et al., 1984; Turner, 1981). These methods can be used to compare the relative water status of different plants, which may allow the identification of specific drought resistance traits.

In alfalfa breeding programs, herbage yield is one of the most important selection criteria, while relative water usage is generally overlooked. Increasing the availability of water through deeper rooting or leaving a greater amount of water in the soil for subsequent growth (via slower or more efficient water use), are two characteristics that alfalfa cultivars could employ for increased drought resistance.

The objectives of this study were to describe seasonal and diurnal water relations in several alfalfa cultivars with divergent genetic backgrounds, during the establishment year, using measurements and calculations of: water potential (Ψw), osmotic potential (π), osmotic potential adjusted to full turgor (π_{100}), root osmotic potential adjusted to full turgor (π_{r100}), osmotic adjustment (OA), turgor potential (P), relative water content (RWC), turgid weight:dry weight ratio (TW:DW), leaf temperature (T_l), canopy temperature (T_c) and conductance (g_l). In addition, water usage and shoot and root productivity of the cultivars were compared. Because natural (outdoor) environments are not always cooperative and may not provide sufficient water deficits for drought studies, container-grown experiments were included so that soil moisture contents could be controlled. An additional objective in the controlled water studies was to determine the impact of a previous drought cycle (i.e., pre-stress conditioning) on water relations during a subsequent drought.

2.0 Literature Review

2.1 Alfalfa: Evolution and Genetic Potential for Drought Tolerance

Alfalfa (*Medicago sativa* L.) is a perennial forage legume that has been domesticated and grown for more than 3300 years (Heichel, 1983). Although originating in the Near East and Central Asia under stressful growing conditions (Heichel, 1983), alfalfa co-evolved in nine various areas (Barnes et al., 1988a). The selection pressures within the various environments produced significantly different sources of germplasm.

Alfalfa is the most popular forage legume in Canada (Anonymous, 1987a). The most widely grown alfalfa cultivars are of the *M. sativa* species, although some cultivars are of the *M. falcata* species, and others are a combination of the two (*M. media* Pers.). Generally, all North American cultivars have been derived from some combination of the nine original sources of germplasm (Barnes et al., 1988a). This has led to the production of alfalfa cultivars with various characteristics.

Although known for its extravagant water use, characteristics of alfalfa allow it to express a degree of drought tolerance. Carter and Sheaffer (1983b) stated that, ". . ., it (alfalfa) seemed able to tolerate extreme drought stress." Hattendorf et al. (1988) stated that, "the exponential, rather than linear, decline in yield to water stress suggests that alfalfa has some mechanism for maintaining biomass production at high stress levels." Due to the evolutionary background, germplasm from the various sources would likely possess varying degrees and mechanisms for drought tolerance.

Crop production in dry areas can be increased through the development of more suitable cultivars. A better understanding of the physiological processes involved in drought tolerance would be of value in order to generate the desirable drought tolerant or resistant cultivars. Two important considerations for developing plants for drought-prone environments are yield and plant survival (Ludlow and Muchow, 1990). Physiological research can help identify potentially useful selection criteria, which can enhance the yield and survival of the plant (Lawn, 1988). Selection criteria using physiological traits have already been incorporated into some breeding programs (Anonymous, 1988). Techniques for assessing the level of water stress in the plant, and how plants respond to stress, have been developed over the last few decades. These parameters will be discussed in section 2.5.

2.2 Drought and Drought Adaptation

Approximately 26% of the world's arable land is subject to periods of drought stress (Blum, 1984). Drought is a meteorological occurrence defined by Kramer (1983) as the absence of rainfall long enough to result in the depletion of soil water and cause injury to plants. Drought can either be permanent, as is the case in desert regions; seasonal, where there are distinct wet and dry periods in a year; or unpredictable, where periods of drought cannot be reliably forecast. Of these three types of drought, unpredictable droughts are the most challenging, but possibly offer the most potential for plant breeding, because the crop must be adaptive to stress conditions, yet highly productive under good growing conditions.

Drought tolerance has been defined by many agricultural researchers (May and Milthorpe, 1962; Kramer, 1983; Turner and Burch, 1983) and can be most commonly described as either drought escape (avoidance) or drought tolerance. Both drought escape and drought tolerance are achieved through morphological or physiological characteristics of the plant, which are controlled genetically.

The types of drought resistance or tolerance characteristics present in a plant can influence its adaptation to seasonal or temporary droughts. Characteristics of plants are listed under their respective drought tolerance classes in Table 2.1. Plants may contain one or several of these traits, which could act in concert to increase the level of drought tolerance.

Table 2.1 Characteristics of plants that enable them to escape or tolerate drought†.

Drought Escape

- (i) Rapid phenological development
- (ii) Developmental plasticity

Drought Tolerance at High Plant Water Status

- (a) Reduction in water loss
  - (i) Increased stomatal and cuticular resistance
  - (ii) Reduced absorption of radiation
  - (iii) Reduced leaf area

- (b) Maintenance of water uptake
  - (i) Increased rooting depth and density
  - (ii) Increased hydraulic conductance

Drought Tolerance at Low Plant Water Status

- (a) Maintenance of turgor
  - (i) Osmotic adjustment
  - (ii) Increased elasticity
  - (iii) Decreased cell size

- (b) Tolerance of dehydration or desiccation
  - (i) Protoplasmic tolerance
  - (ii) Cell wall properties

† From Turner and Burch (1983)

Drought escape, also referred to as drought avoidance, applies to those species or cultivars that can avoid long, seasonal periods of drought through rapid completion of ontogeny (Morgan, 1984). This can apply to annual crops that mature and produce seed before severe drought stress, or to perennial crops, that produce seed while retaining enough reserves to survive a long dormant period. Under good growing conditions, yield generally increases with length of growing season, therefore, there is a tradeoff between higher yield potential and the ability to circumvent potential drought.

Some species or cultivars can tolerate drought conditions while maintaining a high internal plant water status. This drought tolerance can be achieved by a deep root system that can extract available water deeper in the soil profile (e.g., alfalfa), by reducing transpiration, or by some other physiological means.

A second category of drought tolerance includes those plants that can survive drought with a low plant water status. This is also called "resistance to desiccation" and plants in this category can recover and grow rapidly when soil water becomes available (May and Milthorpe, 1962).

2.3 Effects of Drought Stress on Plants

2.3.1 Morphological Modifications

The color of droughted alfalfa leaves changes from a light green, usually associated with rapidly growing plants, to a dark, grayish-green (Brown and Tanner, 1983a). Leaf color can be used to detect water stress, however, by the time visual symptoms appear, yield is already severely reduced.

Petit et al. (1992) found that the leaf to stem ratio (L:S) was higher for alfalfa grown under dry conditions compared with either optimum or wet soil conditions for clay and gravely sandy loam soil types under both warm (25°C daytime minimum, 19°C night) and cool (15°C daytime minimum, 9°C night) temperature regimes. Similarly, Halim et al. (1989a) observed a 20% increase in L:S of alfalfa under drought stress as compared with a well-watered control. The increases in L:S under drought were mainly attributed to proportionally lower stem growth rates (Halim et al., 1989a; Petit et al., 1992). Reduction in stem growth in alfalfa is characterized by a decrease in stem diameter and a reduction in internode length (Vough and Marten, 1971). In a study by Brown and Tanner (1983a), water stress reduced leaf area and internode length by 39 and 48%, respectively, as compared with irrigated alfalfa. Carter and Sheaffer (1983a) reported that growth rates declined sharply with decreasing midday water potential. In their study, growth was slow at moderate plant stress levels (-1.5 to -2.0 MPa), and was negative at water potentials below -2.1 MPa. Negative growth in their study was attributed to leaf loss.

Salter et al. (1984) found that total root mass in alfalfa decreased, but root fibrousness increased under increasing moisture stress. Increasing root fibers may be another mechanism for increasing the plant's ability to extract soil water. Generally, alfalfa's deep root system is an important trait that allows it to withstand drought (Jung and Larson, 1972).

The morphological changes described play an important role in pre-stress conditioning of the plant. For example, reduced leaf area in a droughted plant would decrease ET upon re-watering compared with an unstressed plant. Characterizing the impact of these morphological changes would also help reveal the benefits of incorporating these traits into a breeding program.

2.3.2 Physiological Changes

Several physiological responses result from drought stress. Because few studies have looked specifically at alfalfa, examples from other field crops will be included to help portray some of the possible physiological effects of drought stress.

Net photosynthesis in the upper leaves of alfalfa was reduced by more than 35% in non-irrigated treatments compared with irrigated plants (Nicolodi et al., 1988). The authors suggested that the decrease was due to both stomatal and non-stomatal factors, however, the contributions of stomatal processes and mesophyll resistance were not separated.

Drought stress can alter enzyme activity, which in turn affects other processes within the plant. Mayoral et al. (1981) showed that ribulose-1,5-biphosphate (RuP_2) carboxylase and phosphoenolpyruvate (PEP) carboxylase activity of *Triticum aestivum* decreased immediately with decreases in plant water potential. In a related, but more drought tolerant species, *Triticum kotschyi*, RuP_2 carboxylase activity remained constant to a plant water potential of -2.2 MPa, while PEP carboxylase activity actually increased until plant water potential was reduced below -2.2 MPa. The maintenance of enzyme activity under drought stress may be an important characteristic for some species.

Another important response to drought is osmotic adjustment. The accumulation of solutes within cells is an important mechanism for maintaining turgor potential as water potential decreases. Osmotically active compounds have been shown to increase in leaves of many species such as wheat (*Triticum aestivum* L.) (Johnson et al., 1984), barley (*Hordeum vulgare* L.) (Blum, 1989), sugarbeets (*Beta vulgaris* L.) (McCree and Richardson, 1987), lupins (*Lupinus spp.*) (Turner et al., 1987), cowpea (*Vigna unguiculata* L. Walp.) (McCree and Richardson, 1987), sorghum (*Sorghum bicolor* L.) (Santamaria et al. 1990), and tall fescue (*Festuca arundinacea* Schreb.) (West et al., 1990) when subjected to drought conditions. Osmotic adjustment may occur in alfalfa, but little information is available (Sheaffer et al., 1988).

Specific compounds such as the sesquiterpenoids (abscisic acid, phaseic acid, (E)(E)-farnesol, and xanthoxin) are capable of initiating stomatal closure under drought stress (Harborne, 1989). However, Harborne indicated that any evidence that drought resistant plants contain higher levels of abscisic acid (ABA), is circumstantial; others, such as Tardieu and Davies (1993) feel that ABA plays a critical role in stomatal regulation. Proline accumulates in water-stressed plants and in many species is higher in drought resistant than in drought susceptible varieties (Harborne, 1989). Ford (1984) concluded that pinitol accumulation may indicate the ability of a legume to tolerate low leaf water potentials. Some compounds may remain at higher levels in previously stressed plants, which could form a basis for some of the "pre-stress conditioning effect" seen in plants subjected to drought stress.

2.3.3 Effect on Alfalfa Forage Quality

Alfalfa forage quality is important to beef and especially dairy producers. Peterson et al. (1992) showed that forage quality generally increased under drought conditions. This increase in quality was due to decreased concentrations of neutral detergent fiber, acid detergent fiber and acid detergent lignin, presumably due to an increase in the L:S (Petit et al., 1992).

The effect of water shortages on alfalfa crude protein concentration is not conclusive. Gillford and Jensen (1967) reported increased crude protein (CP) in droughted alfalfa while Halim et al. (1989b) showed that CP decreased if the stress occurred at bud or flower stages. Others (Vough and Marten, 1971; Carter and Sheaffer, 1983a) have reported no effect of drought on alfalfa quality.

2.4 Environmental Effects on Plant Water Status

Various environmental factors play an important role in determining the level of stress experienced by a plant. Boyer (1969) points out, however, that environmental measurements may not reflect the conditions within a plant because the plant is rarely in equilibrium with its surroundings.

Xu et al. (1990) reported that photosynthesis in wheat was mainly affected by air temperature and humidity and only slightly affected by soil water status. In addition, they concluded that the effects of above-ground conditions and soil moisture regime were generally additive, but partly synergistic. This demonstrates that the effects of high temperature stress usually confound the effects of drought stress and are difficult to separate (Kramer, 1969). However, because drought stress and heat stress typically occur in concert (Ludlow, 1980), "drought stress" usually includes the combined effects of soil and atmospheric conditions.

Bula (1972) found, under controlled conditions, that alfalfa stem and leaf growth were greatest at 25°C. But as Tateno and Ojima (1976) found in sorghum, drought stress reduces the optimum day and night temperatures for growth. Although the optimum temperature for root water absorption is generally above 30°C, these high temperatures usually result in plant moisture stress due to greater moisture loss from the leaves (Treshow, 1970).

The effects of temperature on plant water relations and growth are also confounded by an accompanying increase in vapor pressure deficit (VPD) (e_{leaf} - e_{air}). The vapor pressure gradient increases with temperature resulting in increased water loss from leaves (Kramer, 1983). Decreases in relative humidity also increase the VPD and increase water demand from the leaf.

Wind increases transpiration by decreasing the boundary layer, although, at high levels of radiation, transpiration can actually be decreased by a light breeze (Kramer, 1983). Grace and Russell (1977) showed that a simulated constant wind, applied to well-watered grass (*Festuca arundinacea*) plants, increased stomatal density and decreased stomatal size. In addition, they found that plants grown under windy conditions lost their ability to regulate water loss through stomatal closure.

Light can also indirectly affect plant water relations. The type or quality of light can influence stomatal opening or closure (Farquhar and Sharkey, 1982) and increased irradiance can elevate leaf temperature, which would increase the vapor pressure deficit and force increased transpiration unless the stomates close. Gist and Mott (1957) showed that dry matter yield of alfalfa declined under decreasing light intensity treatments over various temperature regimes.

One of the most important environmental factors influencing plant water status is soil moisture. Ash et al. (1992) reported that alfalfa crops grown in the Winnipeg, Manitoba region have an average annual water requirement of 400 mm. However, only 200 mm of growing season precipitation are typically received by the time of the second cut. Relying on stored soil moisture for part of the shortfall, the average plant moisture deficit is still about 100 to 225 mm, although year to year variation could increase or decrease this estimate substantially (Ash et al., 1992).

Soil characteristics can affect water relations in several ways. First, proper nutrient balance will promote root growth and allow extraction of water located deeper in the profile. Second, soil texture will influence the amount of available water, and the water extraction pattern (i.e., water is extracted more evenly at all depths in sandy soils (Christian, 1977)). Third, fine textured soils can restrict root growth because of high soil densities. Fourth, soil type and density can influence the availability of oxygen, with saturated soils, especially clay-textured, often having oxygen limitations.

2.5 The Definition and Measurement of Plant Water Relations

2.5.1 The Meaning of Plant Water Relations

Water relations of a plant are described by parameters that characterize both the water content of the plant as well as the energy status of the water within the plant (Turner, 1981). Both water content and energy status of water in the plant need to be measured because the relationship between the two varies with species, growth conditions and stress history (Turner, 1981).

The energy status of water within a plant is quantified by the measurement of water potential (Ψw). The components of Ψw can be described by the water potential equation:

$$\Psi w = \pi + P + \tau + G \qquad (1)$$

where the symbols designate the components of osmotic potential (π), turgor potential (P), matric potential (τ), and gravitational potential (G), respectively (Boyer, 1969; Kramer, 1983; Jones, 1986). Others (Wiebe, 1966; Tyree, 1967) define the equation similarly, but do not include the gravitational pressure component because it is very small.

Osmotic potential arises from the presence of solutes in cells. It is always negative since the concentration of solutes in plant solutions is never less than that of pure water, which has Ψw by definition (Slatyer and Taylor, 1960). Solutes consist of: soluble sugars, carboxylic acids, potassium, chloride, and amino acids (Turner and Burch, 1983). Other components have been specifically linked to drought resistance and have already been discussed in section 2.3.2.

Turgor potential (P), also known as turgor pressure or pressure potential, is the positive pressure within a cell. As water enters the cell, due to an osmotic gradient, cell volume increases. An equal, but opposite force, is exerted by the cell wall to the interior (Simpson, 1981; Turner and Burch, 1983). As water is lost from the cell, due to drought or heat stress, cell volume and turgor potential decrease. Cell growth is reduced and ultimately ceases as turgor potential is reduced to zero.

Matric potential describes the energy of water held in microcapillaries or in other cell components, or bound on the surface of cell walls (Kramer, 1983). Wiebe (1966) found matric potentials to be -0.01 MPa in plant tissue when 50% of their original water content had been lost, demonstrating the relative insignificance of this component. Similar results were found by Boyer (1967) for sunflower, (*Helianthus annus* L.) while in the same study, rhododendron (*Rhododendron roseum* Rehd.) showed matric potential values of about -0.9 MPa at 50% water content. Passioura (1980) suggests that the matric potential is already accounted for in P or π in the solid phase, or is a mixture of hydrostatic and osmotic pressures that cannot be separated experimentally.

Gravitational potential refers to the downward force of gravity on the plant. It too, is unimportant for most field crop measurements, as the gravitational force is only 0.01 MPa if the crop is 1.0 m tall (Turner and Burch, 1983). The water potential equation is thus composed mainly of negative osmotic pressure and positive turgor potential terms.

2.5.2 Water Potential

Of all the measurements to determine plant water status, water potential (Ψw) is the most popular (Hsiao, 1973) and seems to have the widest application (Kramer, 1988). Several methods can be used to determine Ψw in plant tissue (Barrs, 1968; Slavik, 1974), however, the pressure chamber technique is the most widely used. Because of its ease of use, its speed and reliability, and its lack of temperature control requirements, the pressure chamber is especially well-suited to field measurements (Turner, 1981). This method was made popular after Scholander et al. (1965) reintroduced an idea that Dixon originated in 1914. An extensive review on the methods and applications of Ψw in ecological studies was written by Ritchie and Hinckley (1975).

In a comparative study using a leaf dew-point hygrometer and a pressure chamber to measure Ψw in alfalfa, Brown and Tanner (1981) determined that similar results were observed if the same plant was used for both methods. Additionally, they concluded that large standard deviations about the means for both methods indicated that plant to plant variability is sufficiently large to pose problems when measuring small differences in water potential. Part of the variability was due to variations in establishment-year root development, as variability decreased slightly in the subsequent year (Brown and Tanner, 1981).

Carter and Sheaffer (1983b) determined that Ψw values on well watered, established alfalfa, ranged from -0.1 to -0.4 MPa at dawn to -0.8 to -1.2 MPa at midday. Under severe plant water stress, water potential values dropped to below -2.0 and -4.5 MPa at dawn and midday, respectively. They also showed that for high, medium low and unirrigated water treatments, the lowest Ψw generally occurred between 1400 to 1700 h. Similar findings were reported by Sharratt et al. (1983) who determined that minima for Ψw occurred at 1500 h for both irrigated and non-irrigated treatments of alfalfa. This was also the point of maximum difference between the two water treatments, which were 117 and 25% of extractable soil water, respectively. Water potential for the non-irrigated alfalfa declined at a faster rate, especially near the peak stress period; however, recovery after the midday minimum was also more rapid in the non-irrigated alfalfa.

Hall and Larson (1982) evaluated water relations under greenhouse conditions for two cultivars of *Medicago sativa* L., 'Cody' and 'Sonora' (winter-hardy and non-winter-hardy, respectively), during periods of stress and recovery. They found a significant negative linear relationship between Ψw and days after onset of stress when data was averaged over both cultivars. The cultivars did not differ in water status over increasing water deficits, although after re-watering recovery occurred more rapidly for 'Sonora'. Plants generally attained pre-stress water potential levels within 24 h following re-watering.

Carter et al. (1982) also showed differences in Ψw for three alfalfa cultivars grown in containers. The cultivars varied in winterhardiness and Phytophthora root rot (*Phytophthora megasperma* Drechs. f. sp. *medicaginis*) resistance. The authors reported that cultivars with greater root lengths had lower water potential under drought stress. Estill et al. (1991) found differences between pale and dark alfalfa leaf chlorophyll variants, however, results were inconsistent between years and soil moisture levels.

2.5.3 Relative Water Content

Maintaining a high water content in plants is important for growth and for facilitating many other biochemical processes. In addition, it has been shown that the ability of a plant to maintain a high relative water content (RWC), as Ψw is reduced, is a characteristic of drought tolerance (Frank et al., 1984).

The water content of a plant can be expressed as a percentage of fresh weight, a percentage of dry weight, as a percentage relative to full turgidity (relative water content) or percent water deficit (1 - RWC) (Kramer, 1983). Of these techniques, RWC is the most common term used.

Plant water content can be calculated on a dry weight or fresh weight basis, but problems exist with these methods (Turner, 1981). Dry weights can change seasonally, and even diurnally, making accurate comparisons over time impossible. Calculating water content as a percentage of fresh weight tends to minimize differences between samples as compared with other bases (Turner, 1981), however, the method of relative water content avoids these problems altogether. It is calculated by using the following equation where RWC, FW, TW, and DW, correspond to relative water content, fresh weight, turgid weight, and oven dry weight, respectively.

$$RWC = \frac{(FW - DW)}{(TW - DW)} \qquad (2)$$

This equation was introduced by Weatherley (1950), although at this time it was termed "relative turgidity" rather than "relative water content". The RWC technique does have disadvantages, such as, the considerable time lag between sampling and obtaining a result, and the fact that the required weighing operations are time-consuming (Smart and Bingham, 1974).

Weatherley (1950) reintroduced a method in which leaf disks were used to evaluate relative turgidity. This method is well suited to crops with few, large leaves but would not be the most efficient method for alfalfa. Shimshi (1973) suggested that in addition to using the traditional method of floating leaf disks on water, dipping the petioles of detached leaves into water would also achieve saturation. This method would be superior when working with plants with small leaves, although it does have the disadvantage of being a more destructive measurement. In addition, using whole leaves eliminates injection errors along the cut edges of the leaf disks, which can be substantial in some species (Barrs and Weatherley, 1962). Hewlett and Kramer (1963) found the use of whole leaves to be superior for measuring leaf water content of several hardwood tree species.

The uptake of water into plant tissue is divided into two phases (Barrs and Weatherley, 1962). Phase I refers to the initial rapid uptake of water, while phase II is reflected in a slow persistent uptake that continues as long as the leaf or disk remains healthy. Error in RWC measurements can arise from continued uptake of water after full turgor has been attained or from changes in dry weight during the period of water uptake (Barrs, 1968).

2.5.4 Turgid Weight:Dry Weight Ratio

The turgid weight:dry weight (TW:DW) ratio of plant tissue can be calculated from the data used to calculate RWC. The value derived from this calculation is an indirect measurement of the water-holding capacity of the tissue. Although a single measurement holds little relevance, a decreasing TW:DW ratio over time can indicate a decreasing cell size (Cutler et al., 1977).

Turner et al. (1987) showed a concurrent decrease in the TW:DW ratio with an increase in tissue osmotic pressure using water-deficit-stressed lupins. They attributed the decrease in the TW:DW ratio to the accumulation of osmotically active and osmotically inactive matter in the leaf tissue. They suggested that the TW:DW ratio may be a good screening technique for osmotic adjustment in lupins, due to the correlation of the two over species and environments.

In a study by Ford (1983), several pasture legume species were subjected to water stress. Those species, which had high drought tolerance, exhibited osmotic adjustment. Some of the osmotic adjustment was attributed to a change in water-holding capacity of the cells (decreasing TW:DW ratio) while the remaining effect was ascribed to the accumulation of osmotically active solutes.

2.5.5 Osmotic Potential

Osmotic potential (π) is a measure of the osmotically active solutes in the cell. The ability to decrease π in response to low water potentials is a trait associated with drought tolerance (Frank et al., 1984).

Osmotic potential can be measured using several methods, (Slavik, 1974), however, osmometry or psychrometry are the most commonly used approaches. In either case, plant tissue must be frozen to disrupt cell membranes. Although this step is necessary, it also leads to the mixing of the symplastic and apoplastic water (Kramer, 1983). This results in a π that appears higher than it actually is. The pressure-volume curve technique, which will be further examined in a subsequent section, can also be used to determine osmotic potential. Using the water-release curve (i.e., the pressure-volume technique), one can derive the actual π value, without dilution error. However, the main disadvantage of this method, and the reason that it is not as widely used, is that it is very time consuming.

Wenkert (1980) found that the mixed-sap osmometer method resulted in values 11 to 16% more dilute (higher π) than those derived using the pressure-volume method using leaves of *Zea mays* L. Conversely, Brown and Tanner (1983b) found π from sap expressed from frozen and thawed alfalfa leaf tissue to be 0.21 to 0.89 MPa lower than those obtained from water-release curves. They state that some of this variation may be accounted for by differences in leaf position. In their study, the top leaf was used for the water-release curves, while the next three or four leaves were used for the frozen-tissue method. However, they suggested that leaf position accounted for less than 50% of the variation between the two methods and that the remaining difference resulted from the production of solutes in the thawed tissue by enzymatic hydrolysis of nonstructural carbohydrates. Tissue was thawed for three hours prior to expression of the plant sap, which could account for the large differences observed. Sheaffer et al. (1988) cited unpublished work in Wisconsin that showed that no change in sap π occurs over time if the thawing takes place rapidly, although the exact length of time was not defined.

2.5.6 Adjusted Osmotic Potential and Osmotic Adjustment

Osmotic potential varies with species under a given set of conditions. Consequently, a single point-in-time measurement of osmotic potential provides little information about the stress condition of the plant or how it is reacting to drought conditions. Furthermore, osmotic potential comparisons could be confounded by differing RWC status of the plant materials used. Osmotic potential adjusted to full turgor (π_{100}) accounts for this variation by correcting the π value to that at 100% RWC. The change in π_{100} over time (osmotic adjustment) gives a net change in solute concentration, not an increase due to dehydration of the tissue. Osmotic adjustment is therefore defined as "an increase in solute concentration above that which originates from cell water loss" (Zur et al., 1981).

Osmotic adjustment (OA) or solute accumulation allows turgor maintenance under stress conditions, thus forming a basis for drought tolerance in some species (Turner and Burch, 1983). Leaf solute accumulation has shown to be related to grain yield in sorghum (Santamaria et al., 1990) and in barley (Grumet et al., 1987).

Turner et al. (1987) found increases in OA of up to 0.5 MPa in lupins subjected to a drying cycle. There were differences in OA between lupin species in their study, and although the species with the greatest OA occurred in drier locations, OA did not appear to play a role in the distribution of the species.

Munns and Weir (1981) evaluated the contribution of sugars to the osmotic adjustment of wheat leaves growing under moderate water deficits. They found that the increase in sugars accounted for 70 to 100% of the osmotic adjustment, which was approximately equivalent to the decreased consumption of carbohydrate due to a reduction in growth. The types of sugars involved varied with location in the leaf (elongating or expanded zones), but were mostly glucose or sucrose.

Sharp and Davies (1979) showed that root tip turgor potential was maintained in corn for up to seven days when water was withheld. This turgor maintenance was due to solute accumulation in the root.

2.5.7 Turgor Potential

Positive turgor potential (P) is essential for plant growth (i.e., cell division and elongation). Kirkham et al. (1972) showed cell division was stimulated in radish cotyledons when P was increased from 0.5 to 0.6 MPa, while cell elongation increased above 0.3 MPa. Brown and Tanner (1983a) observed that P was 0.3 ± 0.07 MPa when leaf expansion in alfalfa ceased (Ψw was below -1.0 MPa during this time).

Fluctuations in P likely allow the plant to translate changes in plant water status into metabolic change (Turner and Jones, 1980). Substances, such as the hormone ABA, have shown to accumulate as P approached zero; therefore, the role of ABA in stomatal closure indirectly links stomatal response to P. The ability of a plant to maintain turgor potential under increasing drought stress (decreasing Ψw) reflects how the plant is reacting via osmotic adjustment.

The most popular technique for determining *P* is to calculate it from the difference between Ψw and π measurements (Turner, 1981).

$$P = \Psi w - \pi \qquad (3)$$

Using equation (3) to determine *P* has some drawbacks. Because *P* is calculated from two variables, the errors associated with both Ψw and π are combined (Turner, 1981). This usually results in *P* data being more variable than either Ψw or π data. Dilution errors, associated with π measurements (previously described), or rapid water loss in water potential measurements using the pressure chamber technique, will result in lower turgor values (sometimes causing π to be negative). Turner (1981) stated that both Ψw and π should be measured on the same tissue to minimize errors. He further indicated that the pressure-volume method is preferred for accurate *P* measurements for two reasons: first, dilution errors are avoided, and second, both measurements occur on the same tissue. Although other methods exist, such as using a pressure sensitive transducer that directly measures *P* (Turner, 1981), these are more difficult and time consuming to use.

Several reports in the literature have indicated negative values for turgor potential. Tyree (1976) reinterpreted several of these datasets, plotting the isotherm of $-1/\Psi w$ versus RWC. He concluded that no negative turgor potentials were detectable, however, he did concede that his method may overlook negative values to a few tenths of a MPa. Markhart et al. (1981) tried to further explain even some of the small negative turgor values being reported. They determined that dilution of the protoplast by apoplastic water would explain some of the small negative turgor potentials. They suggested that a species specific correction factor needs to be developed for psychrometric measurements.

2.5.8 Foliage Temperature

The measure of foliage temperature, either leaf temperature (T_l) or canopy temperature (T_c), compared with ambient air temperature (T_a) can be used as an indirect indicator of transpiration rate. Higher foliage temperatures indicate lower rates of transpiration and vice-versa. Canopy temperature, along with other concurrently measured parameters, have been used to calculate a crop water stress index (CWSI), which is closely related to extractable soil moisture (Jackson et al., 1981).

Canopy temperature measurements are usually remotely sensed using an infrared thermometer, while leaf temperature can be directly measured with a thermocouple. Blad and Rosenberg (1976) compared the two methods in alfalfa and found thermocouple readings were up to 3°C higher than infrared measurements between 400 and 600 h, however, values were much closer in the afternoon when ambient temperatures were higher. The infrared thermometer has the advantages of being neither destructive nor disruptive (O'Toole et al., 1984). Thermocouples are non-destructive, but are disruptive. A disadvantage of using the infrared thermometer is the adverse effect of environmental conditions such as wind (O'Toole and Hatfield, 1983) and intermittent periods of cloud, on measurements (Gardner et al., 1992).

Under a range of irrigation treatments, Carter and Sheaffer (1983b) showed that alfalfa canopy temperature increased from sunrise, reached a maximum at about 1500 h, and then declined. Unirrigated treatments had canopy temperatures up to 8.5°C higher than irrigated treatments. Unirrigated treatments frequently reached temperatures above 30°C and had leaf temperatures greater than ambient air temperatures on all four sampling dates. They stated that "this suggests that droughted alfalfa was subjected to heat stress in addition to water stress". Similar diurnal trends were reported by Sharratt et al. (1983) who noted that the maximum difference in canopy temperature between irrigated and non-irrigated alfalfa occurred at 1500 h. Temple and Benoit (1988) found seasonal mean

$T_c - T_a$ ranged from -7.1°C to -2.9°C for normally irrigated alfalfa plots and -6.8°C to 2.3°C for water stressed plots (30% less water than normal).

Clarke and McCaig (1982) reported that leaf temperature was not a suitable screening technique for detecting drought resistance variation in wheat genotypes, as no differences were noted in their study. A similar conclusion was made for alfalfa plants (Anonymous, 1988). However, Hattendorf et al., (1990) determined that $T_c - T_a$ was generally higher for the non-dormant cultivar, 'CUF 101', than the dormant cultivar, 'Vernal', under drought stressed conditions, indicating that under certain circumstances, cultivar differences may occur.

2.5.9 Leaf Conductance

Stomata are the primary control centres for the exchange of both water vapor and carbon dioxide gases. Therefore, any reduction in water loss via stomatal closure is balanced by a reduction in carbon assimilation. In a comparison between sugarbeets, an osmotic adjuster, and cowpea, a stomatal regulator, McCree and Richardson (1987) concluded that there was no carbon gain advantage to either method. The optimization of the relationship between stomatal closure to prevent water loss, and stomatal adjustment to maintain carbon assimilation, is ultimately reflected in the water-use-efficiency ratio. Sensitivity of stomata to plant water stress is often seen in drought tolerant plants, however, this character can also be detrimental to yield under moderate drought conditions. Using a porometer, measurement of stomatal conductance (g_l), or its reciprocal, stomatal resistance, provides a quantitative assessment of stomatal activity by measuring leaf gas exchange via the leaf pores.

Cole and Dobrenz (1970) found in alfalfa, that stomatal densities were greater on the adaxial (upper) surface than the abaxial (lower) surface of leaves. In addition, they noted that terminal leaves had more stomata per unit area than basal leaves. Despite this, Carter and Sheaffer (1983b) found similar g_l values for both upper and lower surfaces in alfalfa during diurnal measurements over a range of soil moisture conditions. This suggests that adaxial stomata are either not as widely open or have a shorter aperture length than abaxial stomata (Heichel, 1983).

Carter and Sheaffer (1983b) reported that the daily cycle of g_l began with a rapid increase after sunrise, reaching maximum values of 3.3 cm s^{-1} (for well-watered alfalfa) between 1000 and 1200 h. Conductance declined throughout the midday and evening, until stomata closed at sunset. In medium-low irrigated plots, conductance was similar to less stressed plants until late in the morning, when stomata partially closed. Late in the afternoon, when evaporative demand decreased, the stomata reopened and conductance resumed the waning pattern of the high irrigated treatment. Conductance of drought-stressed alfalfa remained low (0.1 to 0.3 cm s^{-1}) throughout the day.

Stomatal closure is thought to be mainly influenced by turgor potential (Bennett et al., 1987), and external vapor pressure (Ludlow, 1980); although other factors such as light quality and irradiance, partial pressures of carbon dioxide, mesophyll metabolites (such as ABA) and metabolites from roots (such as cytokinin), can play important roles (Farquhar and Sharkey, 1982). More recent literature (Davies and Zhang, 1991), emphasizes the importance of root signals in regulating stomates. Carter and Sheaffer (1983b) found that conductance declined linearly until Ψw approached -2.5 MPa, then remained steady. They suggest that stabilization at this point indicates either incomplete stomatal closure, or cuticular conductance. Similar linear trends were observed for two greenhouse-grown alfalfa cultivars over a Ψw range from -0.4 to -1.6 MPa (Hall and Larson, 1982). Response of g_l to Ψw in other species has often been curvilinear (Ludlow, 1980; Munger et al., 1987).

Carter and Sheaffer (1983b) also established a positive linear relationship between leaf conductance and canopy temperature for unstressed alfalfa. This relationship may be influenced by an increasing vapor pressure deficit (VPD) as temperatures increased, but no clear resolution was made, because data for VPD were not recorded.

Hall et al. (1988) determined that there was no significant difference in leaf conductance between a non-dormant ('CUF 101') and a dormant ('Agate') alfalfa cultivar. Hattendorf et al. (1990), on the other hand, found that leaf conductance averaged 40% lower for a non-dormant ('CUF 101') versus a dormant ('Vernal') cultivar at a crop water stress index of zero (full transpiration). Because no differences in total water use were found, Hattendorf et al. (1990), theorized that due to the rapid regrowth of 'CUF 101', water loss was distributed differently during the growth cycle and was lower when conductance measurements were taken. Cole and Dobrenz (1970) found that stomatal density was 45% lower for the non-dormant cultivar 'Sonora' versus the dormant cultivar 'Ladak'. If stomatal densities are similar within dormancy types, the above findings would suggest that stomatal density may not be the critical factor in determining differences in stomatal conductance.

2.5.10 Water Loss From Detached Leaves

Measuring water loss rate on rehydrated, detached leaves, can be used to assess drought tolerance of plant tissue. The plot of the logarithmic water content versus time is divided into three phases (Slavik, 1974). Differences in stages I and III can be used to discern genetic superiority for drought tolerance. In phase I, the relationship is linear, because stomata are fully open. During phase II, the line becomes curvilinear, as the stomata respond and begin to close. The line returns to linearity in phase III, when the stomata close and the remaining water loss is conducted through the cuticle. Generally, researchers have used differences in phase III for comparative analysis, but others have compared water loss after 30 minutes (phase I).

The ability of excised leaves to retain water has shown some potential for differentiating drought resistance in wheat cultivars (Dedio, 1975; Clarke and McCaig, 1982). Clarke and McCaig (1982) determined that the drought-hardy durum cultivar, 'Pellisier', had higher pre-anthesis water retention ability as compared with seven other lines. They also determined that early in the season, leaves from irrigated plants of 'Pellisier' had lower water contents than leaves from rain-fed plants after 24 hours of drying. Based on these results, the authors suggested that using drought-hardened material for varietal screening would be more efficient. This observation also points out the importance of pre-stress conditioning for the expression of drought tolerance.

Dedio (1975) found that the water retention ability of the wheat cultivar, 'Pitic 62', increased with age, while the opposite trend was reported by Clarke and McCaig (1982). Considering that leaf position was also significant (Dedio, 1975), it may be necessary to sample at various stages to detect differences between cultivars. This would accommodate deviations resulting from different environmental conditions or different stages of plant growth.

2.5.11 Plant Pressure-Volume Curves

When Scholander et al. (1965) reintroduced the pressure-bomb method, they described a procedure where osmotic potential and intracellular water content are determined from repeated water potential and water content measurements. This procedure, known as the pressure-volume (PV) or water release technique, allows among other things, the point of incipient plasmolysis (zero turgor) to be determined (Ritchie and Hinkley, 1975). The relationship is described by the equation:

$$\frac{1}{P} = \frac{V - V_0}{RTn} \qquad (4)$$

where P is the applied pressure in the chamber, V is the volume of cell sap removed by the pressure, V_0 is the original cell sap volume, R is the universal gas constant, T is the Kelvin temperature, and n is the solute content (Ritchie and Hinkley, 1975). The procedure involves subjecting initially fully turgid plant material to successive pressures, then, either collecting the expressed sap (Scholander et al., 1965) or weighing the plant material at each balancing point, and finally, drying and weighing the material after several pressure and weight cycles. A plot of $1/\Psi w$ versus the water content, yields a curve with two distinct sections (Ritchie and Hinkley, 1975) and is the most common transformation of the data (Stadelmann, 1984). The first part is curvilinear, as the applied pressure is balanced by osmotic and turgor potentials. The line becomes linear (second section) when the turgor potential has been lowered to zero. Least-squares regression analysis of the linear portion of the curve (Wenkert, 1980) produces an equation that can be extrapolated to the ordinate, where $1/P$ = initial osmotic pressure, or to the abscissa, where the water content indicates the apoplastic or "bound" water content (Andersen et al., 1991; Ritchie and Hinkley, 1975; Tyree and Hammel, 1972). The point at which the curvilinear line intersects the linear line is considered the point of incipient plasmolysis. In the turgid area of the curve, bulk modulus of elasticity (BMOE) can be determined. BMOE is the change in cell volume (RWC) per unit change in turgor pressure or applied pressure (Melkonian, 1982) and describes the relative elasticity of the cell wall.

Although the pressure-volume method has been reported as being more accurate for determining osmotic potential as compared with other methods, (Wenkert, 1980; Brown and Tanner, 1983b) it is very time consuming. Tyree et al. (1978) state that it takes between 5 and 20 hours to generate one or up to two simultaneous curves. A modification to the original method by Richards (1973), using a specific period of 10 minutes for each balancing pressure, decreased sampling time considerably; however, Tyree et al. (1978) were critical of the accuracy of the results. A more rapid method has recently been proposed where turgid leaf discs are weighed, suspended over concentrations of salt solutions for 12 hours, re-weighed, then dried and weighed again, which would allow more than 40 curves to be generated in 2 days (Livingston and de Jong, 1988).

Using transformed PV curves, Frank et al. (1984) detected differences for initial turgor loss point, osmotic potential at full hydration, and leaf water potential at zero turgor in three species of wheatgrass (*Agropyron dessertorum* (Fisch.) Schult., *A. smithii* Rydb., and *A. intermedium* (Link) Haloc.). Despite their positive findings, they concluded that due to the high labor requirement, modifications in the methodology are necessary before application in breeding population evaluation is practical.

Brown and Tanner (1983b) determined that the π of sap expressed from frozen alfalfa tissue was lower than that determined using the PV technique. They felt that the freeze-thaw method was less reliable because of starch and sucrose hydrolysis during the thawing process. However, the PV technique has its own problems. For example, Campbell et al. (1979) showed that the apoplastic water fraction, which averaged 30% for wheat, influenced osmotic measurements determined by the PV method. This is supported by Cortes and Sinclair (1985) who also concluded that some apoplastic water diluted the symplast in soybean plants, resulting in anomalously high osmotic fractions, which in some cases, exceeded 100%.

2.6 Alfalfa Water Use and Efficiency of Dry Matter Production

2.6.1 Water Use in Alfalfa

Alfalfa has long been acknowledged as being an extravagant user of water as compared with other crops (Christian, 1977; Sheaffer et al., 1988). Its need for large amounts of water arises from its long growing season (Sheaffer et al., 1988) and its consumption of advected sensible heat (Blad and Rosenberg, 1976; Williams and Stout, 1981).

Carter and Sheaffer (1983a) reported from a study in Minnesota, that water use by well-watered alfalfa ranged from 5.3 to 10.0 mm d^{-1} during July, August and early September, but fell to 2.4 mm d^{-1} for late September and October as plant growth was slowing for fall dormancy. In a study in western Nebraska, averaged over three years, Daigger et al. (1970) found that daily water use increased from May to early August, then declined, and was 4.2, 5.5 and 5.9 mm d^{-1} for the first, second and third cuts, respectively. Temple and Benoit (1988) determined that seasonal water use in California averaged 8.9 mm d^{-1} for normally irrigated plots (watered at 50% available soil water (ASW)) and 6.1 mm d^{-1} for drought-stressed plots (watered at 75% ASW), while pan evaporation (Epan) averaged 5.9 mm d^{-1}. In a diurnal study conducted at the University of Minnesota, Sharratt et al. (1983) showed that evapotranspiration (ET) for irrigated and non-irrigated alfalfa was similar at sunrise and reached a maximum difference of 0.2 mm h^{-1} at 1500 h. Maximum ET for the irrigated and non-irrigated treatments was 0.78 and 0.58 mm h^{-1}, respectively.

Hattendorf et al. (1990) found that the daily water use of a non-dormant alfalfa cultivar ('CUF 101') was 5.2 mm d^{-1} over the first 10 days after harvest, compared with two dormant cultivars, which averaged 3.9 and 3.1 mm d^{-1}, however, seasonal water use values were similar. They attributed the initially greater water use rate for 'CUF 101' to faster regrowth. Cole et al. (1970) found significant differences in water use between alfalfa cultivars at both the seedling and mature plant stages. They also noted that greater variation existed within cultivars than among cultivars. McElgunn and Heinrichs (1975) attributed slower growth rates to the lower water use of *Medicago falcata* L. genotypes, as compared to genotypes of *M. media* Pers. or *M. sativa* L.

2.6.2 Soil Water Depletion Patterns for Alfalfa

The deep taprooting characteristic of alfalfa gives it the ability to extract water deep within the profile. Brun and Worcester (1975) concluded that established alfalfa, grown in soils of various textures, significantly extracted water to a depth of 3 to 4 m. In addition, they found that alfalfa extracted water held at tensions greater than -1.5 MPa. Kohl and Kolar (1976) found that established alfalfa obtained about 80% of its water supply from the first metre of soil, with the remaining being obtained from the 1 to 2.3 m profile. They also reported that water was removed in the lower portion of the profile at a soil matric potential of -0.7 to -1.0 MPa, while moisture was more available in the upper profile (-0.2 MPa). These results contrast those of Cohen and Strickling (1968) who concluded that alfalfa plants extracted little, if any water, below 0.7 m.

2.6.3 Evapotranspiration Efficiency in Alfalfa

Alfalfa evapotranspiration efficiency (ETE) is defined as the biomass yield per unit area per unit of ET (Sheaffer et al., 1988). Under dry, sub-humid conditions, Bauder et al. (1978) determined that alfalfa dry matter yield (kg ha^{-1}) is a linear function of ET for values of ET between 150 and 750 mm. Calculating from their regression equation, it was determined that 115 mm of water are required to produce 1000 kg ha^{-1}. This supports earlier work by Daigger et al. (1970) who reported alfalfa water use at 114 mm per metric ton or about 8.7 kg ha^{-1} mm^{-1}. However, Bauder et al. (1978) reported much higher ETE figures with a range of 12.1 to 23.1 kg ha^{-1} mm^{-1} under four irrigation treatments over four years, while Wright (1988) reported ETE values of 17.2 kg ha^{-1} mm^{-1} for irrigated alfalfa. Similarly, work by Carter and Sheaffer (1983a) showed ETE values ranging from 9.7 to 30.1 kg ha^{-1} mm^{-1} over the third and fourth growth cycles. The low

ETE values in their study were from unirrigated treatments, while the irrigated treatments showed greater ETE. Bauder et al. (1978) also concluded that irrigated plots generally had higher ETE, and Daigger et al. (1970) reported lower ETE under less favorable growing conditions.

Despite alfalfa's reputation for high water use, Cohen and Strickling (1968) concluded that alfalfa was as evapotranspiration efficient as tall fescue grass (*Festuca arundinacea* Schreb.). Bolger and Matches (1990) found alfalfa to be more evapotranspiration efficient that sainfoin (*Onobrychis vicaefolia* Scop.), while Fairborn (1982) previously demonstrated that, although some differences in ETE were apparent between alfalfa cultivars, they were generally not significantly different from cicer milkvetch (*Astragalus cicer* L.), alsike clover (*Trifolium hybridum*) or sainfoin. McElgunn and Heinrichs (1975) found no differences in ETE in their evaluation of several diverse alfalfa cultivars.

2.7 Conclusions

Drought stress is the most prevalent form of environmental stress to plants (Blum, 1984). Ash et al. (1992) confirmed that alfalfa crops are generally subject to water limitations in the eastern prairie region of Western Canada (southern Manitoba and south-eastern quarter of Saskatchewan).

Although some forage quality factors may increase under drought stress, the overall impact of drought stress is negative for biomass yield. A better understanding of the physiological or morphological responses to drought stress is an important first step in improving alfalfa cultivars for water-limiting environments.

To develop an understanding of plant water relations, one must appreciate the complexity of the processes involved, including various environmental and genetic factors. Several techniques exist to help physiologists describe plant water relations. However, few of these methods reliably discern differences within a species. Final conclusions must be based on the overall analysis of several water relations parameters, their interaction with the environment, and the efficiency and effectiveness of the plant to extract and utilize water for the production of dry matter.

3.0 Materials and Methods

3.1 Field Experiments

3.1.1 General

Field experiments were conducted at the University of Manitoba, Winnipeg Field Research Lab, 'Point' location (49.9°N, 97.2°W) on a Riverdale silty loam soil. The soil has been previously characterized by Mohr (1996) as being composed of 13% sand, 45% silt and 42% clay and having an EC of 0.30 dS m^{-1} and a pH of 7.4. In 1991, plots were seeded on an area of land previously cropped to space-planted ornamental flowers, while in 1992, the plots were seeded on an area of land previously cropped to solid-seeded Triticale. Preparation of the soil included fall and spring tillage operations and spring harrowing (twice) to smooth and pack the seedbed.

The experimental design was a randomized complete block with four replications and six alfalfa cultivars as treatments. Plot size was 2 x 6 m in 1991 and 3 x 6 m in 1992. 'Excalibur' alfalfa was seeded at both ends of each block to reduce border effects. In addition, a fallow plot was randomly located at one end of each block to act as a control for soil water extraction analysis.

A diverse range of alfalfa cultivars was used in this study with fall dormancy (FD) ratings ranging from 1 to 8 (also in Appendix B). This group of cultivars included: 'Alfagraze' (FD=2), 'Excalibur' (FD=4), 'Legend' (FD=4), 'Nitro' (FD=8), 'Rangelander' (FD=1), and 'Wilson' (FD=6). The FD rating scale ranges from 1 to 9, where a "1" indicates a fall dormant cultivar that would produce minimal fall growth in Manitoba after early September and a "9" indicates a non-dormant cultivar that continues to grow in the fall until a killing frost.

'Alfagraze' (*M. sativa* L.) was selected in Georgia for persistence under grazing and is not only suitable for grazing, but also for hay and silage production (Bouton et al., 1991). 'Excalibur' (*M. sativa* L.) is best suited for hay production. A multi-foliate leaf is a distinctive trait for 'Legend' alfalfa (*M. sativa* L.). Non-dormant fall growth, root mass and root nitrogen concentration were selection criteria used in developing 'Nitro' (*M. sativa* L.) for use as a special 1-year hay source and plow-down green manure crop (Barnes et al., 1988b). 'Rangelander' alfalfa (*M. media* Pers.), which was developed at Swift Current, Saskatchewan, was selected for persistence, has a creeping-root system and is suited for dryland production (Heinrichs et al., 1979). 'Wilson' alfalfa (*M. sativa* L.) was developed for improved performance under deficit levels of irrigation and its intended use is for hay production in New Mexico (Anonymous, 1987b).

Plots were hand seeded on 17 May 1991 and on 14 May 1992, at a rate of 350 viable seeds m^{-2}. The procedure involved mixing the seed with about 2 litres of sand, blocking the plot into smaller quadrants, and sprinkling the mixture uniformly over the plot. The sand was slightly damp to help maintain a uniform mixture and had been previously run through a 6 mm sieve to remove stones and other foreign material. The seed was inoculated prior to seeding with 'Dormal' in 1991 and 'Nugold' in 1992; both were clay based inoculum. After seeding, the plots were hand-raked to incorporate the seed and to remove the larger soil clods. The soil surface was then firmed using a 90 cm steel packer. Final seed placement was generally 10-15 mm below the soil surface, although some seeds remained at the surface or between the surface and the 10 mm depth.

To ensure uniform and rapid germination of the seed, irrigation was initiated on 25 May in 1991 and on 21 May in 1992. The sprinkler system applied approximately 7 mm h^{-1} and was run for 0.5 to 2 h at a time, usually at daily intervals. Containers to measure water application were randomly placed in the plot area. Total irrigation was 39.5 mm in 1991 and 36.8 mm in 1992.

Stand counts were taken on a 0.25 m^2 area, in both the front and back halves of the plot, to show uniformity in establishment of the cultivars. Counts were made on 29 June and 7 August, on the plots established in 1991, and on 12 August, on the plots established in 1992. Counts prior to the first harvest were not completed in 1992. Mean results of the four replications are listed in Table 3.1. Plant population densities on 29 June 1991 were non-significant, indicating an equivalent plant density. Plant population densities after the first harvest in both years indicated differences in plant stands. However, this may not be a concern. Tesar and Marble (1988) suggest that approximately 150 to 250 plants' m^2 are necessary to obtain maximum yields in the year after seeding. Mean results show that densities of all cultivars fell within this range.

Table 3.1 Mean alfalfa plant counts per m^2†.

	Field Experiment		
	One		Two
Cultivar	29 June 1991	7 Aug. 1991	12 Aug. 1992
Alfagraze	266	232 a	188 ab
Excalibur	271	219 ab	166 bc
Legend	231	204 abc	196 a
Nitro	283	196 bc	157 c
Rangelander	244	216 ab	198 a
Wilson	174	178 c	175 abc
LSD	70	30	28

† - Non-destructive counts. Some plant coalescence probably occurred.
Means within a column followed by the same letter are not significantly different at alpha=0.05 (LSD).

Aluminum access tubes were installed in the center of each plot on 22 May 1991 and on 15 May 1992. The tubes were 5 cm in diameter, 150 cm long in 1991 and 240 cm long in 1992. In addition, 240 cm tubes were installed on 10 October 1991 in the center of the back half of the plot to allow measurements deeper in the soil profile. A trailermounted auger was used to excavate the soil for installation of the tubes. Application of dishwashing liquid to the exterior of the tube facilitated penetration into the clay soil.

A summerfallow plot was established randomly at one end of each block to act as a control plot for water extraction comparisons. Plot dimensions of the summerfallow plots were 4 x 6 m in 1991 and 6 x 6 m in 1992. Similarly, aluminum access tubes were installed in the center of the plot. The surface was kept free of vegetation by hand weeding and chemical application of glyphosate ('Roundup'; Monsanto Canada Inc.) at a rate of 356 g ai ha^{-1}.

Weeds were controlled in the plot area with one application of imazethapyr ('Pursuit', American Cyanamid Company) at a rate of 50 g ai ha^{-1}, on 11 June in 1991 and on 19 June in 1992. 'Assist' (Hoechst Canada Inc., Regina, Saskatchewan) was added at a rate of 2.5% v/v, and applied together in 113 l ha^{-1} of water. Most plants had at least four leaves at time of spraying.

Daily mean, minimum, and maximum air temperatures, and the times of their occurrence, mean soil temperature, precipitation and solar radiation were recorded using a CR10 data recorder (Campbell Scientific Inc., Logan, Utah), which was located within 100 m from the plot area. Daily pan evaporation and wind run were recorded by Environment Canada at the Winnipeg International Airport (49.9°N, 97.2°W). Mean weekly values are presented in Figures 4.1 through 4.6, while daily environmental data during water relations measurements are documented in Table 4.10.

3.1.2 Forage Yield

Square metre samples for yield were removed from the back half of the plots on an area that had been spared from water relations sampling. Yield samples were obtained on 31 July and 8 October 1991 and on 11 August and 13 October 1992. All plots were essentially at full flower at the time of first cut, but were at various vegetative stages on the final harvest dates. The samples were placed in paper bags and dried in a forced air oven at 65°C for at least 48 h. The remainder of the stand was cut to an approximate height of 5 cm using a small plot forage harvester.

3.1.3 Soil Water Measurements

Soil water in the top 10 cm was measured gravimetrically. A bulb planter (approximately 7 cm in diameter) was used to obtain two soil samples from each plot on the dates listed in Table 3.2. Samples were dried for 48 h at 65°C to 75°C and soil water content was calculated as a percentage of dry soil mass. Bulk density samples were collected for each replication periodically throughout the season to adjust soil water data to the volumetric form. Mean bulk densities ranged from 0.97 to 1.16 g cm^{-3} over both years. Bulk density samples were obtained by tapping a sharpened 10 × 5 cm piece of aluminum tubing into the soil until the top of the tube was flush with the soil surface. After the sampling tube was carefully dug out of the soil, the soil was removed and placed in a plastic bag. The soil was oven dried for 48 h and the corresponding bulk density was calculated. Gravimetric soil moisture was converted to volumetric form by multiplying the gravimetric value by the bulk density.

Volumetric soil water content between 10 and 110 cm in 1991 and between 10 and 205 cm in 1992 was determined using a neutron soil moisture probe (Troxler Laboratories Model 3330; Triangle Park, North Carolina). Measurements were conducted at 20 cm intervals beginning at 20 cm below the soil surface. Sampling dates were the same as those for gravimetric samples and are listed in Table 3.2.

Table 3.2 Sampling dates for soil water measurements.

1991	1992
6 June	15 June
17 June	7 July
28 June	23 July
8 July	12 August
20 July	26 August
7 August	10 September
21 August	22 September
13 September	13 October
10 October	

3.1.4 Water Use and Water Use Efficiency

Water use (mm) in each plot was determined for each harvest by summing the differences in soil water content at each depth interval, using measurements taken at the beginning and end of each growth cycle. Total irrigation, if any, and rainfall during this period were also added to this amount. Loss of water due to runoff and deep drainage were assumed to be negligible. Evapotranspiration efficiency was calculated from the above ground dry matter production divided by the water used during that growth cycle and was expressed as kg ha^{-1} mm^{-1}. Effective rooting depth was determined as the deepest level at which soil moisture was significantly lower in the alfalfa plots versus the summerfallow plots (Entz and Fowler, 1988).

3.1.5 Physiological Parameters

3.1.5.1 General

Sampling dates for the physiological parameters in the field study are listed in Table 3.3. Methodology will be individually discussed for each parameter in the following sections.

Table 3.3 Sampling dates and times for field study measurements.

		Parameter / Sampling Time (h)		
Year	Date	Ψw, RWC, π, P	T_c	g_l
1991	22 July	1030-1530[w]		
	23 July	1030-1500[x]	1550-1610	
	25 July		1615-1640	950-1550
	30 July	1000-1540	1550-1610	
	20 August	1000-1610	1620-1635	
	29 August	1030-1630	1450-1505	
1992	23 July		1515-1545	
	29 July		1420-1435	
	30 July	1100-1600	1625-1645	
	4 August			1330-1650
	7 August		1630-1645	
	8 August		1600-1615	
	9 August		1615-1630[y]	1430-1640
	10 August	1040-1600	1400-1420	
	4 September	1400-1600[z]		
	24 September	1430-1700	1715-1730	
	30 September		1255-1310	
	1 October		1450-1500	

[w] - only blocks 1 and 2 were completed.
[x] - only blocks 3 and 4 were completed.
[y] - for blocks 1, 2, and 3; block 4: 1705-1710.
[z] - Ψw was not measured; P could not be calculated.

3.1.5.2 Water Potential

A Scholander-type pressure chamber (PMS Instrument Company Model 1002; Corvallis, Oregon) was used for Ψw determinations (Turner, 1988). Alfalfa stems approximately 10 cm in length (Nicolodi et al., 1988) were randomly selected from the front half of the plot area. The stem was enclosed in a plastic sheath (Turner and Long, 1980) and excised from the plant using a scalpel. A spring-type paper clip was used to secure the top of the plastic sheath around the stem with about 2 cm of the stem protruding out of the sheath. The cut end of the stem was inserted through a rubber diaphragm using the insertion tool supplied by the manufacturer so that the length of stem protruding out of the chamber was typically less than 0.5 cm (Millar and Hansen, 1975). Putty was placed around smaller stems to prevent gas leakage around the stem. The rubber diaphragm and plant material were placed in the chamber top, which was subsequently secured to the pressure chamber. The chamber was pressurized at a rate less than 0.01 MPa sec^{-1}, with a slightly slower pressurization near the endpoint. This was similar to Brown and Tanner (1981), who used a rate of about 0.006 MPa sec^{-1} with alfalfa. Duration from leaf excision until a balancing pressure was obtained ranged from approximately 2 to 6 minutes. Four stems from each plot were sampled on each date. In 1992, the top of the chamber was modified to accommodate two alfalfa stems to reduce the sampling time.

3.1.5.3 Relative Water Content and Turgid Weight:Dry Weight Ratio

The youngest, fully expanded leaf from three randomly selected plants within each plot was used for RWC determination. Each leaf was placed in a separate hermetically sealed vial (Turner, 1981) and placed in an insulated container to maintain temperature near ambient conditions. Initial mass for each vial and leaf was recorded to the nearest milligram within 2 to 7 h. The leaves were removed from the vials, placed in test-tubes containing distilled water, and set in the dark at room temperature. After about 20 hours, the leaves were removed, surface-dried, and returned to their original vials for re-weighing. The leaves were dried for 48 h at 65°C to obtain dry weights. RWC was calculated using the equation described in 2.5.2. The mean of the three values was used for statistical analysis. The TW:DW ratio was calculated from values generated in obtaining RWC.

3.1.5.4 Osmotic Potential and Turgor potential

Leaves from the top 10 cm of two randomly selected alfalfa stems were removed and placed into disposable 5 ml syringes. Syringes were quickly sealed and packed in ice. Within 2 hours, samples were moved to a freezer at -20°C. At a later date, osmotic potential was determined using a vapor pressure osmometer (Wescor Model 5500XR; Logan, Utah). The osmometer was calibrated using recommended salt solutions prior to measurement of each sampling date and was verified after measurement of each replication. The frozen samples were thawed for approximately 30 minutes. After thawing, similar force was used to express the sap from the syringes. A filter paper disk was dipped into the sap, blotting any excess liquid, to ensure a similar sample size of approximately 10µl. Osmotic potential was adjusted to full turgor by multiplying the π by the RWC. Turgor potential was calculated as the difference between Ψw and π as described in section 2.5.7.

Root osmotic potential at full turgor OP_{r100} was measured in the 1992 field experiment. Tap-root samples were obtained from four randomly selected plants. A section of each tap-root, 2-5 cm below the crown, was removed and placed in a test-tube containing distilled water for a period of 24 h. Preliminary analysis had indicated that 24 hours was sufficient to obtain full turgor (Appendix C). After rehydration, two sections of root from each plot were inserted into a 5 ml disposable syringe, sealed and placed in a freezer at -20°C. OP_{r100} was determined using the vapor pressure osmometer as described above.

3.1.5.5 Canopy Temperature

Canopy temperatures were measured on field plots using an Everest Interscience Model 112 hand-held infrared thermometer (IRT). Sampling dates and times are listed in Table 3.3. The IRT was held at about 1.2 m above the ground and about 2.75 m from the target area, giving a measurement angle of 15° above the canopy. Given the distances and a field of view of 4° for the IRT, the area assessed with each measurement would be approximately 0.13 m^2 (O'Toole and Real, 1984). Three measurements per plot were taken, facing westerly in 1991 and easterly in 1992. The mean value of the three measurements was used for statistical analysis.

3.1.5.6 Stomatal Conductance and Leaf Temperature

Stomatal conductance and leaf temperature were measured using a steady-state porometer (Li-Cor Inc. Model LI-1600; Lincoln, Nebraska). Average cuvette humidity was set to the ambient canopy level for each replicate. Measurements included: leaf and cuvette temperatures, relative humidity, irradiance, and conductance. Transpiration was also measured in the 1992 field trial. Four measurements were made per plot. Mean values per plot were used for statistical analysis.

3.1.6 Statistical Analysis

All data were subjected to analysis of variance (ANOVA) using the Proc GLM procedure (Statistical Analysis Systems Institute, 1986). In cases where the data indicated significant cultivar difference, a Fisher Protected least significant difference (LSD) test was performed for means separation. Significant differences were assumed at the $P \leq 0.05$ level unless otherwise indicated. Before data were combined, Chi-square analysis was conducted to verify that the error variances were homogeneous (Steel and Torrie, 1980).

Orthoganal contrast analysis was used to test for differences in soil water content between the summerfallow and cropped plots.

3.2 Controlled Water Experiments

3.2.1 General

In 1991, controlled water experiments were conducted outdoors at Winnipeg under a "rainout" shelter (experiment one), while in 1992, two experiments were conducted under greenhouse conditions at the University of Manitoba campus (experiments two and three). The experimental design was a 2 × 6 factorial in experiments one and three (2 × 3 factorial in experiment two), with water treatment as the main plot and cultivar as the subplot factor. The water treatments and their management are described in 3.2.2. In experiments one and three, six alfalfa cultivars: 'Alfagraze', 'Excalibur', 'Nitro', 'Rangelander', 'South African' and 'Wilson' were used, while in experiment two, only three of the cultivars were included ('Excalibur', 'Nitro' and 'Rangelander'). 'South African' (*M. sativa* L.), which was not previously described in section 3.1.1, was labeled as such because it was a drought tolerant selection from South Africa and did not have a cultivar name (S.R. Smith, pers. comm.) Although 'South African' has not been tested for fall dormancy, it is believed to be near the upper end of the scale (FD=7-8). The tests were replicated three times in experiments one and three and four times in experiment two.

Deep containers were used in this study to more closely replicate a natural soil profile, while also providing a larger soil volume to permit a slower onset of drought stress (Pennypacker et al., 1990). The treatments were grown in 55 cm deep containers, which were constructed using 20 cm diameter PVC pipe. The bottom end of the pipe was covered with a commercial PVC end cap and fastened in place with two screws. The cap was sealed onto the pipe using a silicone sealant to prevent water leakage. In the 1992 experiments, a 25 mm diameter PVC pipe was placed in the middle of the container to facilitate a more uniform moisture distribution when small amounts of water were added at low soil moisture levels. The 75 cm long pipe was drilled with 2 mm (approximately) holes between the 5 and 45 cm heights. The bottom of the pipe was plugged and two layers of cotton broadcloth were attached to the outside of the pipe to act as a wick and also to prevent soil erosion around the pipe when water was added.

An Almissipi very fine sandy loam soil, which had been collected near Elm Creek, Manitoba, was passed through a 6 mm screen to remove clods and other foreign material. Soil was added to the containers to a depth of about 50 cm. The containers were dropped lightly to compress the soil in an effort to maintain similar bulk densities between containers. Soil bulk densities averaged 1.28, 1.36 and 1.30 and ranged from 1.26 to 1.30, 1.34 to 1.39, and 1.29 to 1.32 g cm^3 for experiments one, two and three, respectively.

Alfalfa seeds were inoculated and pre-germinated in petri-dishes. When the seeds had germinated to a root length of about 1 cm, they were planted in the containers at a depth of 0.5 to 1 cm. Twelve, equally spaced seedlings were planted in each container at a radius of 7 cm. Plants that did not survive transplanting were replaced within the first two weeks of the experiment.

In experiment one, the containers were placed in a growth chamber under a 18°C day, 14°C night and 16 hour photoperiod regime from planting date, 26 May 1991, until 11 June 1991, when they were moved to a greenhouse to increase the rate of establishment. Irradiance ranged from about 200-300 $\mu E\ m^{-2}\ sec^{-1}$ in both environments, although temperatures were generally warmer in the greenhouse. On 27 June, the containers were moved outdoors under the "rain-out" shelter similar to the one described by Poppe (1991). The shelter was approximately 2 m in height and was covered with a heavy clear plastic. Side panels of the shelter were generally rolled up unless rain was imminent. Under the shelter, irradiance was reduced by approximately 300 μE m^{-2} sec^{-1} as compared with direct sunlight (i.e., intensity on a sunny day was 1600 μE m^{-2} sec^{-1}, while direct sunlight produced 1900 μE m^{-2} sec^{-1}). On 16 July, the containers were placed in holes dug into the ground, so that the soil within the container was flush with the surrounding soil surface. Fiberglass insulation was placed around the upper 15 cm of the container to keep the soil temperature within the container near the ambient temperature of the surrounding soil. In 1992, the plants were grown under greenhouse conditions for the duration of each experiment. Seedlings were planted on 31 January 1992 for experiment two and on 9 June 1992, for experiment three. In experiment three, the greenhouse roof was coated with a "whitewash" to help control internal temperatures. The "whitewash" reduced irradiance to about 300-350 μE m^{-2} sec^{-1}.

3.2.2 Water Treatments

Soil moisture was increased to field capacity at the beginning of each experiment. A volumetric field capacity of 34.0 to 37.4% (depending on the corresponding bulk density of each experiment) after 24 hours was determined. To accomplish this, tubes approximately 4 cm (diamter) by 16 cm (length) were filled about two-thirds full with soil to a bulk density similar to the containers (Dr. C. F. Shaykewich, pers. comm.). The tube was then filled with water. A porous bottom on the tube allowed excess water to flow through the soil. After 24 hours, the soil was removed from the tube, weighed, oven dried, and then reweighed. The graimetric water content was calculated from the weights and converted to the volumetric form by multiplying by the bulk density of the sample.

Soil fertility was adjusted according to soil test recommendations using K_2HPO_4, K_2SO_4 and $ZnSO_4$ on 14 June 1991 and at the beginning of each experiment in 1992, as advised by Dr. K. Vessey (pers. comm.).

Soil moisture was monitored by weighing the containers at various intervals during the growth cycle. A Toledo hanging spring-type scale (capacity 45 kg, 200 g increments) was used in 1991 and a platform-type digital scale (readout to the nearest gram) was used in 1992. A rope and pulley system was used to lift the containers out of the ground in 1991, while in subsequent experiments, special hooks were constructed to manually lift the containers.

During Phase 0, which was the plant establishment period, soil moisture was generally maintained above 70% of field capacity in both treatments. Water was added directly to the soil surface in 1991, but was mostly added through the 25 mm pipes (as described above) in 1992. Phase I of the experiment began when the two water treatments were treated differently and was initiated on 24 July 1991; 25 March 1992 and 11 August 1992. In Phase I, soil moisture was increased to field capacity in the well-watered treatment 44 hours in advance of water relations measurements in 1991. Water was essentially withheld from the droughted treatment (only 1 L added) until 44 hours prior to the 8 August sampling date. At this time, soil moisture content was increased to 40% of field capacity in the droughted treatment. On 13 August, soil moisture was increased to 30% of field capacity (about 44 hours prior to the final sampling in Phase I, 1991). In experiment two, soil moisture was increased to field capacity about 20 hours prior to water relations measurements in the well-watered treatment, while soil moisture content was increased at the same time to 32, 30, 25, and 20% of field capacity prior to the 8, 16, 23 and 30 April sampling dates, respectively. The same approach was used in experiment three, although the soil moisture contents were standardized at 30, 21 and 21% of field capacity prior to the 20 August, 3 September and 10 September sampling dates, respectively. At the end of Phase I, soil moisture contents were increased to field capacity. Phase II of the experiments began on 17 August 1991; 1 May 1992; and 11 September 1992 when soil moisture contents in both treatments were increased to field capacity. Following this, water was withheld from both treatments in 1991, while in experiment two and three, drought was gradually and equally imposed in all treatments. This was accomplished by adding water about every two days to containers with lower soil moisture to equal that of the container with the highest soil moisture content. In experiment two, soil moisture contents were equilibrated to 73, 30, 23 and 20% of field capacity about 20 hours prior to water relations measurements on 7, 9, 25 and 28 May, respectively, while in experiment three, soil moisture contents were standardized to 30, 23 and 22% of field capacity prior to the 30 September, 9 October and 14 October sampling dates, respectively.

At the end of Phase II in experiment two, remaining volumetric soil moisture content was 5.7%, which was similar to results of a separate permanent wilting point determination of 5.8%. Permanent wilting point was determined by taking daily growth measurements of alfalfa grown in containers. When growth had ceased, the soil was removed from the containers, weighed, and then dried in an oven at 65°C.

Total ET was calculated as the summation of water added plus the water that would be required to restore the soil to field capacity at the conclusion of each experiment. Total usage in litres was converted to millimetres to make the units comparable with field results. The daily water use during each phase was calculated as the water used in each phase divided by the duration of each phase. Water use efficiencies were calculated by dividing the shoot dry matter production (for Shoot ETE) or the sum of both shoot and root dry matter production (for Whole Plant ETE) by the total ET.

3.2.3 Shoot and Root Dry Matter Production

At the end of each experiment the alfalfa plants were clipped approximately 0.5 cm from the soil surface, placed in a paper bag, and dried in an oven at 65°C for 48 hours. Senesced leaves, which still remained in the container, were also included. Following drying, the dry matter was weighed to the nearest 10 mg. The soil was carefully removed from the containers so that the roots would remain intact. Using a misting action with a garden nozzle, the soil was washed away from the roots. Root production was determined by drying and weighing the material as described above for the shoots. The R:S ratio was calculated as the proportion of root production as compared with shoot production.

3.2.4 Water Relations Measurements

Water potential, RWC, π_{100}, *P*, TW:DW, π_{T100}, and conductance were measured or calculated as described in previous sections, 3.1.5.2 to 3.1.5.6. Although the procedures were the same, the number of samples per treatment was sometimes different. For example, only two samples for Ψw and π were taken from each container on each sampling date to conserve plant material. Water relations measurements were taken between 1000 and 1600 hours on each sampling date.

3.2.5 Water Loss from Detached Leaves

Tests to measure water loss in detached alfalfa leaves were conducted on 25 August, 1 September and 11 September 1992 (Phase I, experiment three). Four recently expanded leaves were cut from each treatment on the previous evening and placed in test-tubes containing distilled water. The test-tubes were placed in the dark at room temperature to allow the leaves to hydrate to full turgor. On the following morning, the leaves were removed from the test-tubes, blotted dry, placed in pre-weighed petri dishes and re-weighed. The petri dishes containing the leaves were placed on the laboratory counter at room temperature (approximately 25°C) under normal indoor lighting. After 1, 2, 4, 6 and 8 hours the petri dishes were re-weighed. The leaves were then dried in an oven at 65°C for 24 hours. The total water content (as determined from turgid and dry weights) and the fraction of water loss at each interval were calculated. The mean water loss fraction for each treatment was used for statistical analysis.

3.2.6 Pressure-Volume Curves

After several attempts to refine a pressure-volume technique for alfalfa, a workable methodology was developed. This procedure is described below.

A segment of alfalfa stem, approximately 10 cm in length, was cut under water from the upper canopy of droughted 'Nitro' and 'Rangelander' treatments in experiment two. The end of the stem segment was placed in a test tube containing distilled water at room temperature for about 12 hours under dark conditions to allow the plant material to reach full turgor. Just prior to beginning the pressure-volume measurements, the stem was removed from the test tubes, blotted dry, and then placed into a special rubber diaphragm as described in section 3.1.5.2. An initial weight was taken after the material was ready and then placed into the pressure chamber. The pressure chamber was over-pressurized to 0.4 MPa and any sap expressed out of the stem was absorbed with a tissue. At times, minor leakage necessitated the addition of gas to the chamber to maintain the pressure. After it was deemed that sap exudation had ceased, the material was removed and reweighed. This process was repeated at pressures of 0.8, 1.2, 1.6, 2.0, 2.4 and 2.8 MPa. The whole procedure took about 6 to 10 hours to complete. Following the final weighing, the plant material was placed in an oven at 65°C for 48 hours to obtain a dry weight. The stopper, putty and plastic sheath were also weighed. From these measurements, the fraction of sap expressed at each balancing pressure could be calculated. The first two replications were measured on 26 May 1992 and the remaining two replications were measured on 27 May 1992. The third replication of 'Rangelander' was not used in the statistical analysis because the data appeared to be incorrect. It was likely that mechanical forces crushed the stem when turgor pressure was lost, which left subsequent sap loss measurements erroneously low. Osmotic potential at full turgor and the portion of apoplastic water were calculated for each cultivar (Turner, 1988). The point of incipient plasmolysis was estimated and π at zero turgor was also calculated.

Bulk modulus of elasticity was calculated using the equation from Melkonian et al. (1982), which is given by the equation:

$$\varepsilon = V_w (\Delta P / \Delta V)$$

where V_W is the volume of water in the leaf at full turgor, ΔV is the volume expressed between balancing pressures and ΔP is the difference between balancing pressures. Bulk modulus of elasticity was calculated for pressure ranges 0 to 0.4 MPa and for 0 to 0.8 MPa. Values for both were included in the statistical analysis.

3.2.7 Statistical Analysis

All data were subjected to analysis of variance (ANOVA) using the Proc GLM procedure (Statistical Analysis Systems Institute, 1986). In cases where the data indicated significant cultivar differences, a Fisher Protected least significant difference (LSD) test was performed for means separation. Significant differences were assumed at the $P \leq 0.05$ level unless otherwise indicated in the discussion. Before data were combined, Chi-square analysis was conducted to verify that the error variances were homogeneous (Steel and Torrie, 1980). Linear and quadratic regression analyses for relationships between water relations variables were conducted on the entire dataset from all three tests using Proc GLM (Statistical Analysis Systems Institute, 1986). Linear regression analysis was conducted on the last four measurements of the pressure-volume procedure, which was visually determined to be the linear portion of the curve.

4.0 Results and Discussion

4.1 Field Studies

4.1.1 Introduction

A field experiment was conducted to evaluate differences in productivity (herbage yield), water use (evapotranspiration; ET), and plant water relations among six alfalfa cultivars in the establishment year. Field experiments were used to provide a representation of this crop under typical growing conditions. This allowed the examination of cultivar response to soil water conditions in a deep soil profile, which permitted a slow onset of plant stress. To determine physiological responses to soil water depletion, several parameters such as Ψw, RWC, π, T_c, T_l, g_l were measured and π_{100}, OA and P were calculated from the data.

4.1.2 Aerial Dry Matter Production

Two harvests were taken from the field plots each year. In 1991, significant cultivar differences ($P<0.05$) were observed for the second and combined total harvests, although differences were also significant for the first harvest at $P=0.0653$ (Table 4.1). In 1992, significant cultivar differences were observed on both harvest dates, as well as for the seasonal total. Combined year analysis is not presented because of a significant year × cultivar effect (data not shown).

Yields tended to be higher in 1991 than in 1992, probably because of higher (more optimal) temperatures during the growing season. In 1992, mean weekly air and soil temperatures, as well as solar radiation, were lower throughout most of the season compared with 1991 (Figures 4.1, 4.2 and 4.3) (1992 was one of the coolest seasons on record). In 1992, total yields of 'Nitro', 'Rangelander' and 'Wilson' were lower than the other three cultivars. 'Nitro' and 'Wilson' also had the lowest first-cut yields. These cultivars, which are non-dormant and semi-dormant, respectively, may have been more affected by the lower (less optimal) temperatures in 1992, especially in the first few weeks of establishment. 'Wilson', which was selected under much warmer conditions in New Mexico, does not seem to perform well in our climate (S.R. Smith, pers. comm.). A differential temperature response for stem and leaf weights (i.e., yield) among diverse alfalfa cultivars has been previously documented (Bula, 1972). 'Alfagraze' and 'Legend' generally had the highest combined total yields over both years.

Table 4.1 Aerial dry matter production (Mg ha^{-1}) of field grown alfalfa at Winnipeg.

Year	Cultivar	1st cut†	2nd cut‡	Total
1991	Alfagraze	5.19 a	2.92 bc	8.11 ab
	Excalibur	4.95 ab	2.89 bc	7.83 bc
	Legend	5.29 a	3.29 a	8.58 a
	Nitro	4.75 ab	3.15 ab	7.90 abc
	Rangelander	4.98 ab	2.65 c	7.63 bc
	Wilson	4.42 b	2.98 b	7.40 c
	LSD (0.05)	0.58	0.29	0.69
1992	Alfagraze	4.74 a	2.87 ab	7.61 a
	Excalibur	4.33 ab	3.19 a	7.52 a
	Legend	4.53 ab	3.03 ab	7.56 a
	Nitro	3.47 c	2.78 bc	6.24 b
	Rangelander	4.25 b	1.80 d	6.05 b
	Wilson	3.34 c	2.46 c	5.80 b
	LSD (0.05)	0.47	0.35	0.59
1991	Mean	4.93 a	2.98 a	7.91 a
1992	Mean	4.11 b	2.69 b	6.80 b
	Overall Mean	4.52	2.83	7.35

† - 1st cut dates were 31 July and 11 August in 1991 and 1992, respectively.
‡ - 2nd cut dates were 8 October and 13 October in 1991 and 1992, respectively.
Means within a continuate column followed by the same letter are not significantly different at alpha=0.05 (LSD).

The significant cultivar × environment (i.e., year) interaction for the second cut and combined harvests, which arose from different cultivar rankings for some cultivars, may be related to the cultivar fall dormancy (FD) ratings, as conditions in 1992 were much cooler during the regrowth period after the first harvest. 'Nitro' is a non-dormant cultivar (FD = 8), 'Wilson' is semi-dormant (FD = 6), while the FD for the other cultivars ranges from 2 - 4 (Certified Alfalfa Seed Council, 1992). Also, 'Wilson' was selected at the New Mexico State University under warm, dry conditions and therefore may not be ideally suited for a cooler temperature regime. 'Rangelander' is a highly fall dormant cultivar (FD = 1 ; S.R. Smith, pers. comm.), therefore, regrowth was slow after the first cut with cooler fall temperatures and shorter daylength. As a result, 'Rangelander' produced the lowest second-cut yields in both years. Comparing the two grazing tolerant cultivars, 'Alfagraze' showed significantly more late-season regrowth than 'Rangelander' (Table 4.2). The data reveals how dramatically regrowth was reduced for 'Rangelander' as compared with the other cultivars in 1992.

Table 4.2 Plant heights of field-grown alfalfa at final harvest date (13 October 1992) at Winnipeg. Represents regrowth that occurred after 11 August 1992.

Cultivar	Height (cm)
Nitro	57.8a
Excalibur	51.0b
Wilson	50.8b
Legend	46.1c
Alfagraze	42.0d
Rangelander	22.6e
LSD (0.05)	3.0

Means within a column followed by the same letter are not significantly different at alpha=0.05 (LSD).

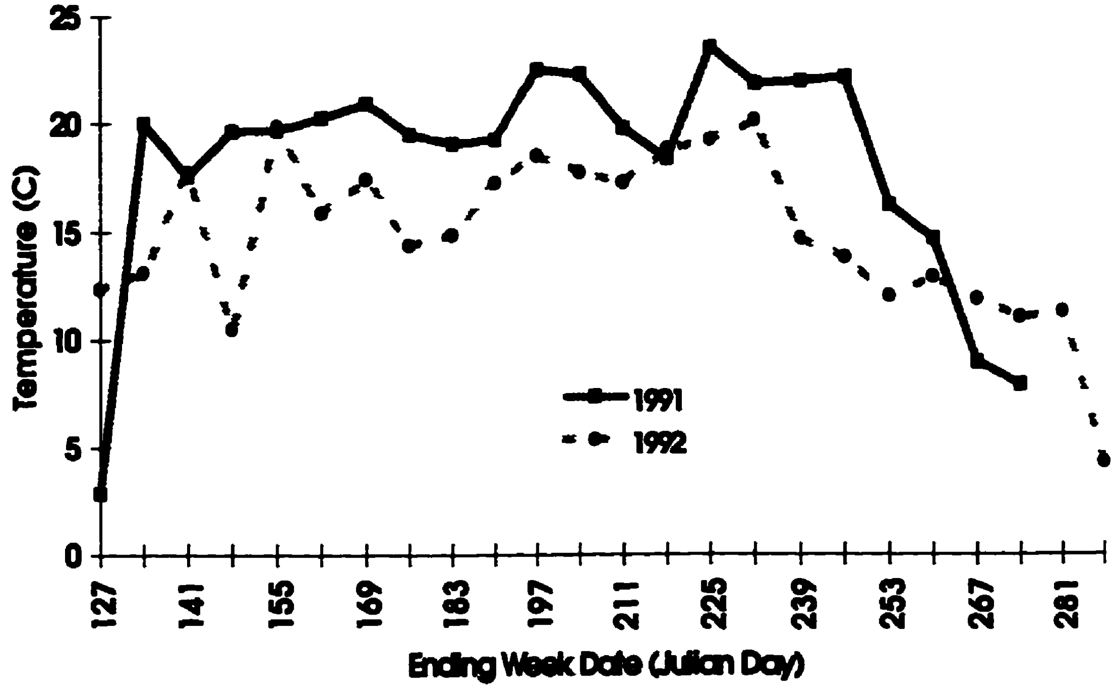

Figure 4.1 Mean weekly air temperatures at Winnipeg.

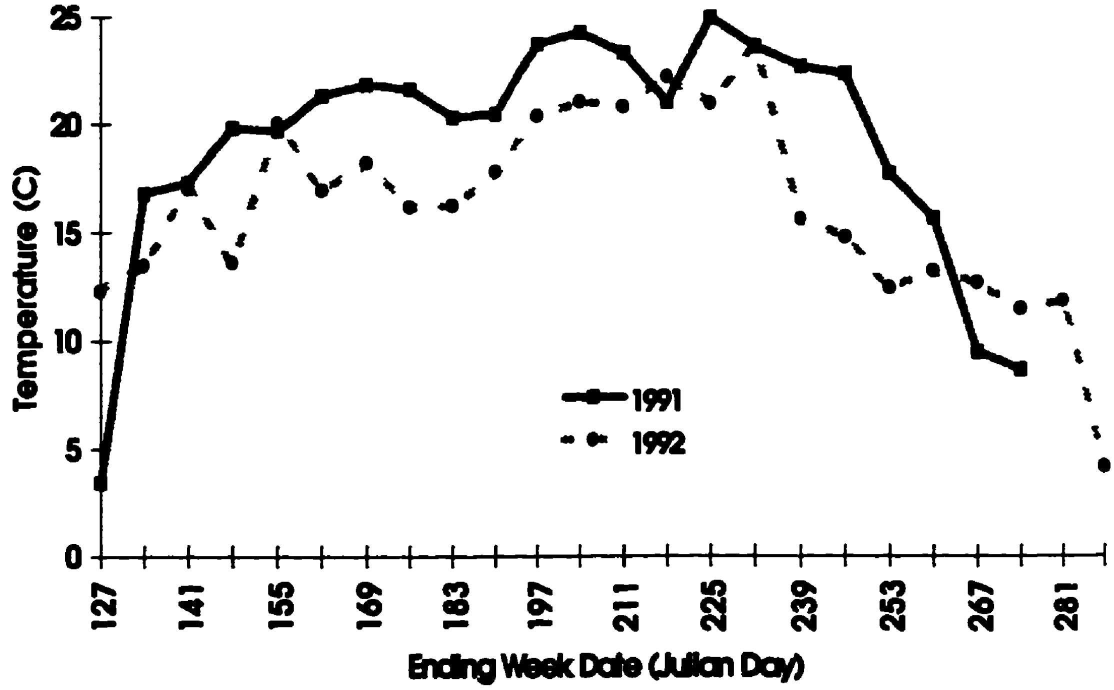

Figure 4.2 Mean weekly soil temperatures at Winnipeg.

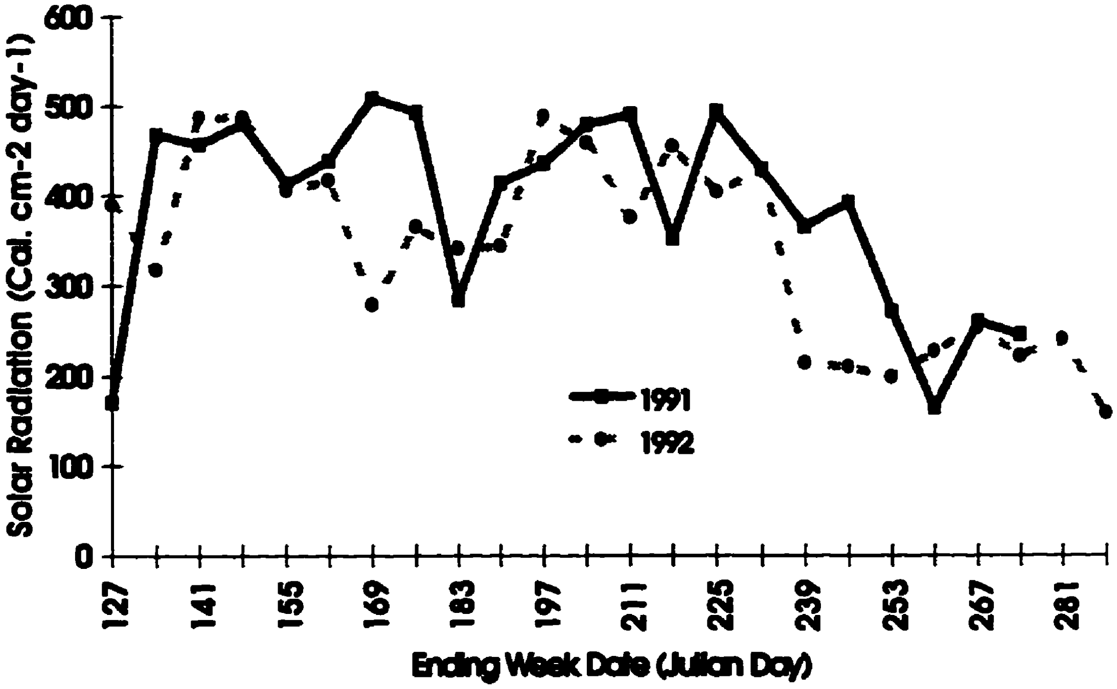

Figure 4.3 Mean weekly solar radiation at Winnipeg.

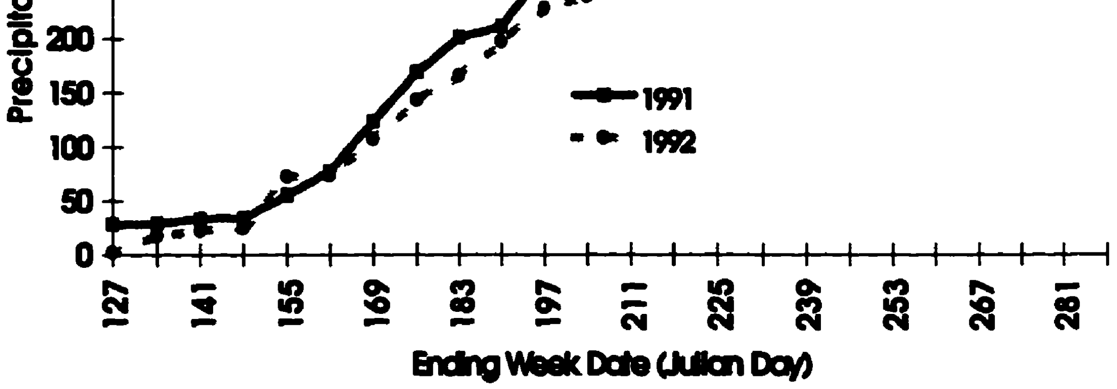

Figure 4.4 Cumulative weekly precipitation at Winnipeg.

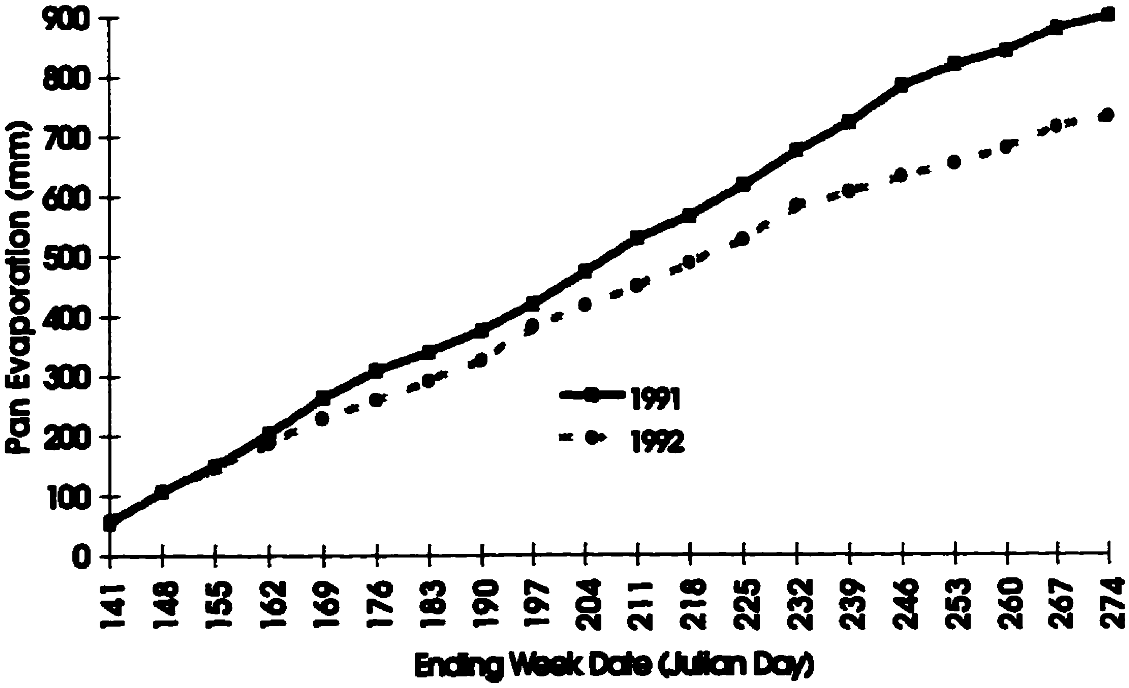

Figure 4.5 Cumulative weekly pan evaporation at Winnipeg.

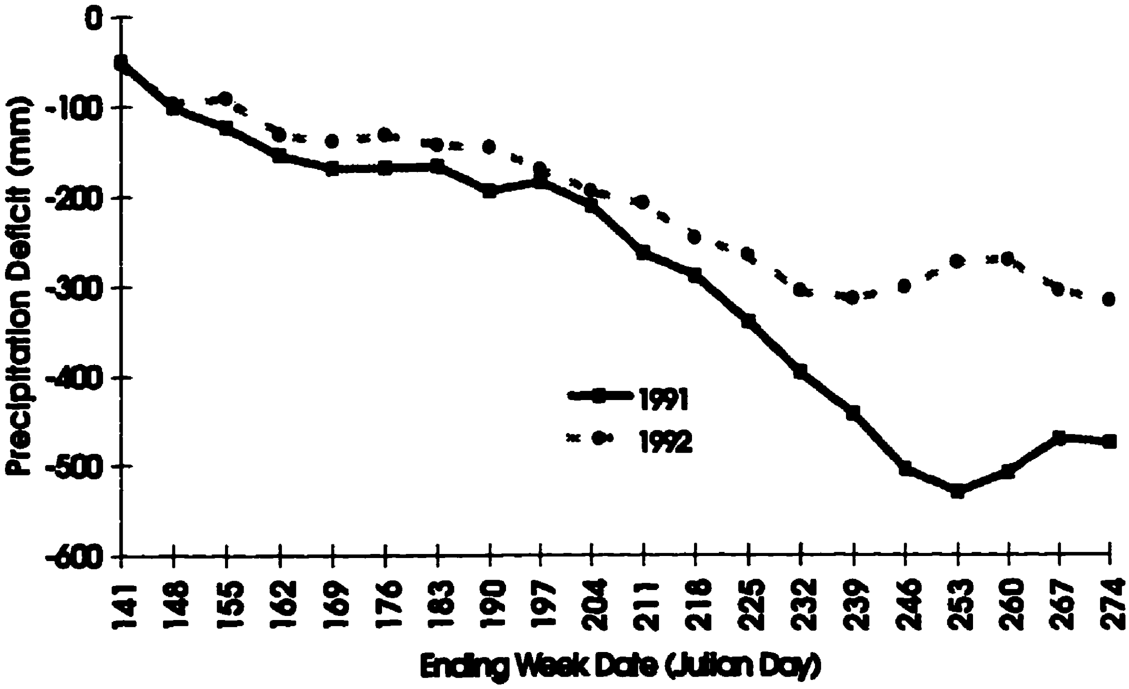

Figure 4.6 Cumulative weekly precipitation deficit (precipitation - pan evaporation) at Winnipeg.

In regression analysis using combined year data, relationships for first-cut yield and ET and total yield and ET (Table 4.3) were significant. Linear relationships between alfalfa dry matter yield and ET have previously been documented by Bauder et al. (1978) and Grimes et al. (1992). At least 28% of the variability was explained by the linear relationships in the present study. However, this does leave room for other factors, such as differences in ETE between the cultivars. When regression analyses were conducted separately for each cultivar, a larger amount of variability was explained. Where relationships were significant, 53 to 86% of the variability was accounted for by the linear function. In all three sets of analysis, only 'Rangelander' had a consistent, significant relationship. Two explanations may help rationalize this consistency. First, factors that could decrease the yield variability within a small sample size would form a stronger relationship. These could include a similar or higher (possibly more optimal) plant counts (Table 3.1) and a potentially more uniform plant stand (because 'Rangelander' is a creeping rooted cultivar). Second, other factors, such as temperature, may have had a lesser impact on 'Rangelander' than the other cultivars.

It is important to remember that the above yield/ET relationships were calculated for establishment year alfalfa where initial soil moisture levels were relatively high. Established alfalfa is likely to have lower soil moisture at the beginning of a given season and would have faster initial growth rates, which would change the dynamics of the relationship.

Table 4.3 Linear regression equations for alfalfa forage yield (Y) as a function of ET at Winnipeg (data from both 1991 and 1992 were included).

Harvest Period	Cultivar	Equation	n	P > F	R^2
1st cut	Alfagraze	Y = -165.0 + 1.67 ET	8	0.1523	0.31
	Excalibur	Y = -288.9 + 1.95 ET	8	0.1556	0.31
	Legend	Y = -261.8 + 1.91 ET	8	0.2054	0.25
	Nitro	Y = -750.0 + 2.92 ET	8	0.0676	0.45
	Rangelander	Y = -640.1 + 2.86 ET	8	0.0012	0.85
	Wilson	Y = -1198.0 + 4.09 ET	8	0.0261	0.59
	All	Y = -483.1 + 2.39 ET	48	0.0001	0.28
2nd cut	Alfagraze	Y = 301.6 - 0.06 ET	8	0.8887	0.00
	Excalibur	Y = 428.2 - 0.57 ET	8	0.0420	0.53
	Legend	Y = 190.0 + 0.59 ET	8	0.0391	0.54
	Nitro	Y = 117.9 + 0.86 ET	8	0.1367	0.33
	Rangelander	Y = -74.1 + 1.37 ET	8	0.0010	0.86
	Wilson	Y = 161.9 + 0.49 ET	8	0.2821	0.19
	All	Y = 193.8 + 0.42 ET	48	0.0837	0.06
Total	Alfagraze	Y = 263.4 + 0.86 ET	8	0.1595	0.30
	Excalibur	Y = 530.7 + 0.39 ET	8	0.3744	0.13
	Legend	Y = 23.6 + 1.29 ET	8	0.0389	0.54
	Nitro	Y = -844.1 + 2.56 ET	8	0.0048	0.76
	Rangelander	Y = -446.3 + 1.88 ET	8	0.0012	0.85
	Wilson	Y = -389.8 + 1.72 ET	8	0.0504	0.50
	All	Y = -115.1 + 1.40 ET	48	0.0001	0.32

4.1.3 Evapotranspiration and Soil Water Extraction

Significant cultivar differences for ET were apparent in the seasonal total ET (ET_t) in 1992 and from seeding to first harvest date (ET_1) in the combined year analysis (Table 4.4). In both cases, 'Rangelander' used less water than the other cultivars. McElgunn and Heinrichs (1975) found that *Medicago falcata* L. genotypes (individual plants) used less water per day than *M. sativa* L. genotypes, ascribing the difference to their slower growth rate. 'Rangelander' is a synthetic cultivar with several *M. falcata* L. parental plants in its genetic background (Heinrichs et al., 1979). Similarly, lower ET for 'Rangelander' in the present study is likely due to slower initial growth and reduced fall growth (Table 4.2). 'Alfagraze', 'Legend' and 'Nitro' generally had the highest ET to the first harvest date, but ET trends in ET_2 and ET_t were not as clear.

Evapotranspiration to the first cut was significantly higher in 1991 than 1992, and was attributed to higher temperatures (Figure 4.1). McElgunn and Heinrichs (1975) found a significant cultivar × soil temperature interaction for ET; however, in the present study the cultivar × year interaction was not significant for ET_1, although soil temperatures differed between the two study years (Figure 4.2). Either the soil temperature differences were not large enough to cause an interaction with ET, or the cultivars used in the present study react similarly to different soil temperatures. Combined year analysis for ET between first and second cuts (ET_2) and ET_t could not be completed due to heterogeneity of error variances.

Daily mean ET levels, which were similar to values reported by Carter and Sheaffer (1983a), ranged from 4.3 to 4.9 mm d^{-1} during ET_1 and from 3.1 to 3.6 mm d^{-1} during ET_2 (Table 4.4). Higher daily ET rates earlier in the season were attributed to warmer temperatures, greater solar radiation, greater precipitation levels, (Figures 4.1 through 4.4) and more available soil moisture (Figures 4.7 and 4.8) (Abdul-Jabbar et al., 1983; Carter and Sheaffer, 1983a).

Table 4.4 Daily (mm d^{-1}) and growth cycle[z] (mm) ET for establishment year alfalfa cultivars at Winnipeg.

Year	Cultivar	ET_1	ET_2	ET_t
1991	Alfagraze	410 a	229 a	639 a
	Excalibur	397 a	244 a	641 a
	Legend	404 a	231 a	635 a
	Nitro	408 a	222 a	630 a
	Rangelander	398 a	246 a	644 a
	Wilson	397 a	241 a	639 a
	LSD (0.05)	ns	ns	ns
1992	Alfagraze	384 a	190 a	574 ab
	Excalibur	375 a	193 a	568 bc
	Legend	384 a	194 a	578 ab
	Nitro	387 a	192 a	580 ab
	Rangelander	372 a	186 a	557 c
	Wilson	379 a	206 a	584 a
	LSD (0.05)	ns	ns	16
Combined	Alfagraze	397 a	210	607
	Excalibur	386 c	218	604
	Legend	394 ab	213	607
	Nitro	398 a	207	605
	Rangelander	384 c	216	600
	Wilson	388 bc	223	611
	LSD (0.05)	8	NA	NA
1991	Mean	402 a	236 a	638a
1992	Mean	380 b	193 b	574b
	Overall Mean	391	215	606
1991	Daily Mean	4.9	3.6	4.3
1992	Daily Mean	4.3	3.1	3.8

z - ET_1, ET_2, and ET_t denote evapotranspiration to the first cut, second cut and seasonal total, respectively.

Means within a continuate column followed by the same letter are not significantly different at alpha=0.05 (LSD).

ns - F-test not significant at alpha=0.05.

NA - not applicable, data could not be combined.

In 1991, the soil profile in spring was essentially filled to field capacity (Figure 4.7 a). Volumetric field capacity in soil samples taken from the top 10 cm was determined to be 43.5%. In 1992, the upper 30 cm was dry initially, but soil water levels were near field capacity at lower depths (Figure 4.8 a). Soil water depletion patterns between seeding and first cut (Figures 4.7 b and 4.8 b) indicated water extraction to a depth of 110-130 cm in 1991 and 70-90 cm in 1992. This was validated with orthoganal contrasts between the summerfallow and alfalfa plots (Tables 4.5 and 4.6; 7 August in 1991 and 12 August in 1992). After the final harvest, water depletion patterns indicated soil water extraction to a depth of 170-190 in 1991 and 130-150 cm in 1992 (Figures 4.7 c and 4.8 c). Highlights of the statistical analysis in tables 4.5 and 4.6 verify the water extraction to this depth. Because initial soil moisture (Figures 4.7 a and 4.8 a) and the amount and timing of precipitation were similar in 1991 and 1992 (Figure 4.4), differences in effective rooting depth between the two years were likely more related to above ground growing conditions such as air and soil temperature and solar radiation, than soil moisture.

Relatively few cultivar differences in soil water extraction were noted in this study (Tables 4.7 and 4.8). On 20 July 1991, 'Rangelander' had extracted the least amount of water from the 30-50 cm depth, while 'Alfagraze' had extracted the most (data not shown). At the end of the season, 'Wilson' extracted more water at the 170-190 cm depth than all the other cultivars except "Rangelander'. (data not shown). In 1992, more differences were apparent, with similarities between dates late in the season. Sampling dates from August through September indicated that soil moisture was highest for 'Rangelander' and 'Excalibur', and lowest for 'Nitro'. At the end of the season, 'Wilson' had removed the most water at the 90-110 cm depth, 'Rangelander' and Excalibur had removed the least, while 'Nitro' was not significantly different from any of the cultivars (data not shown). 'Rangelander' and 'Excalibur' were previously shown to have the lowest ET (Table 4.4).

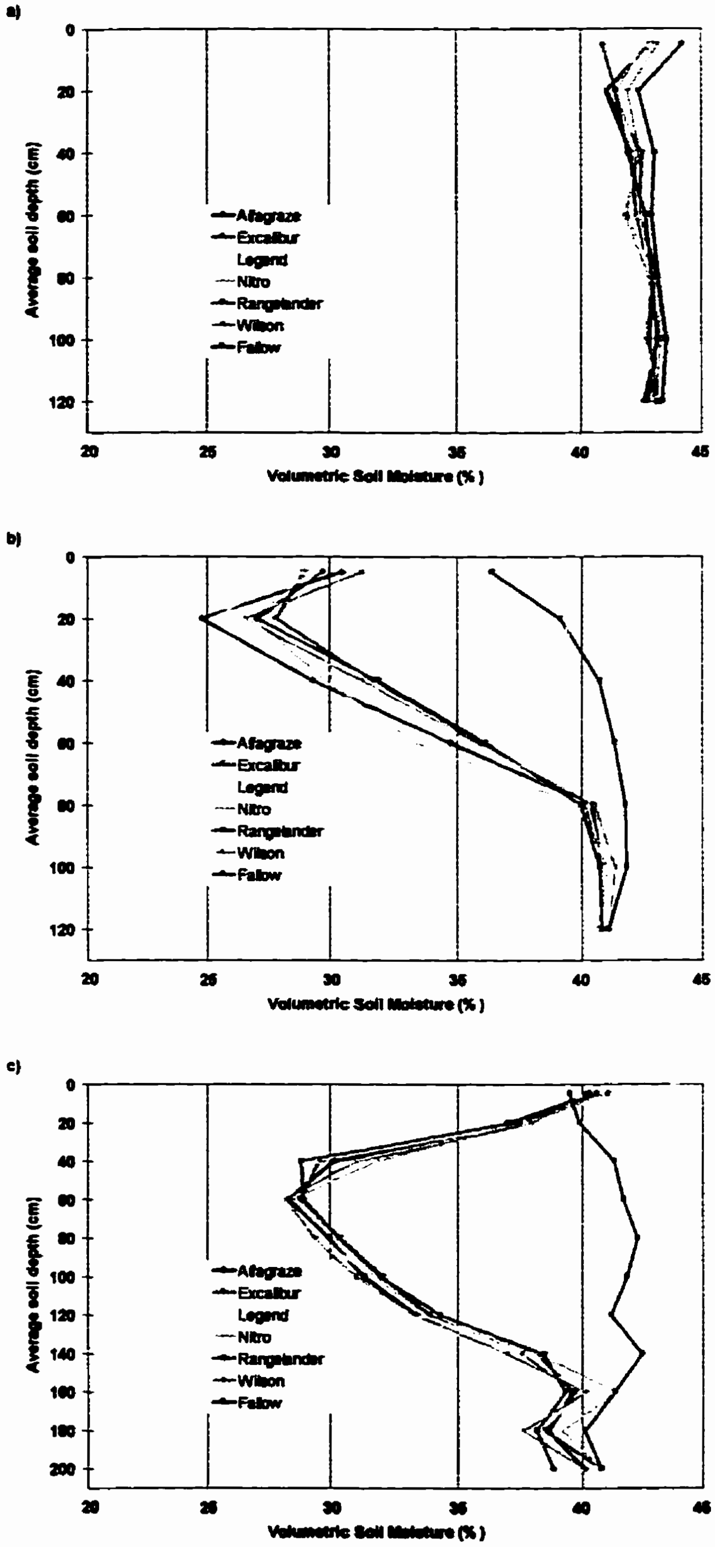

Figure 4.7 Soil water depletion patterns for six alfalfa cultivars and summerfallow plots in 1991 after: a) seeding, b) 1st cut, and c) final harvest at Winnipeg.

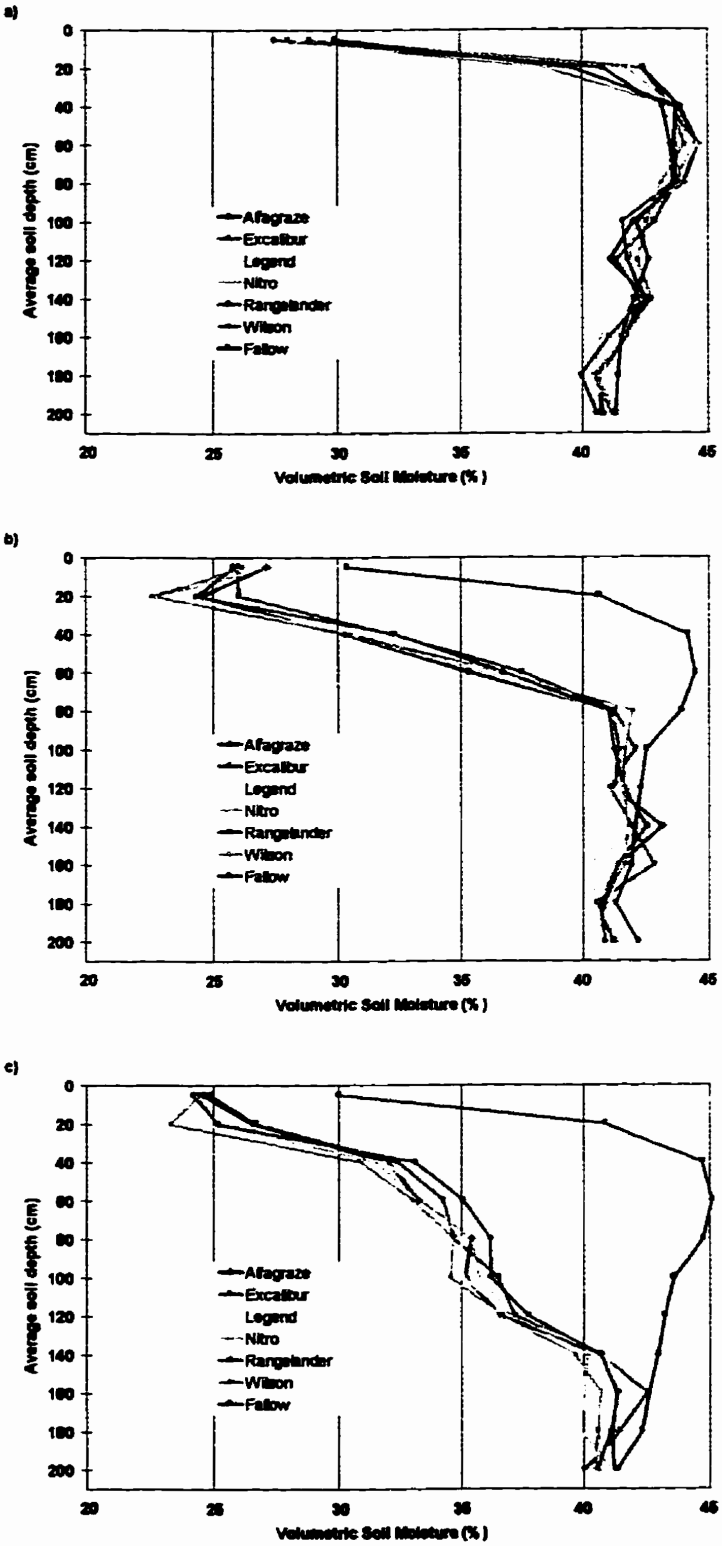

Figure 4.8 Soil water depletion patterns for six alfalfa cultivars and summerfallow plots in 1992 after: a) seeding, b) 1st cut, and c) final harvest at Winnipeg.

Despite significant water extraction to a depth of as much as 180 cm, soil water reserves accounted for a relatively small portion of the total ET. Soil water accounted for 25.6, 32.4, 28.0, 22.0, 20.9 and 21.6% of the total ET during the first and second growth cycles and combined total, for 1991 and 1992, respectively. Soil water reserves provided a greater portion of the total ET in 1991, even though greater precipitation was recorded. This was likely because cumulative pan evaporation (Figure 4.5) was higher and the net precipitation deficit (Figure 4.6) was greater in 1991 than in 1992.

Table 4.5 Summary of orthoganol contrast analysis for soil water extraction between all six alfalfa cultivars and the summerfallow plots at Winnipeg in 1991.

	Date								
	June			July		August		Sept.	Oct.
Depth	6	17	28	8	20	7	21	13	11
0-10	ns	ns	ns	**	**	**	**	**	**
10-30	ns	*	*	ns	**	**	**	**	**
30-50	ns	*	ns	ns	**	**	**	**	**
50-70	ns	ns	*	ns	**	**	**	**	**
70-90	ns	ns	**	ns	**	**	**	**	**
90-110	ns	ns	ns	ns	*	**	**	**	**
110-130	ns	ns	ns	ns	ns	**	**	**	**
130-150									**
150-170									*
170-190									**
190-210									ns

*,** - F-test significant at alpha=0.05 and 0.01 levels, respectively.
ns - no significant difference.

Table 4.6 Summary of orthoganol contrast analysis for soil water extraction between all six alfalfa cultivars and the summerfallow plots at Winnipeg in 1992.

	Date							
	June	July		August		September		Oct.
Depth	15	7	23	12	27	10	22	13
0-10	*	ns	*	**	*	ns	ns	**
10-30	ns	ns	**	**	**	**	**	**
30-50	ns	ns	**	**	**	**	**	**
50-70	ns	ns	ns	**	**	**	**	**
70-90	ns	ns	ns	**	**	**	**	**
90-110	ns	ns	ns	ns	*	**	**	**
110-130	*	ns	ns	*	**	**	**	**
130-150	ns	ns	ns	ns	ns	ns	ns	**
150-170	ns	ns	ns	ns	ns	ns	ns	ns
170-190	ns	ns	ns	ns	ns	*	*	ns
190-210	ns	ns	ns	**	ns	*	ns	ns

*,** - F-test significant at alpha=0.05 and 0.01 levels, respectively.
ns - no significant difference.

Table 4.7 Summary of soil water extraction ANOVA between alfalfa cultivars at Winnipeg in 1991.

	Date								
	June			July		August		Sept.	Oct.
Depth	6	17	28	8	20	7	21	13	11
0-10	ns	ns	ns	ns	ns	ns	ns	ns	ns
10-30	ns	ns	ns	ns	ns	ns	ns	ns	ns
30-50	ns	ns	ns	ns	*	ns	ns	ns	ns
50-70	ns	ns	ns	ns	ns	ns	ns	ns	ns
70-90	ns	ns	ns	ns	ns	ns	ns	ns	ns
90-110	ns	ns	ns	ns	ns	ns	ns	ns	ns
110-130	ns	ns	ns	ns	ns	ns	ns	ns	ns
130-150									ns
150-170									ns
170-190									**
190-210									ns

*,** - F-test significant at alpha=0.05 and 0.01 levels, respectively.
ns - no significant difference.

Table 4.8 Summary of soil water extraction ANOVA between alfalfa cultivars at Winnipeg in 1992.

	Date							
	June	July		August		September		Oct.
Depth	15	7	23	12	27	10	22	13
0-10	ns	ns	ns	ns	ns	ns	ns	ns
10-30	*	*	ns	ns	ns	ns	ns	ns
30-50	ns	ns	ns	ns	*	ns	ns	ns
50-70	ns	*	ns	ns	*	*	ns	ns
70-90	ns	ns	ns	ns	ns	ns	ns	ns
90-110	ns	ns	ns	ns	ns	ns	*	*
110-130	ns	ns	ns	ns	ns	ns	ns	ns
130-150	ns	ns	ns	ns	ns	ns	ns	ns
150-170	ns	ns	ns	ns	ns	ns	ns	ns
170-190	ns	ns	ns	ns	ns	ns	ns	ns
190-210	ns	ns	ns	ns	ns	ns	ns	ns

* - F-test significant at alpha=0.05.
ns - no significant difference.

4.1.4 Crop Evapotranspiration Efficiency

A wide range of ETE values were observed in this study (8.8 to 16.6 kg ha^{-1} mm^{-1}; Table 4.9). For example, second-cut ETE for 'Excalibur' was almost double that of first-cut 'Wilson' in 1992. However, values in the present study were similar to those reported by Carter and Sheaffer (1983a) (i.e., 9.7 and 13.1 kg ha^{-1} mm^{-1}) for unirrigated alfalfa with similar total herbage yields (6.8 Mg ha^{-1}).

Significant differences in ETE between cultivars occurred in all cases except for the first cut material in 1991. Generally, 'Alfagraze', 'Excalibur', and 'Legend' utilized water most efficiently. Notable exceptions are 'Nitro' (ETE$_2$) in 1991 and 1992 and 'Rangelander' (ETE$_1$) in 1992, where performance was equal to the three previously mentioned cultivars. On the other hand, the lowest ETE was exhibited by 'Nitro' and

'Wilson' in the establishment period (to 1st cut) in 1992. Although there was no significant difference during this period in 1991, 'Nitro' and 'Wilson' once again had the lowest ETE. The combined year analysis for this period showed significant differences, with 'Nitro' and 'Wilson' having lower ETE than the other cultivars. These two cultivars possibly have less stomatal regulation under limited stress, such as during the two establishment periods, and are more extravagant in their water use under these conditions.

Significant year × cultivar interactions were evident for ETE_2 and ETE_t. For ETE_2, the interaction was likely due to a decrease in ETE for 'Wilson' in 1992, while the other cultivars had higher ETE in 1992 compared with 1991. In 1992, ETE_t was higher than in 1991 for 'Alfagraze', 'Excalibur', and 'Legend', but lower for 'Nitro', 'Rangelander', and 'Wilson', thereby causing the interaction. The basis for these interactions may be explained by the same argument used for the cultivar × environment interaction for aerial dry matter production; the cultivars responded differently to the cooler temperatures and reduced solar radiation in 1992.

Cultivar differences in ETE (Table 4.9) were mainly due to yield variation (Table 4.1), as similar rankings were observed. Correlation values for ETE with its components, yield and ET, support this hypothesis (Table 4.10). Yield accounted for 61 to 98% of the variation in ETE, while ET was correlated with ETE in only one instance. In this one case, (1991, ETE_2) correlation values for yield and ET were similar in magnitude. These results imply that, under adequate to moderate drought stress conditions, differences in ETE are generally not dependent upon the plant's ability to uptake water, but depend on its ability to utilize water most efficiently in the production of dry matter.

Table 4.9 ET efficiency (kg ha^{-1} mm^{-1}) of field-grown alfalfa at Winnipeg.

Year	Cultivar	1st cut	2nd cut	Combined
1991	Alfagraze	12.7 a	12.8 abc	12.7 ab
	Excalibur	12.5 a	11.8 cd	12.2 bc
	Legend	13.1 a	14.3 a	13.5 a
	Nitro	11.7 a	14.2 ab	12.5 abc
	Rangelander	12.5 a	10.7 d	11.8 bc
	Wilson	11.1 a	12.6 bc	11.6 c
	LSD (0.05)	ns	1.6	1.0
1992	Alfagraze	12.3 a	15.1 a	13.3 a
	Excalibur	11.6 a	16.6 a	13.2 a
	Legend	11.8 a	15.6 a	13.1 a
	Nitro	9.0 b	14.5 a	10.8 b
	Rangelander	11.4 a	12.0 b	10.9 b
	Wilson	8.8 b	9.7 c	9.9 b
	LSD (0.05)	1.3	2.2	1.1
Combined	Alfagraze	12.5 a	13.9	13.0
	Excalibur	12.0 a	14.2	12.7
	Legend	12.4 a	15.0	13.3
	Nitro	10.3 b	14.4	11.7
	Rangelander	12.0 a	10.2	11.3
	Wilson	10.0 b	12.3	10.7
	LSD (0.05)	0.9	NA	NA
1991	Mean	12.2 a	12.7 a	12.4 a
1992	Mean	10.8 b	13.9 a	11.9 b
	Overall Mean	11.5	13.3	12.1

Means within a consecutive column followed by the same letter are not significantly different at alpha=0.05 (LSD).

ns - F-test not significantly different at alpha=0.05.

NA - not applicable; data could not be combined due to a significant year × cultivar interaction.

Table 4.10 Simple correlation coefficients between ET efficiency and its components, yield and evapotranspiration (ET), for six alfalfa cultivars grown in field trials at Winnipeg.

Year	Growth cycle†	Yield	ET
1991	ETE_1	0.96^{**}	0.03
	ETE_2	0.78^{**}	-0.73^{**}
	ETE_t	0.91^{**}	-0.32
1992	ETE_1	0.99^{**}	-0.19
	ETE_2	0.97^{**}	-0.23
	ETE_t	0.99^{**}	-0.32

† ETE_1, ETE_2, and ETE_t correspond to ET efficiency over the first, second and combined growth cycles, respectively.
** - significant at alpha=0.01.

Snaydon (1972) determined that ETE in established alfalfa was greatest at a ET:E_{pan} ratio of 0.5, where ET was defined as total rainfall and irrigation, although research by Jensen et al. (1988) suggests a ratio of about 0.75 is optimal. Using Snaydon's definition in the present study, evaporation ratios were 0.57, 0.44, 0.57 and 0.71 for the first and second growth cycles in 1991 and 1992, respectively. Lower ETE levels in the first compared with the second growth cycle was partly due to incomplete ground cover for a significant portion of the first growth cycle. The ET:E_{pan} ratio and ETE value for the second growth cycle was higher in 1992 than in 1991, which supports the results of Jensen et al. (1988). Lower pan evaporation for the second growth cycle in 1992 than in 1991 (215 mm and 361 mm, respectively) likely allowed more optimal use of available water (i.e., higher ETE).

4.1.5 Plant Water Relations

Seasonal trends for water potential, osmotic potential, and the resulting turgor potential, are presented in Figure 4.9 with mean values for sampling dates and cultivars listed in Table 4.11. A summary of the analysis of variance for the plant water relations measurements is documented in Table 4.12.

Water potential declined as the season progressed in 1991 and declined from the first to second measurement in 1992. Final Ψw values in 1992 (24 September) were relatively high, despite lower soil moisture levels. This is likely because of plant acclimation, slower growth rates (prior daytime temperatures were cooler), and reduced environmental stress; specifically, reduced solar radiation, as mean temperature, ET_{pan}, and wind run values were similar to those recorded on previous sampling dates (Table 4.13). Mean Ψw values for each sampling date, which are similar to those reported by Brown and Tanner (1981), Carter et al. (1982) and Carter and Sheaffer (1983b; ML treatment), ranged from -0.84 to -1.99 MPa. Peake et al. (1975) observed that visible leaf wilting occurred at a Ψw of -1.5 to -2.5 MPa, while Carter and Sheaffer (1983a) reported that the relative growth rate of alfalfa became negative when the mean midday Ψw was below -2.0 MPa. This implies that, generally, the alfalfa plants in the present study were only moderately stressed, but on 29 August 1991, they were severely stressed.

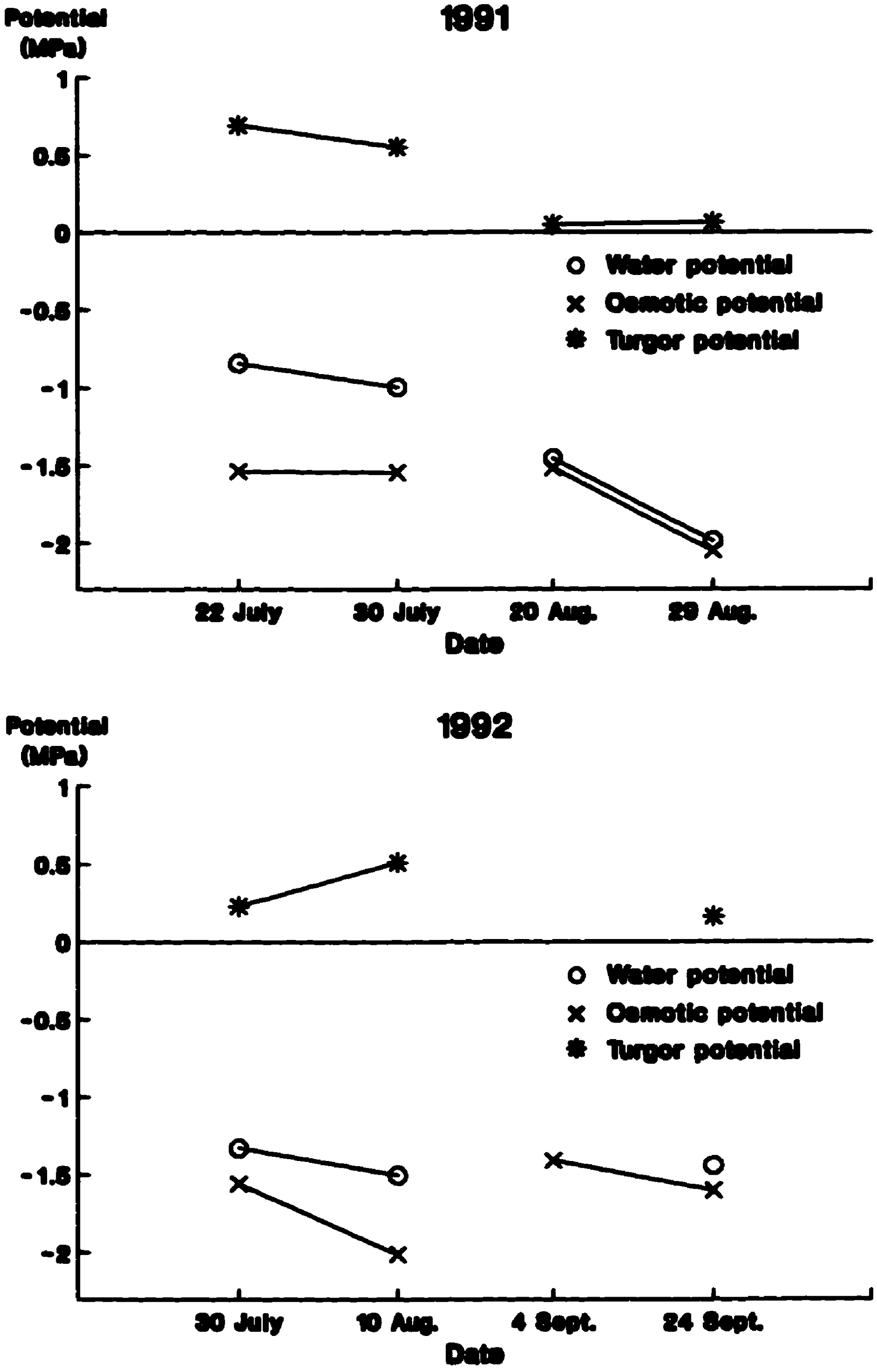

Figure 4.9 Mean values of water potential, osmotic potential and turgor potential for field-grown alfalfa at Winnipeg in 1991 and 1992.

Table 4.11 Mean water relations values by sampling date and cultivar for field-grown alfalfa at Winnipeg in 1991 and 1992.

Year	Date	RWC (%)	Ψ_w (MPa)	P (MPa)	π (MPa)	π_{100} (MPa)	π_{T100} (MPa)	TW:DW(g g^{-1})
1991	22 July	84.6	-0.84	0.69	-1.54	-1.30		5.33
	30 July	84.4	-1.00	0.55	-1.55	-1.31		4.56
	20 August	72.5	-1.46	0.05	-1.51	-1.09		4.97
	29 August	68.4	-1.99	0.06	-2.05	-1.40		4.66
1992	30 July	81.8	-1.33	0.23	-1.56	-1.28	-0.76	5.29
	10 August	75.6	-1.51	0.51	-2.01	-1.52	-0.99	5.04
	4 September	80.6			-1.41	-1.14	-0.70	5.63
	24 September	83.8	-1.44	0.16	-1.60	-1.34	-1.14	5.17
	Cultivar							
	Alfagraze	79.4	-1.33	0.37	-1.67	-1.32	-0.89	5.07
	Excalibur	78.4	-1.45	0.24	-1.66	-1.29	-0.89	5.04
	Legend	79.2	-1.41	0.28	-1.65	-1.30	-0.92	5.08
	Nitro	78.6	-1.26	0.41	-1.64	-1.28	-0.93	4.98
	Rangelander	78.8	-1.43	0.26	-1.66	-1.30	-0.83	5.13
	Wilson	79.3	-1.32	0.36	-1.64	-1.29	-0.93	5.17

Table 4.12 **Summary of the ANOVA for components of plant water relations in field-grown alfalfa cultivars.**

	Date							
	1991				1992			
	July		August		July	August	September	
Parameter	22	30	20	29	30	10	4	24
Ψw	ns	ns	ns	**	ns	**	NA	ns
RWC	ns	ns	ns	ns	*	*	ns	ns
π	*	ns	ns	ns	ns	ns	ns	ns
$\pi 100$	ns	ns	ns	ns	ns	ns	ns	ns
$\pi_T 100$					ns	ns	ns	ns
P	ns	ns	ns	ns	ns	**	NA	ns
TW:DW	ns	ns	ns	ns	ns	ns	ns	ns

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*,** F-test significant at alpha=0.05 and 0.01 levels, respectively.

ns - no significant difference.

NA - not applicable because Ψw measurements were not taken on this date and *P* could not be calculated.

Table 4.13 Environmental conditions on water relations and canopy temperature sampling dates at Winnipeg in 1991 and 1992.

Year	Date		Mean Temp. (°C)	Min. Temp. (°C)	Time at Min. Temp. (h)	Max. Temp. (°C)	Time at Max. Temp. (h)	Soil Temp. (°C)	Ppt. (mm)	Solar Rad. (cal. cm^{-2})	Pan Evap. (mm)	Wind Run (km)
1991	22	July	21.0	15.9	2336	24.9	1606	23.9	0.0	520	9.9	343
	23	July	19.7	13.1	532	26.9	1621	22.5	0.0	540	9.2	294
	25	July	18.1	11.7	541	24.2	1654	22.0	0.0	473	3.4	121
	30	July	21.9	15.4	609	30.4	1624	24.3	2.3	543	10.0	307
	20	August	24.6	17.5	740	32.5	1655	24.3	0.0	417	9.2	256
	29	August	26.9	17.8	724	34.4	1534	24.7	0.0	445	14.1	248
1992	23	July	20.3	11.4	557	26.7	1421	26.1	0.0	541	8.0	221
	29	July	17.1	9.7	453	24.3	1642	22.9	0.0	565	3.6	122
	30	July	18.8	11.4	430	25.7	1634	25.0	0.0	512	8.0	149
	4	August	18.8	11.0	549	24.9	1434	22.8	0.0	507	7.2	176
	7	August	21.4	15.9	554	26.8	1629	22.8	0.0	338	4.4	129
	8	August	24.7	16.8	536	32.6	1835	25.8	1.5	504	6.6	178
	9	August	23.2	15.5	2356	28.5	1620	22.4	13.7	404	8.0	232
	10	August	18.0	13.4	625	24.3	1418	19.8	0.0	462	6.6	223
	4	September	17.3	12.3	732	23.1	1543	19.2	0.0	351	6.2	243
	24	September	20.0	14.3	17	26.0	1624	19.4	0.0	243	9.8	440
	30	September	12.4	2.8	747	25.1	1647	13.5	0.0	286	3.2	139
	1	October	18.4	7.7	12	32.4	1709	17.5	0.0	270	NA	NA

NA - not available

Differences in cultivar Ψw were only significant on 29 August 1991 and 10 August 1992. Values for these dates are presented in Table 4.14. It was also on these two dates that the lowest mean Ψw was recorded for each year. Brown and Tanner (1981) concluded that between plant variability is large enough to pose problems when measuring small differences in alfalfa Ψw. Moderate to high stress levels, therefore, may be required to help discern cultivar differences. Data from all sampling dates were analyzed together with results indicating cultivar differences similar to those found on the individual dates where significant cultivar differences were detected (Table 4.14). In general, 'Nitro' had the highest Ψw, while 'Excalibur', 'Rangelander' and 'Legend' had the lowest.

Table 4.14 Water potential (MPa) in field-grown alfalfa cultivars when significant differences were detected (at Winnipeg in 1991 and 1992).

Cultivar	29 Aug. 1991	10 Aug. 1992	Combined Dates^z
Nitro	-1.75 a	-1.35 a	-1.26 a
Wilson	-1.92 ab	-1.51 ab	-1.32 ab
Alfagraze	-1.94 ab	-1.39 a	-1.34 bc
Legend	-2.10 bc	-1.62 bc	-1.41 cd
Rangelander	-2.19 c	-1.44 ab	-1.43 d
Excalibur	-2.03 bc	-1.72 c	-1.45 d
LSD (0.05)	0.21	0.20	0.07

Means within a column followed by the same letter are not significantly different at alpha=0.05 (LSD).
z - data from all seven sampling dates combined.

Some cultivar differences in Ψw may be accounted for by examining differences in root morphology. 'Nitro' was selected for its large root mass (Barnes et al., 1988b). A larger rooting system may give 'Nitro' the ability to extract soil water more effectively. This is challenged by Carter et al. (1982), who found that cultivars with greater root lengths had lower Ψw under moisture stress. However, in their controlled study, equal amounts of water were applied to each cultivar. The larger rooted cultivars would be at a disadvantage, and would display greater stress (lower Ψw), if they used more water. In another study, Carter and Sheaffer (1983c) showed that nitrogenase specific activity decreased with decreases in plant water potential. Some of 'Nitro's ability to accumulate greater amounts of root nitrogen (Barnes et al., 1988b) may be linked to its higher mean midday Ψw. 'Rangelander', on the other hand, was shown to have lower root mass under controlled conditions in the present study (see Table 4.24). This characteristic may make 'Rangelander' less effective in extracting soil moisture and may account for its lower ET.

Mean midday RWC of alfalfa leaves was in the low to mid 80% range (Table 4.15). On dates when stress was greater, as indicated by lower Ψw, mean RWC values dropped as low as 68%.

Table 4.15 Leaf relative water content (%) in field-grown alfalfa cultivars when significant differences were detected (at Winnipeg in 1992).

Cultivar	30 July 1992	10 Aug. 1992
Alfagraze	83.4 a	76.5 ab
Wilson	83.2 a	73.4 bc
Rangelander	82.5 a	79.1 a
Nitro	81.9 a	75.2 bc
Legend	81.2 ab	75.7 bc
Excalibur	78.8 b	73.2 c
LSD (0.05)	2.8	3.2

Means within a column followed by the same letter are not significantly different at alpha=0.05 (LSD).

Cultivar differences in RWC were significant only on the first two sampling dates in 1992 (Table 4.15). On these two dates, 'Excalibur' had the lowest RWC, while 'Rangelander' generally had the highest. On other dates, ranking of the cultivars varied (data not shown), however, there were no significant differences in RWC. Combined analysis failed to prove significant differences between cultivars (data not shown).

There were no cultivar differences detected for the leaf TW:DW ratio on any of the sampling dates (data not shown). Due to heterogeneity of error variances, an overall combined analysis over both years was not conducted. However, a combined analysis of the first three sampling dates in 1992 was significant at P=0.0825. In this case, 'Nitro' had a lower TW:DW ratio than 'Rangelander', 'Excalibur' or 'Wilson' (Table 4.16). A smaller TW:DW ratio would indicate a smaller cell size.

Table 4.16 Leaf TW:DW ratios in field-grown alfalfa cultivars (combined data from all sampling dates at Winnipeg in 1991 and 1992).

Cultivar	TW:DW
Rangelander	5.51 a
Excalibur	5.46 a
Wilson	5.36 a
Alfagraze	5.32 ab
Legend	5.28 ab
Nitro	5.00 b
LSD (0.05)	0.34

Means within a column followed by the same letter are not significantly different at alpha=0.05 (LSD).

No cultivar differences for π_{100} were detected on any sampling date (data not shown). Due to heterogeneity of error variances, only dates within years could be combined. In these analyses, no cultivar differences were noted in either year.

Osmotic potential adjusted to full turgor was variable across the dates, although the lowest π_{100} (-1.40 and -1.52 MPa for 29 August 1991 and 10 August 1992, respectively) was recorded when the alfalfa was under the most stress (i.e., had the lowest Ψw of the season) and the maturity stage was mid- to full flower. Samples taken from regrowth material after the first harvest had the highest π_{100} in each year. Differences in

π_{100} between sampling dates indicate that alfalfa has the ability to osmoregulate. However, as both plant age and stress varied between sampling dates, it is not possible to determine how much of the change in π_{100} was due to each factor.

Osmotic adjustment in the field study was calculated as the difference in π_{100} between the second and first sample taken during the establishment period or between the second and first sample taken on regrowth material. Cultivar differences in OA were not significant for any of the sampling intervals in 1991 or 1992. However, analysis of combined OA data from the second interval in 1991 and the first and second intervals in 1992, resulted in significant differences (Table 4.17). The first interval from 1991 was not included in the combined analysis because stress levels were low and no OA occurred during this interval. Results showed that 'Rangelander' had the most OA, while 'Excalibur' exhibited the least. Because Ψw was sometimes different for cultivars during these sampling periods, the results may be confounded. However, because 'Excalibur' and 'Rangelander' had similar overall Ψw values (Table 4.14), the differences in OA do appear to be valid. Also, because 'Excalibur' was under the most stress, as indicated by the lowest mean Ψw, and osmoregulated the least, 'Excalibur' appears to have an inferior ability to osmoregulate than the other cultivars.

The most extreme OA values occurred for 'Legend', ranging from +0.13 to -0.40 MPa during the first and second sampling interval of 1991, respectively. These values are similar to those reported for barley (Blum, 1989), where values ranged from +0.17 to -0.46 MPa under moderate stress conditions (Ψw ranged from -1.37 to -1.51 MPa, which was comparable to sampling conditions in the present study). Daily change in OA averaged -0.0006, -0.035, -0.022, and -0.010 MPa d^{-1} between the first and second sampling date and between the third and forth sampling date for 1991 and 1992, respectively. With little change in plant stress during the first interval in 1991 there was only a slight increase in π_{100}. From this information, it can be assumed that most of the increase in OA in subsequent intervals, was mainly due to drought stress with very little change in π_{100} attributable to plant age.

Table 4.17 Osmotic adjustment^z (MPa) in leaf tissue of field-grown alfalfa cultivars (combined data from all sampling dates at Winnipeg in 1991 and 1992).

Cultivar	Osmotic Adjustment
Rangelander	-0.32 a
Legend	-0.30 ab
Nitro	-0.25 abc
Alfagraze	-0.25 abc
Wilson	-0.23 bc
Excalibur	-0.19 c
LSD (0.05)	0.08

Means within a column followed by the same letter are not significantly different at alpha=0.05 (LSD).

z - the difference in π_{100} between the second and first sample taken during the establishment period or between the second and first sample taken on regrowth material

Root osmotic potential adjusted to full turgor (π_{r100}) was significantly higher for 'Rangelander' than the other cultivars during the first and second samplings in 1992 (P=0.0559 and 0.0606, respectively; Table 4.18). Combined analysis of the first three sampling dates also indicated the same results. These results were unexpected, since no differences in leaf π_{100} were detected.

Because of continued fall growth, differences in root solute concentrations between dormant and non-dormant lines could be expected. However, based on orthogonal contrasts, π_{r100} for 'Nitro' and 'Wilson' were shown to be similar to the other cultivars on the final sampling date of the season. In the combined analysis, the non-dormant cultivars did have lower π_{r100} (P=0.0485) as compared with the other cultivars (-0.84 and -0.81 MPa, respectively) although mean differences were smaller than on the final date when π_{r100} was -1.20 and -1.11 MPa for the non-dormant and dormant cultivars, respectively. The combined analysis had a smaller C.V. (5% as compared with 11% for 24 September), which may explain part of the difference in significance.

Table 4.18 Root osmotic potential (MPa) at full turgor in field-grown alfalfa cultivars at Winnipeg in 1992.

Cultivar	30 July 1992	10 Aug. 1992	4 Sept. 1992	24 Sept. 1992	Combined Analysis^z
Rangelander	-0.64 a	-0.84 a	-0.69 a	-1.13 a	-0.73 a
Alfagraze	-0.76 b	-1.03 b	-0.72 a	-1.06 a	-0.84 b
Nitro	-0.78 b	-1.03 b	-0.73 a	-1.20 a	-0.84 b
Legend	-0.78 b	-1.02 b	-0.72 a	-1.18 a	-0.84 b
Excalibur	-0.79 b	-1.04 b	-0.65 a	-1.09 a	-0.82 b
Wilson	-0.81 b	-1.01 b	-0.72 a	-1.20 a	-0.84 b
LSD (0.05)	0.11	0.13	0.09	0.19	0.06

Means within a column followed by the same letter are not significantly different at alpha=0.05 (LSD).

z - only 30 July, 10 August, and 4 September data could be combined due to heterogeneity of error variances.

Root osmotic potential adjusted to full turgor (π_{T100}) decreased from the first to the second sampling date, then increased on the third date (Table 4.11). This increase was likely due to a net movement of carbohydrates into the regrowth tissue. There was a substantial decrease in π_{T100} from the third to the final sampling date (-0.70 to -1.14 MPa) likely because the alfalfa plants accumulated reserves for winter survival and spring regrowth.

Turgor potential is important, not only for maintaining structure, but also for facilitating growth. Brown and Tanner (1983a) found that leaf expansion in alfalfa ceased when P fell below 0.3 MPa. In the present study, mean P was above this critical level on three of the seven dates (Table 4.11). On two of these dates, 22 July and 30 July 1991, high P was attributed to low stress levels (Ψw above -1.00 MPa; Table 4.11). On the third date, 10 August 1992, despite moderate stress levels (Ψw = -1.51 MPa), P was maintained through osmotic adjustment and tissue dehydration. Mean *P* ranged from 0.05 to 0.23 MPa on the other more stressful dates. On these dates, some samples indicated negative turgor potential. Dilution of the protoplast from apoplastic water in the determination of π, may have been be the cause of these small negative values (Markhart et al., 1981).

Only on 10 August 1992 were cultivar differences in *P* detectable. 'Alfagraze' and 'Nitro' had the highest *P*, while 'Excalibur' had the lowest (Table 4.19). In the analysis of all dates combined, 'Alfagraze' and 'Nitro' maintained the highest *P*, while 'Excalibur' and 'Rangelander' had the lowest *P*. As Turner (1981) suggests, *P* data were much more variable than either Ψw or π data. In the present study, C.V. were near 300% on two occasions. The large C.V. values likely contributed to the difficulty in detecting significant cultivar differences.

Table 4.19 Turgor potential (MPa) in field-grown alfalfa cultivars when significant differences were detected (at Winnipeg in 1991 and 1992).

Cultivar	10 Aug. 1992	All Dates Combined
Alfagraze	0.67 a	0.37 a
Nitro	0.65 a	0.41 a
Rangelander	0.57 ab	0.26 c
Wilson	0.50 ab	0.36 ab
Legend	0.39 bc	0.28 bc
Excalibur	0.27 c	0.24 c
LSD (0.05)	0.20	0.09

Means within a column followed by the same letter are not significantly different at alpha=0.05 (LSD).

4.1.6 Relationships Between Water Relations Variables

Relationships between water relations variables have been used to detect and better understand drought tolerance in some species. For example, Noy-Meir and Ginzburg (1969) and Ihe and Thurtell (1981) reported that the ability of a plant to maintain high RWC at a reduced Ψw indicates drought tolerance.

The linear relationship between RWC and Ψw was not significantly different for the alfalfa cultivars, therefore, the entire dataset was analyzed together. The results of the regression analysis are presented in Figure 4.10. A large portion of the variation was explained by the linear effect. Some of the remaining factors contributing to the variation may include small cultivar effects and differences in leaf π_{100} between sampling periods. Using the drought-tolerance theory of Noy-Meir and Ginzburg (1969) and Ihe and Thurtell (1981), based on the relationship between RWC and Ψw, it would appear that alfalfa is less drought tolerant than other crops such as wheatgrass (*Agropyron sps.*), where RWC ranged from about 75-90% at a Ψw of -2.0 MPa (Frank et al., 1984).

The relationship of *P* versus RWC in alfalfa was also linear (Figure 4.11), although the predictability of *P* from RWC is poor ($R^2 = 0.22$). From the equation, it was determined that the point of zero turgor occurred at 63% RWC. This is much lower than values obtained for several species of wheatgrass (88-94%; Frank et al., 1984). Coyne et al. (1982) and Richter (1978) determined that plants with low cell wall elasticity had a higher RWC at zero *P* than plants with high cell wall elasticity, which implies that cell wall elasticity is much greater in alfalfa than wheatgrass. Highly elastic cells may limit the ability of alfalfa to tolerate drought, as more rigid cell walls have been associated with drought tolerance in wheat (Melkonian et al., 1982).

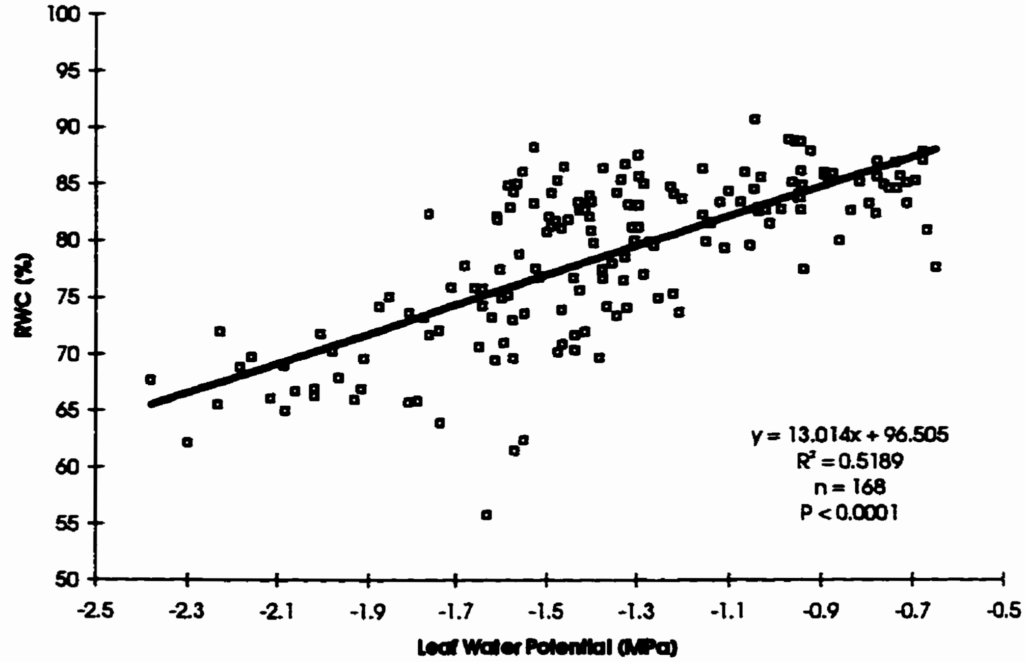

Figure 4.10 Relationship between relative water content and water potential for six alfalfa cultivars grown under field conditions at Winnipeg in 1991 and 1992.

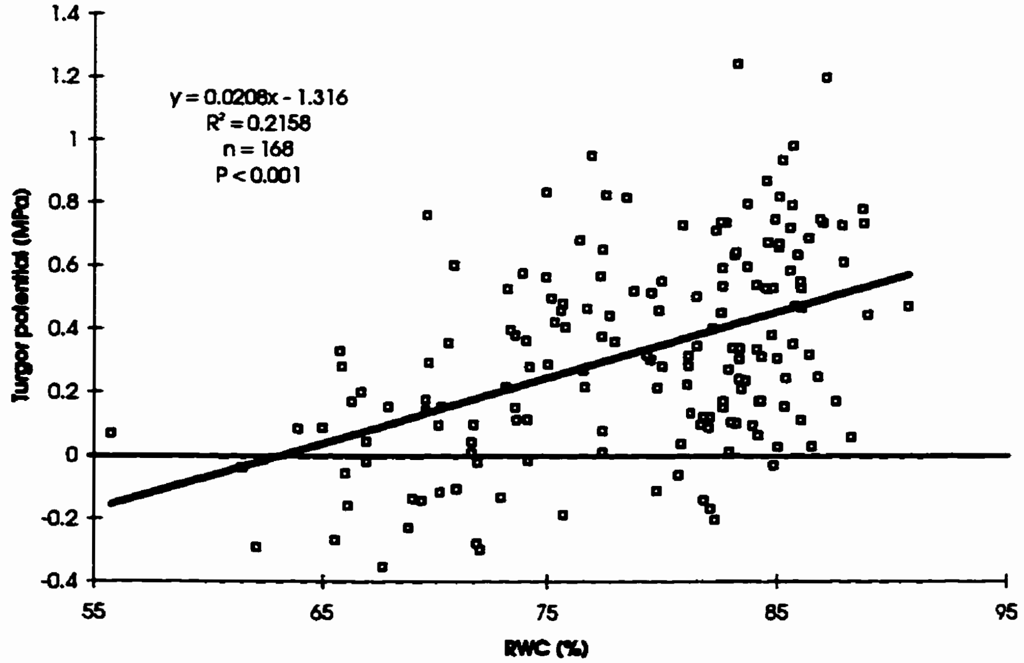

Figure 4.11 Relationship between turgor potential and relative water content for six alfalfa cultivars grown under field conditions at Winnipeg in 1991 and 1992.

The relationship between Ψw and *P* reflects the ability of a plant to maintain turgor potential (necessary for growth) as stress levels increase (i.e., Ψw decreases). The curvilinear relationship for Ψw and *P* from the field data is shown in Figure 4.12. From the equation, it was determined that the point of zero *P* was reached at a Ψw of about -1.9 MPa. In tall fescue (*Festuca arundinacea* Schreb.), the point of zero turgor was reached between -2.0 and -2.6 MPa (White et al., 1992), implying that growth could be maintained at a relatively lower Ψw in fescue than in alfalfa.

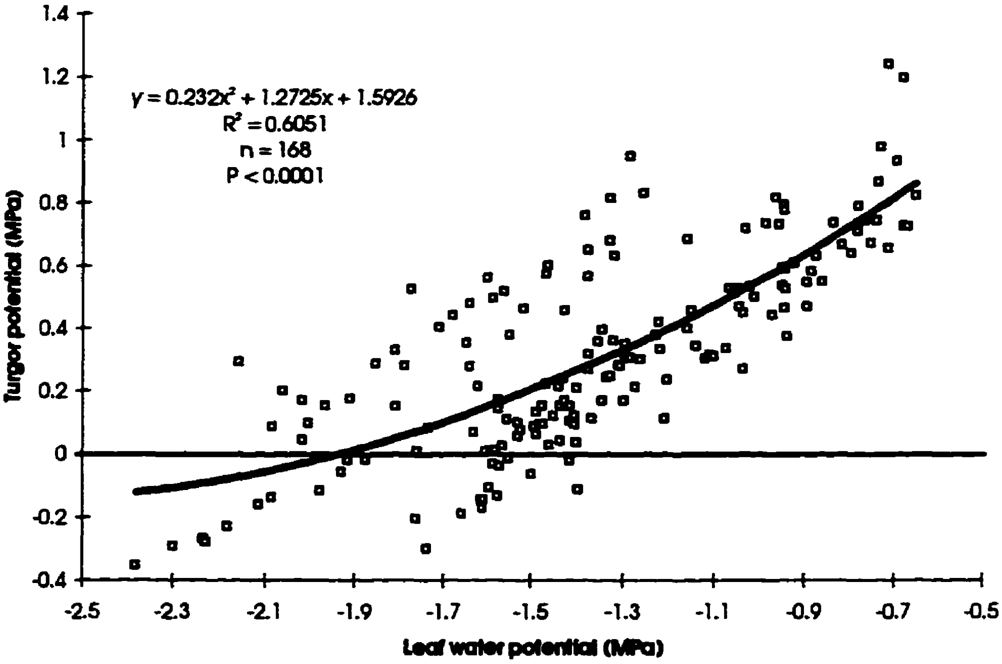

Figure 4.12 Relationship between turgor potential and water potential for six alfalfa cultivars grown under field conditions at Winnipeg in 1991 and 1992.

Carter and Sheaffer (1983a) showed that the relative growth rate of alfalfa reached zero at a midday Ψw of -2.1 MPa. The loss of turgor near this point in the present study supports their findings.

4.1.7 Relationships Between Water Relations and Environmental Variables

Plant water relations are influenced by conditions above and below the soil surface. Using linear regression analysis, mean daily RWC and *P* were shown to be significantly related to maximum daily air temperature (Table 4.20). Maximum air temperature was also the variable that was most closely related to mean daily Ψw ($P = 0.0502$). The absence of a significant relationship with soil moisture indicates that average soil water content did not significantly contribute to plant water relations differences in this study.

Xu et al. (1990) concluded that the diurnal pattern of photosynthesis in wheat was primarily decided by above-ground environmental conditions, while Weatherly (1951) reported that RWC was solely affected by atmospheric conditions as long as soil moisture remained above a critical level. In the present study, only maximum daily air temperature was linearly related to the plant water relations variables (Table 4.20). This observation can be explained by considering the time of day the data was obtained. Because the water relations variables were measured from about 1000 to 1600 h, they will be most closely related to the environmental parameter that describes the conditions during that time. All the other above-ground environmental variables represent an accumulation of daily (ET, SR, and MAT) conditions or depict conditions prior to the sampling time (AT_{Min}). Although it is likely that other factors, such as, air vapor pressure, wind speed and net radiation (Idso et al., 1981), affected plant water status, air temperature seemed to have the greatest effect.

Table 4.20 Linear regression equations for daily mean RWC, Ψw and P as a function of environmental variables on each sampling date at Winnipeg in 1991 and 1992.

Water Relations Variable	Environmental Variable†	Equation‡	n	$P > F$	R^2
RWC	ET_{PD}	$y = 83.9 - 0.65x$	8	0.4246	0.11
	ET_{SD}	$y = 87.6 - 0.94x$	8	0.3520	0.15
	MSM	$y = 54.6 + 0.74x$	8	0.1513	0.44
	MAT	$y = 102.8 - 1.13x$	8	0.1033	0.38
	AT_{Min}	$y = 98.6 - 1.33x$	8	0.1971	0.26
	AT_{Max}	$y = 109.5 - 1.15x$	8	0.0094	0.70
	SR	$y = 78.0 + 0.00x$	8	0.9348	0.00
Ψw	ET_{PD}	$y = -1.29 - 0.01x$	7	0.8887	0.00
	ET_{SD}	$y = -0.74 - 0.07x$	7	0.3666	0.16
	MSM	$y = -1.60 + 0.01x$	7	0.7939	0.03
	MAT	$y = -0.22 - 0.05x$	7	0.3050	0.21
	AT_{Min}	$y = -0.80 - 0.04x$	7	0.6231	0.05
	AT_{Max}	$y = 0.31 - 0.06x$	7	0.0502	0.57
	SR	$y = -2.05 + 0.00x$	7	0.3592	0.17
P	ET_{PD}	$y = 0.23 + 0.01x$	7	0.8283	0.01
	ET_{SD}	$y = 0.71 - 0.04x$	7	0.4276	0.13
	MSM	$y = 0.10 + 0.01x$	7	0.8433	0.02
	MAT	$y = 1.20 - 0.04x$	7	0.2450	0.26
	AT_{Min}	$y = 0.71 - 0.03x$	7	0.6190	0.05
	AT_{Max}	$y = 1.53 - 0.04x$	7	0.0344	0.62
	SR	$y = -0.32 + 0.00x$	7	0.1912	0.31

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† - Previous day ET, Sampling day ET, Mean soil moisture to 90 cm, Mean air temperature, Minimum air temperature, Maximum air temperature, and Solar radiation, respectively.

‡ - X and Y denote the appropriate environmental and water relations variables, respectively.

4.1.8 Diurnal Water Relations Responses

Diurnal water relations variables measurements were conducted on two cultivars, 'Rangelander' and 'Excalibur', on 5 August 1992. 'Rangelander' and 'Excalibur' were selected because they represented two distinct alfalfa types, grazing and hay, respectively, and because some water relations differences were detected between the two cultivars in prior field and controlled water experiments. Only RWC and Ψw will be discussed.

The diurnal response of leaf RWC is shown in Figure 4.14. RWC was surprisingly low (80-84%) at hour 800 (daylight savings time) given that leaves are generally quite turgid early in the morning. However, Weatherly (1951) found that the RWC for cotton could be as low as 87% at 630 h when vapor pressure deficits were high. The RWC decreased until hour 1700 then increased by hour 2200 to values similar to those found at hour 1100.

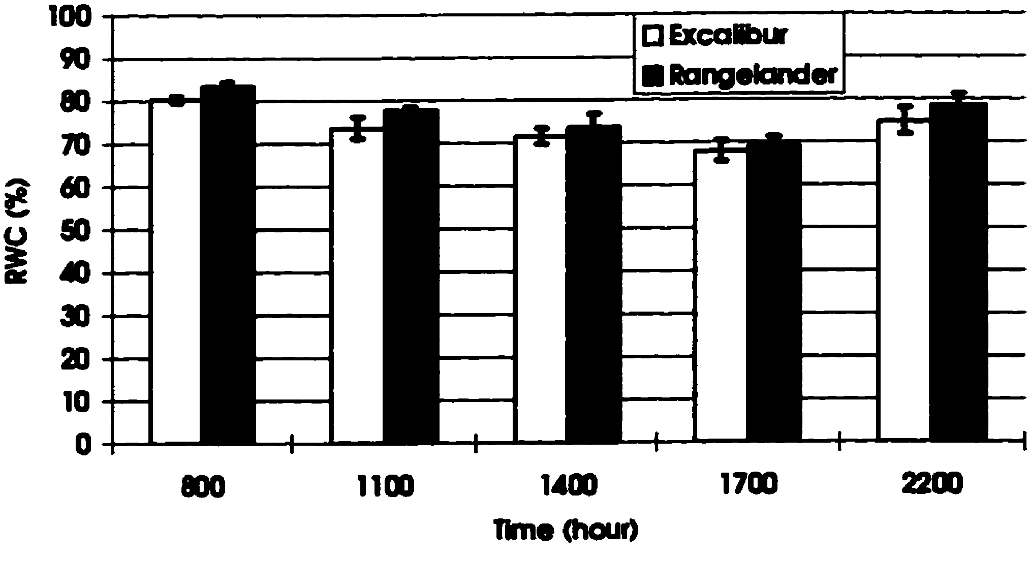

Figure 4.13 The diurnal RWC response in two alfalfa cultivars under field conditions at Winnipeg on 5 August 1992. Mean values with standard error bars are shown. Plants were at full bloom when measurements were taken.

'Excalibur' consistently had lower RWC values than 'Rangelander'; however, differences were only significant at hours 800 and 1100. As the vapor pressure deficit increases, factors such as leaf positioning and angle may become more important (Reed and Travis, 1987) and result in a greater range in RWC levels (i.e., higher SE values) within and between alfalfa plants.

The diurnal pattern of Ψw values was generally similar to that for RWC, with some exceptions. The Ψw values at hour 1400 and hour 1700 were similar (Figure 4.14), whereas the RWC continued to decrease until hour 1700. In addition, the Ψw at hour 2200 was even greater than the Ψw at hour 1100, which may indicate that Ψw recovers more quickly than RWC in alfalfa. This is logical because Ψw is theoretically the driving force for water transport in plants (Schulze et al., 1988). No differences were significant between the two cultivars, although the Ψw for 'Rangelander' was higher at all times except hour 1700.

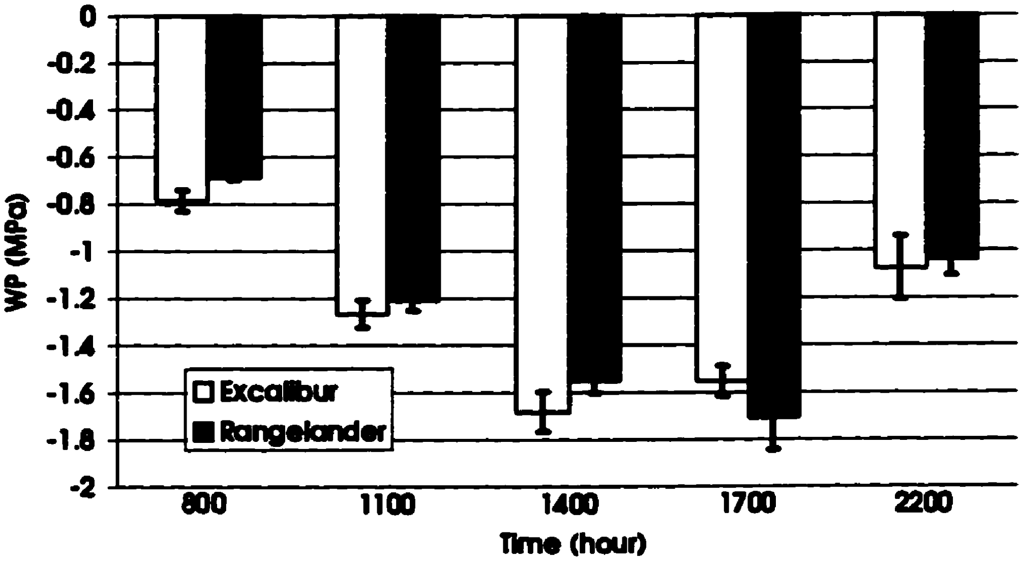

Figure 4.14 The diurnal Ψw response of two alfalfa cultivars under field conditions at Winnipeg on 5 August 1992. Mean values with standard error bars are shown. Plants were at full bloom when measurements were taken.

The decrease in Ψw between 800 and 1400 hours was rapid (-0.17 and -0.13 MPa hour^{-1} between hour 800 and 1100 and hour 1100 and 1400, respectively). This is an important consideration when taking measurements across replicates. The recovery of Ψw was equally amazing, as Ψw increased at a rate of 0.19 MPa hour^{-1} from 1700 to 2200 hours.

4.1.9 Leaf and Canopy Temperatures

Alfalfa cultivar differences in leaf (T_l) or canopy temperature (T_c) may indicate differences in transpiration rates (Hattendorf et al., 1990). Differences in transpiration rates are a result of differential stomatal regulation and may be a mechanism for drought tolerance or drought avoidance in alfalfa.

Mean T_c in this study ranged from 18.4°C on 30 September 1992 to 31.0°C on 29 August 1991. Cultivar differences were significant on 2 of 5 measurements in 1991 and 2 of 15 in 1992. Fewer cultivar differences in 1992 may be explained, in part, by the lower overall air temperatures. The mean T_c recorded for 1992 was 23.5°C, while in 1991 it was 27.0°C.

Differences in cultivar T_c are listed in Table 4.21. 'Nitro' generally had the highest canopy temperature, although it was not usually significantly higher than 'Alfagraze'. 'Legend' had a relatively low T_c in 1991, however, on 30 September 1992, it had the highest T_c. 'Rangelander' had the lowest T_c on 24 September 1992, but like 'Wilson', most often ranked in the middle of the group. 'Excalibur' usually had the lowest canopy temperature, implying a higher transpiration rate.

The implied transpiration rates from T_c data do not correspond with the seasonal ET data reported in Table 4.4. From the T_c data, lower water use would be expected for 'Nitro' and 'Alfagraze'; however, the opposite was noted. Furthermore, 'Excalibur' could be expected to be the most extravagant water user, but was shown to use the least amount of water from soil water extraction data. An explanation for these inconsistencies might be that because T_c was usually measured at midday, it only represents transpiration rates when they were at their lowest. Transpiration rates for 'Nitro' and 'Alfagraze' may have been higher than for 'Excalibur' during parts of the day when the plants are under less stress. Because 'Excalibur' had lower ET levels and an implied higher midday transpiration rate, it may have an inferior ability for stomatal regulation.

Table 4.21 Canopy temperature (°C) of field-grown alfalfa cultivars when significant differences were detected (at Winnipeg in 1991 and 1992).

Cultivar	25 July 1991	30 July 1991	Combined Dates 1991	24 Sept. 1992	30 Sept. 1992
Nitro	22.9 a	29.1 a	27.3 a	21.4 a	18.5 ab
Alfagraze	22.9 a	28.9 a	27.1 ab	21.1 ab	18.5 ab
Legend	22.5 b	28.3 b	26.8 c	21.0 ab	18.7 a
Rangelander	22.6 b	29.0 a	27.1 ab	20.4 c	18.4 abc
Wilson	22.4 b	28.8 ab	27.0 bc	21.1 ab	18.1 c
Excalibur	22.6 b	28.7 ab	27.0 bc	20.8 bc	18.2 bc
LSD (0.05)	0.3	0.5	0.3	0.5	0.4

Means within a column followed by the same letter are not significantly different at alpha=0.05 (LSD).

Leaf temperature (T_l) minus ambient temperature (T_a), as measured with thermocouples on the porometer unit, allowed an additional opportunity to evaluate cultivar leaf temperatures. Cultivar differences occurred on one of three sampling dates and were also significant in a combined analysis of the 1992 data (Table 4.22). Results indicated that T_l - T_a was highest for 'Wilson' and lowest for 'Excalibur'. Temperature differences for the remaining cultivars were similar to either 'Wilson' or 'Excalibur'. These results again imply that 'Excalibur' was transpiring at a higher rate than the other cultivars.

The 1991 measurement date could not be combined due to heterogeneity of error variances.

Table 4.22 Leaf minus cuvette temperature (°C) measured in field-grown alfalfa cultivars when significant differences were detected (at Winnipeg in 1992).

Cultivar	4 Aug. 1992	Combined Dates 1992
Wilson	1.29 a	0.98 a
Rangelander	1.06 ab	0.80 ab
Nitro	0.70 bc	0.60 bc
Legend	0.82 bc	0.60 bc
Alfagraze	0.90 abc	0.53 bc
Excalibur	0.62 c	0.46 c
LSD (0.05)	0.41	0.27

Means within a column followed by the same letter are not significantly different at alpha=0.05 (LSD).

Results for the other cultivars were not entirely consistent with previous canopy temperature conclusions. For example, T_l - T_a was highest for 'Wilson', yet T_c results were often lower, and T_c for was generally highest for 'Nitro', although T_l - T_a results were intermediate. However, the consistent results for 'Excalibur' permit greater confidence in the previous conclusion of higher transpiration rates for this particular cultivar.

The linear relationship between T_l - T_a and leaf conductance was not strong, largely due to variable conductance data (C.V. = 43.2%), although it was statistically significant (Figure 4.15). The linear trend, however, does substantiate the previous assumption that lower leaf temperatures relative to cuvette temperatures, imply higher transpiration rates.

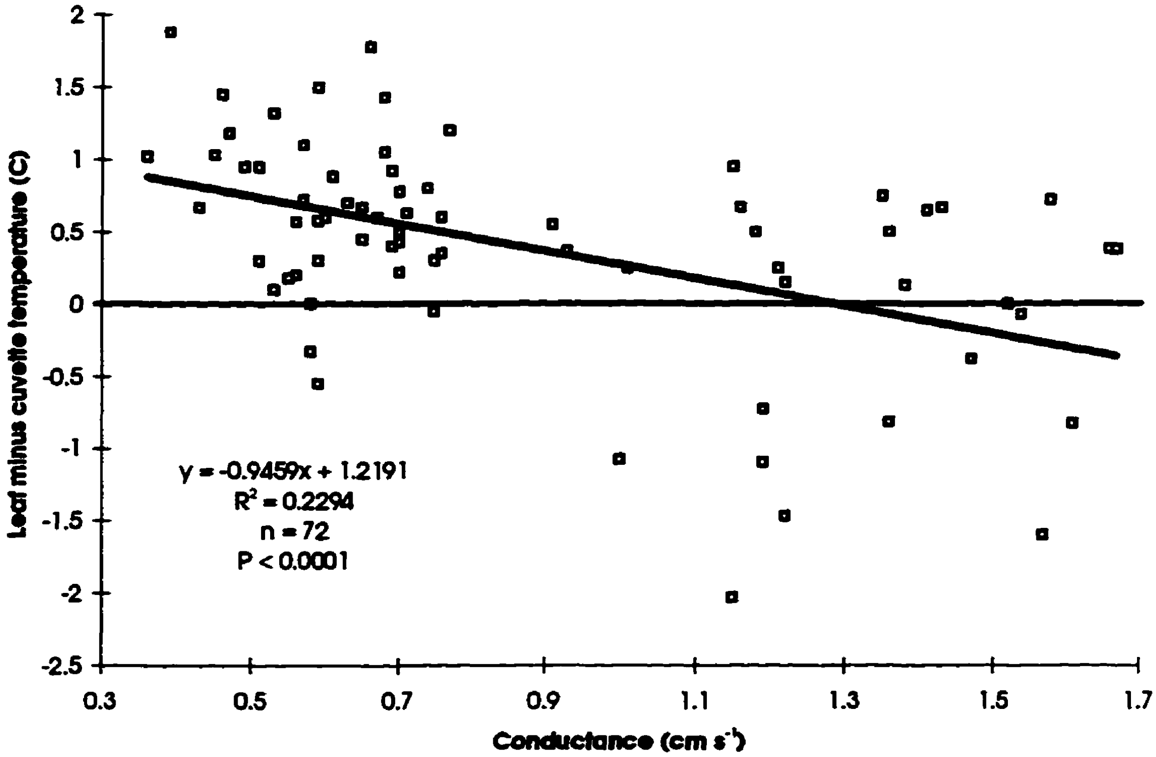

Figure 4.15 The relationship between leaf temperature minus cuvette temperature and leaf conductance under various conditions at Winnipeg in 1991 and 1992, averaged over six alfalfa cultivars.

4.1.10 Leaf Conductance

Leaf conductance (g_l) values in the present study ranged from 0.55 to 1.42 cm s^{-1} (Table 4.23). Values within this range were reported by Carter and Sheaffer (1983b) for medium-low irrigation treatments, with higher and lower values recorded in high and no irrigation treatments, respectively. Carter and Sheaffer (1983b) found that when plants were under little stress ($\Psi w \geq 0.9$ MPa), g_l increased with canopy temperature. Because conductance was lower in 1992 than in 1991, despite higher mean leaf temperatures (26.8 °C, 30.2°C, and 23.6°C for 4 and 9 August 1992 and 25 July 1991, respectively), plant stress must have been a limiting factor. In 1991, Ψw measured on 22 and 23 July was -0.84 MPa, while in 1992, Ψw measured on 10 August was -1.51 MPa. Therefore, these results support the findings of Carter and Sheaffer (1983b) that g_l in alfalfa decreases with decreases in Ψw.

No significant cultivar differences in g_l were noted in the present study (Table 4.23). Hattendorf et al. (1990) had previously shown that a non-dormant alfalfa cultivar, 'CUF-101', had lower rates of g_l than more dormant cultivars, 'Vernema' and 'Vernal', under good moisture conditions. As stress increased, however, differences in stomatal conductance were not detectable. Because plants in the present study were at least moderately stressed during periods of g_l measurement, cultivar differences may also have been more difficult to detect.

Table 4.23 Leaf conductance (cm s^{-1}) in field-grown alfalfa cultivars (at Winnipeg in 1991 and 1992).

Cultivar	25 July 1991	4 Aug. 1992	9 Aug. 1992
Alfagraze	1.33 a	0.60 a	0.65 a
Excalibur	1.39 a	0.70 a	0.69 a
Legend	1.42 a	0.73 a	0.63 a
Nitro	1.39 a	0.62 a	0.65 a
Rangelander	1.37 a	0.61 a	0.62 a
Wilson	1.24 a	0.55 a	0.52 a
LSD (0.05)	0.32	0.21	0.20

Means within a column followed by the same letter are not significantly different at alpha=0.05 (LSD).

4.2 Controlled Water Experiments

4.2.1 Introduction

Controlled water experiments were conducted to further evaluate differences in productivity, water use (evapotranspiration), and plant water relations between alfalfa cultivars. Five of the cultivars were the same as those grown in the field, while 'South African' was substituted for 'Legend' in the controlled water study. Controlled water experiments were used because they permitted the control of soil moisture; something that was not possible in our field trials. The experiment was divided into three Phases. During Phase 0, which was the establishment period, both water treatments were treated equally. In Phase I, the differential watering took place, as treatments were either droughted or well-watered. At the end of Phase I, the water content of both treatments was increased to field capacity, which was then followed by Phase II where both water treatments were droughted. One purpose of Phase I was to evaluate alfalfa water relations under two water regimes. However, the main objective was to compare water relations responses and productivity between alfalfa cultivars and to investigate possible interactions between the cultivars and water treatment. The objective of Phase II was to determine whether pre-stressing alfalfa affected subsequent water relations and whether this pre-stress conditioning effect was different between alfalfa cultivars. These experiments were conducted in 1991, in early 1992 and in mid-1992, and will be discussed as experiments one, two and three, respectively.

4.2.2 Shoot and Root Dry Matter Production

The well-watered treatment yielded the largest amount of aerial dry matter in all three experiments. Above-ground yields from the droughted treatment were 58, 64, and 69% of the well-watered treatment in experiments one, two and three, respectively. This appeared to be correlated with the percentage of days the droughted treatment was differentially under stress (i.e., length of Phase I), which was 26.5, 25.3, and 23.4% for experiments one, two and three, respectively. However, analysis indicated a non-significant relationship between yield and drought-days, despite a highly negative correlation coefficient ($r=-0.98$).

In experiment one, 'Nitro' was the highest yielding and 'Wilson' the lowest yielding cultivar (Table 4.24). The other cultivars were equal to 'Wilson' except for 'Excalibur', which was equal to 'Nitro'. A significant water treatment × cultivar interaction was observed in experiment three. In this experiment, 'Wilson' yielded less than the other cultivars in the well-watered treatment, while all cultivars produced similar amounts of above-ground dry matter in the droughted treatment (data not shown). No cultivar differences were observed in experiment two.

In terms of cultivar performance, some similarities between the field and controlled water experiments were apparent. 'Wilson' had the lowest first-cut yield in both years of the field experiment and in both cases of the controlled water experiment where it was included. In addition, 'Excalibur' yielded well (i.e., not significantly different from the highest yielding cultivar) in both the field and controlled experiments. The relatively low yields for 'Alfagraze' in the controlled experiment, however, were not consistent with field experiments, where yields of this cultivar were generally high. Perhaps, the productivity of 'Alfagraze' is superior only when the benefits of its prolific rooting system (Table 4.24) are realized (Table 4.1).

Significant differences in root production were evident between water treatments and between cultivars (Table 4.24). Root yields were always lower in the droughted treatment and were 70, 68, and 65% of the non-stressed treatment yield in experiments one, two and three, respectively. Correlation analysis of these values with the percentage of days the droughted treatment was under stress until the end of Phase I (26.5, 25.3, and

23.4% for experiments one, two and three, respectively), yielded a significant and very strong relationship ($r=1.0$). Of particular interest was the fact that shoot weight decreased, while root weight increased with increased duration of stress. Although uncontrolled environmental factors may have influenced the relationships in some manner, the opposing trends suggest that, under stress, more assimilate was directed toward the roots. This is not unexpected, as this is probably a stress response mechanism. For example, grazing tolerant plants were distinguished from intolerant by their ability to maintain root total nonstructural carbohydrates during severe grazing in Georgia (S.R. Smith, pers. comm.).

Cultivar differences for root dry matter production were significant in all three experiments. 'Alfagraze' produced the most root dry matter in experiments one and three, while 'Rangelander' produced the least root dry matter in all three experiments. Brummer and Bouton (1992) previously demonstrated the ability of 'Alfagraze' to produce root dry matter equal to or greater than 'Florida 77' and 'Travois', especially under frequent clippings. The 'South African' cultivar also produced a root mass equal to that of 'Alfagraze'. Lower root production for 'Rangelander' may be related to slower growth rates for *M. falcata* cultivars, even though McElgunn and Heinrichs (1975) were unable to consistently show lower root production for *M. falcata* cultivars. 'Wilson' produced a lower root mass than many of the cultivars in experiment one and was equal to 'Rangelander' in experiment three. 'Nitro' produced a large root mass in experiments one and two; however, in experiment three, its root production was less than average. A large root mass is expected for 'Nitro', as this was one of the selection criteria used in its development (Barnes et al., 1988b). Although Carter et al. (1982) found a significant cultivar × moisture interaction for root weight in their study, the interaction was not significant in any of the three present experiments.

Table 4.24 Dry matter production (g container^{-1}) of shoots and roots and the ratio of root mass to shoot mass for container-grown alfalfa under controlled watering at Winnipeg.

Experiment	Treatment/ Cultivar	Shoot Yield	Root Yield	Total Yield^{z}	R:S Ratio
One	Well-watered	19.13 a	31.27 a	50.40 a	1.65 b
	Droughted	11.11 b	21.88 b	32.98 b	1.97 a
	LSD (5%)	1.02	1.83	2.49	0.13
	Alfagraze	14.28 c	30.78 a	45.06 a	2.21 a
	Excalibur	16.13 ab	26.08 bc	42.21 ab	1.67 c
	Nitro	16.74 a	29.25 a	45.99 a	1.78 bc
	Rangelander	14.72 bc	20.30 d	35.02 c	1.43 d
	South African	14.72 bc	28.65 ab	43.37 a	2.00 ab
	Wilson	14.11 c	24.38 c	38.49 bc	1.77 bc
	LSD (5%)	1.76	3.16	4.32	0.23
	Mean	15.12	26.58	41.69	1.81
Two	Well-watered	33.90 a	42.07 a	75.97 a	1.24 a
	Droughted	21.80 b	28.44 b	50.24 b	1.31 a
	LSD (5%)	3.14	5.18	7.36	0.15
	Excalibur	28.62 a	35.49 ab	64.11 ab	1.26 ab
	Nitro	28.56 a	39.79 a	68.34 a	1.40 a
	Rangelander	26.38 a	30.48 b	56.86 b	1.16 b
	LSD (5%)	3.85	6.34	9.02	0.19
	Mean	27.85	35.25	63.10	1.27
Three	Well-watered	24.97 a†	32.78 a	57.75 a	1.31 a
	Droughted	17.35 b	21.30 b	38.65 b	1.23 a
	LSD (5%)	1.60	3.22	4.53	0.09
	Alfagraze	20.68 bc	31.32 a	52.00 ab	1.52 a
	Excalibur	23.90 a	29.11 abc	53.01 a	1.20 bc
	Nitro	21.22 abc	25.49 bcd	46.71 abc	1.18 bc
	Rangelander	21.70 ab	22.69 d	44.39 bc	1.06 c
	South African	20.85 bc	30.02 ab	50.87 ab	1.42 a
	Wilson	18.61 c	23.63 cd	42.24 c	1.25 b
	LSD (5%)	2.77	5.57	7.85	0.16
	Mean	21.16	27.04	48.20	1.27

Means within a continuate column followed by the same letter are not significantly different at alpha=0.05 (LSD).

† - water treatment by cultivar interaction is significant at alpha=0.05.

z - sum of shoot and root yields.

Variation in root yield between cultivars was much greater than cultivar differences for shoot yield. Shoot yield varied by 17, 8 and 25% for experiments one, two and three, respectively, while root yield for the same experiments varied by 39, 26 and 32%, respectively. Shoot yield is an important criteria in developing new cultivars, while root production is rarely considered. This is likely the reason for the differences in variation.

Water treatment and cultivar differences were also significant for the combined root and shoot yields (Table 4.24). As expected, total yield was greatest for the well-watered treatment in all three experiments. Generally, cultivar rankings were similar between the three experiments, although some minor differences were noted.

In experiment one, 'Alfagraze', 'Nitro' and 'South African' yielded the most total dry matter. 'Rangelander' yielded the least, but was not significantly different than 'Wilson'. 'Excalibur' yielded slightly less than the top three cultivars, but was not significantly different from any of them. Although experiment two had only three cultivars, similar variation was observed. For example, 'Nitro' produced the greatest amount of dry matter, although the production of 'Excalibur' was similar. 'Rangelander' was much lower at only 83% of that for 'Nitro'. In experiment three, 'Excalibur' had the highest total production, although, 'Alfagraze', 'Nitro' and 'South African' were not significantly different. 'Rangelander' was intermediate and not significantly different from the highest and lowest yielding cultivars. 'Wilson' had the lowest total production, mainly due to much lower shoot yields.

Ratios of root yield to shoot yield (R:S) ranged from 0.72 to 2.49 over the three experiments (Table 4.24). Jodari-Karimi et al. (1983) previously reported mean R:S ratios in alfalfa, ranging from 0.2 to 0.8, for three water treatments during different times of the year. Other research has indicated that R:S ratios increase from about 0.5 to 1.0 within 2-4 months from seeding (Gist and Mott, 1958; Matches et al., 1962). Higher R:S ratios in the present study may have been due to the following reasons. First, clipping of the shoots for water relations samples would likely reduce shoot production more than root production. Second, leaves lost due to senescence were largely unaccounted for, whereas dead roots were most often recovered. This was especially important in the "rain-out" shelter experiment, where wind likely removed many leaves. Third, some insect damage may have reduced shoot production more than root production. On the other hand, McElgunn and Heinrichs (1975) found that higher soil temperatures increased alfalfa shoot production more than root production (i.e., lowered the R:S ratio). In the present study, soil temperatures were likely slightly higher than typical field conditions for the "rain-out" shelter experiments, and were likely much higher in the two greenhouse experiments. Based on the higher R:S ratio results in the present study, the importance of the first and second reasons given above, outweighed any potential effect of higher soil temperatures.

In experiment one, drought stress resulted in a higher R:S ratio. This is consistent with Jodari-Karimi et al. (1983), who found that greater R:S ratios in alfalfa were associated with increased stress, although Gist and Mott (1957) reported decreasing R:S ratios with increased drought stress in container-grown seedlings.

Two factors likely contributed to the lack of significant differences between water treatments in 1992. First, because initial growing conditions were warmer in 1992 (data not shown), most of the root growth may have occurred before Phase I was initiated. Second, with the absence of wind in the greenhouse, a greater number of senesced leaves would have remained attached and would be included in the shoot dry matter yields.

The cultivar effect was significant for R:S ratio in all three experiments (Table 4.24). 'Alfagraze' had the highest R:S ratio in experiments one and three, but was not significantly greater than 'South African'. In experiment two, 'Nitro' had the highest R:S ratio, but was not significantly greater than 'Excalibur'. 'Rangelander' had the lowest R:S ratio in all three experiments, mainly due to lower root production.

A high R:S ratio implies a relatively larger root area from which water can be gathered to supply the shoot and transpiring leaves. However, root production does come at a cost. Passioura (1983) speculated that the cost in water of producing root dry matter was at least double that for the shoot. Therefore, cultivars that produce an abundance of root dry matter, when moisture is non-limiting, would have lower ETE. Passioura also suggested that reducing the R:S ratio may be a means to marginally increase the ETE in some crops. Following this logic, we would expect 'Rangelander' to be more water-use-efficient, while cultivars such as 'Alfagraze', would be less water-use-efficient under non-limiting soil moisture conditions. This was the case in experiment one (Table 4.26), where it could be assumed that root production was not a limiting factor because of the limited soil volume in the containers. However, in the field study, ETE was similar for both cultivars during the establishment period (Table 4.9), when soil moisture was most adequate.

4.2.3 Evapotranspiration and Evapotranspiration Efficiency

Figures 4.16 through 4.18 show the average cumulative amount of water added to each of the water treatments for experiments one, two and three, respectively. All figures show that the initial uptake of water was quite low. This slow uptake continued for the first 45 to 55 days, reflecting the delayed establishment of the plants under artificial conditions. Part of the slow establishment was due to lower temperatures and/or reduced light, as compared with normal field conditions. The final data point represents the amount of water that would have been required to bring the soil water content up to field capacity, which was the starting moisture level.

Total evapotranspiration (ET) was higher in the well-watered treatment compared with the droughted treatment in each year, which confirms that the water treatments were dissimilar (Table 4.25). Droughted treatments received 33, 37 and 39% less water than the well-watered treatments in experiments one, two and three, respectively. This indicates that the ratio between the two water treatments remained relatively consistent, despite large differences in total mean ET between the experiments. For example, the mean water use in experiment two was 70% higher than in experiment one. Greater water use in 1992 was probably due to a longer experiment duration (about 25% longer).

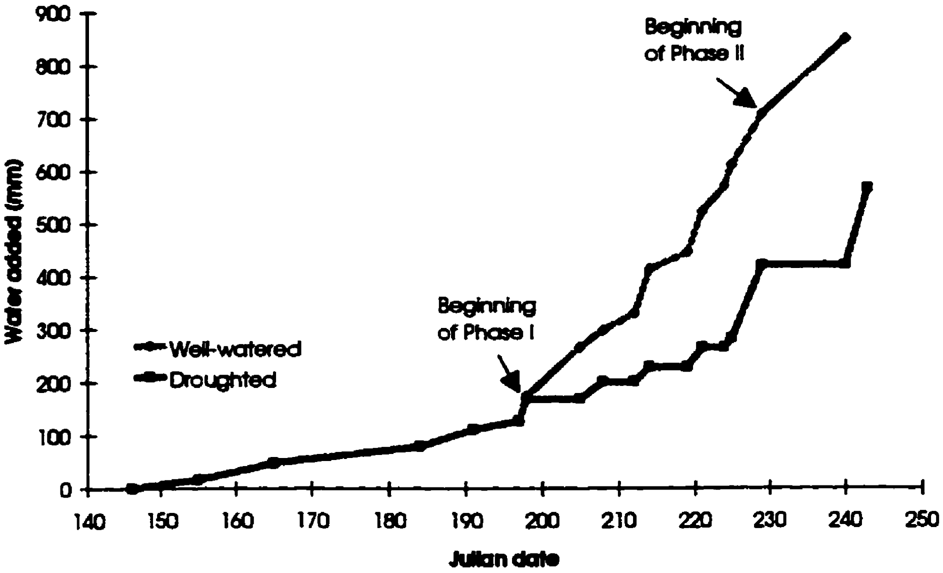

Figure 4.16 Average cumulative amount of water added (mm) to the well-watered and droughted treatments in experiment one.

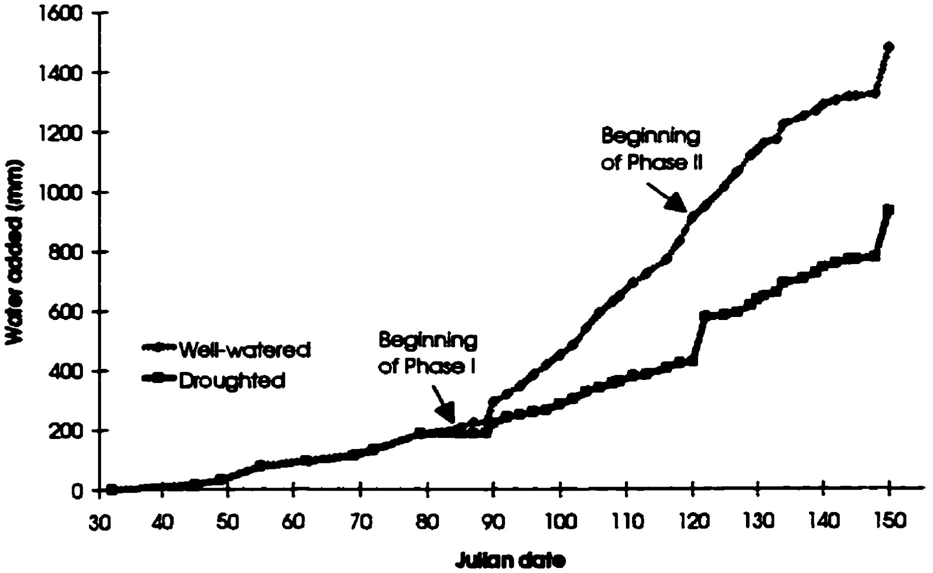

Figure 4.17 Average cumulative amount of water added (mm) to the well-watered and droughted treatments in experiment two.

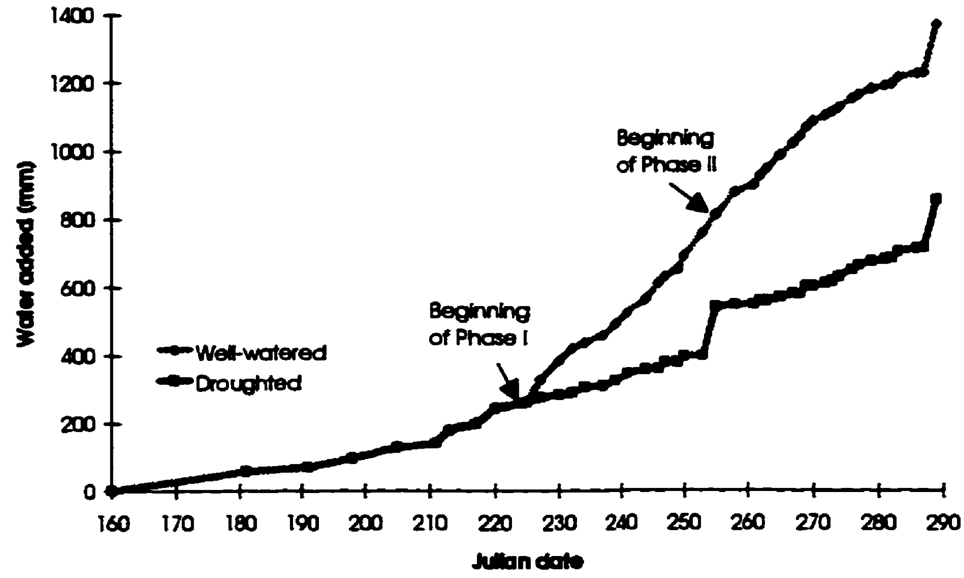

Figure 4.18 Average cumulative amount of water added (mm) to the well-watered and droughted treatments in experiment three.

Cultivar differences in ET were significant in experiments one and two, however, no differences were found in experiment three. 'Nitro' used the most water, while 'Rangelander' used the least water in experiments one and two. This is consistent with the results from the combined analysis of the first growth cycle in the field study (Table 4.4). The other cultivars were intermediate in their ET with 'Excalibur' equal to 'Nitro' and 'Wilson' equal to 'Rangelander' in experiment one and 'Excalibur' equal to 'Rangelander' in experiment two.

Table 4.25 Total (mm) and daily evapotranspiration (mm d^{-1}) during Phase 0, I and II for container-grown alfalfa under controlled watering at Winnipeg.

Experiment	Treatment/ Cultivar	Total ET	Daily ET Phase 0	Daily ET Phase I	Daily ET Phase II	Difference between I and II ^{z}
One	Well-watered	849 a	4.5 a	20.0 a†	12.9 a	7.1 a†
	Droughted	565 b	2.9 b	11.4 b	10.4 b	1.0 b
	LSD (5%)	26	0.2	0.7	0.2	0.7
	Alfagraze	709 bc	3.8 a	15.7 b	11.5 a	4.2 bc
	Excalibur	715 abc	3.6 a	16.2 ab	11.7 a	4.5 ab
	Nitro	758 a	3.9 a	17.4 a	11.7 a	5.7 a
	Rangelander	652 d	3.4 a	14.0 c	11.4 a	2.3 d
	South African	727 ab	3.9 a	16.1 b	11.8 a	4.3 bc
	Wilson	679 cd	3.5 a	14.9 bc	11.7 a	3.2 cd
	LSD (5%)	45	0.4	1.3	0.4	1.2
	Overall Mean	707	3.7	15.7	11.6	4.1
Two	Well-watered	1478 a	6.2 a	23.0 a	19.0 a	4.0 a
	Droughted	930 b	4.0 b	13.5 b	12.7 b	0.8 b
	LSD (5%)	96	0.5	2.3	1.2	1.9
	Excalibur	1204 ab	5.1 b	17.8 a	16.2 a	1.7 a
	Nitro	1280 a	5.7 a	19.4 a	16.1 a	3.3 a
	Rangelander	1128 b	4.5 b	17.5 a	15.2 a	2.3 a
	LSD (5%)	118	0.6	2.8	1.5	2.3
	Overall Mean	1204	5.1	18.2	15.8	2.4
Three	Well-watered	1372 a	5.9 a	17.9 a	17.0 a†	0.9 a
	Droughted	839 b	3.8 b	11.4 b	9.6 b	1.9 a
	LSD (5%)	86	0.5	1.8	0.9	1.6
	Alfagraze	1140 a	5.0 a	16.1 a	13.0 a	3.1 a
	Excalibur	1129 a	4.7 a	15.0 a	14.1 a	0.8 a
	Nitro	1141 a	5.2 a	15.2 a	13.3 a	1.9 a
	Rangelander	1083 a	4.6 a	14.5 a	13.4 a	1.1 a
	South African	1092 a	4.9 a	13.9 a	13.3 a	0.6 a
	Wilson	1047 a	4.8 a	13.4 a	12.4 a	0.9 a
	LSD (5%)	148	0.8	3.1	1.5	2.7
	Overall Mean	1105	4.9	14.7	13.3	1.4

Means within a continuate column followed by the same letter are not significantly different at alpha=0.05 (LSD).

† - water treatment by cultivar interaction is significant at alpha=0.05.

z - Daily ET Phase I minus Daily ET Phase II.

Although some similarities between the field and controlled water studies were observed, as previously mentioned, some distinctions between cultivars may not be apparent using a limited soil volume. By the end of each experiment, root growth had reached the bottom of the container; therefore, possible differences in root length may have had little effect on the capacity to extract water. This could create some differences between the relative performance of cultivars in the field versus controlled water studies. For example, 'Excalibur' was shown to have lower ET than 'Nitro' in the field, but was similar to 'Nitro' in the first controlled water experiment. 'Excalibur' was also shown to have a shallower depth of water extraction in the 1992 field experiment. If this was the main limitation to its ability to extract water, similar differences in a controlled study may not have been detected. On the other hand, because 'Rangelander' had the lowest ET among cultivars in both the controlled and field studies, characteristics other than root length likely limited, at least in part, its ability to extract soil water. Other traits that would limit soil water extraction include high axial resistance to flow in the roots (Passioura, 1983) and lower root density.

Differences in mean daily ET were significant between water treatments in all experiments over all three time periods (Table 4.25). In Phase 0, mean daily water use was lower for droughted treatments because it included the period of time that soil moisture was being lowered to stress levels. During Phase I, water use was lower for droughted treatments because water application was restricted. In Phase II, despite both treatments being droughted, water use was still greater in the previously well-watered treatment, due to greater shoot and root mass. This is an important consideration when interpreting the concept of pre-stress conditioning. The reduction in daily water use (difference between Phase I and Phase II) was greater for the well-watered treatment in experiments one and two, however, there was no significant difference in experiment three.

Daily water use during Phase II was reduced by a greater extent in experiment one than in experiments two and three. This difference is explained by the way the two years were treated. In experiment one, water was withheld from all containers; therefore, in previously well-watered containers, soil moisture was used quickly and plants were under stress for a longer time. In experiments two and three, water was added to those containers that used water more quickly, so that soil moisture was maintained at consistent levels for all containers. Although the previously well-watered treatments in experiments two and three were under stress for a longer period of time, it was proportionately shorter than in experiment one.

The daily rates of water use were generally much higher than those reported for field studies. Carter and Sheaffer (1983a) reported values of up to 10 mm d^{-1} under non-limiting soil moisture conditions, while in the field experiments of the present study, values ranging from 3.1 to 4.9 mm d^{-1} were recorded. The mean values of the well-watered treatments ranged as high as 23.0 mm d^{-1} in Phase I, experiment two. These results are opposite those of Fairbourn (1982), who found that evapotranspiration was 100 to 200% higher in the field compared to greenhouse studies for various forage species. Higher rates of daily ET can be partly attributed to: a) greater leaf exposure to light and wind for potted plants, b) higher soil temperatures, and c) the high conductivity of a fine textured soil, which was constantly damp for the well-watered treatment in Phase I.

Hattendorf et al. (1990) had previously shown that the water consumption of a non-dormant alfalfa cultivar was initially higher after harvest as compared with dormant cultivars. In the present study, mean daily water use was analyzed to determine if the cultivars used water differently over the three assigned periods. Cultivar differences in mean daily water consumption were only significant in the well-watered treatment of Phase I, experiment one; Phase 0, experiment two; and in the well-watered treatment of Phase II, experiment three (Table 4.25). In Phase I, experiment one, the well-watered treatment of 'Nitro' had the highest rate of daily water use, 'Rangelander' had the lowest, and the other cultivars were intermediate. 'Nitro', again, had a higher rate of water use in experiment two. In experiment three, the rate of daily water use was lower for 'Wilson' than the other previously well-watered cultivars. All significant interactions, as indicated in Table 4.25, resulted because there were cultivar differences in the well-watered treatment, but no differences between cultivars in the droughted treatment. The lack of consistent cultivar differences for water use during specific periods made it difficult to form any concrete conclusions. However, in cases where the water use rates were different between the cultivars, the rankings were similar to the cultivar ranking for the total ET for each of the experiments.

Some differences in both shoot and whole plant evapotranspiration efficiency (ETE) between the droughted and well-watered treatments were noted from the analysis, although results were not consistent between experiments (Table 4.26). In experiment one, the well-watered treatment had greater shoot ETE, while in experiment two, the droughted treatment had greater whole plant ETE and in experiment three, both the shoot and whole plant ETE were higher for the droughted treatment. Results from experiment three contrast findings of Carter and Sheaffer (1983a) that irrigated alfalfa had a higher ETE than rain-fed alfalfa.

Evapotranspiration efficiency values were much lower in the controlled experiments compared with the field experiments. The mean value for the three controlled experiments converted to 6.75 kg ha^{-1} mm^{-1}, which was about half of the mean values reported in the field study. Reduced lighting, having a soil surface that was often wet, thus allowing greater evaporation, and clipping a higher portion of dry matter for water relations samples were factors that contributed to lower ETE in the controlled water experiments.

Table 4.26 Evapotranspiration efficiency (mg mm^{-1}) of container-grown alfalfa under controlled watering at Winnipeg.

Experiment	Treatment/Cultivar	Shoot ETE	Whole Plant ETE
One	Well-watered	22.6 a	59.2 a
	Droughted	19.7 b	58.3 a
	LSD (5%)	1.0	1.8
	Alfagraze	20.0 c	63.7 a
	Excalibur	22.3 a	59.1 bc
	Nitro	21.9 ab	60.8 ab
	Rangelander	22.0 ab	52.9 d
	South African	20.0 c	59.6 b
	Wilson	20.4 bc	56.4 c
	LSD (5%)	1.8	3.0
	Mean	21.1	58.7
Two	Well-watered	23.0 a	51.2 b
	Droughted	23.6 a	54.1 a
	LSD (5%)	2.0	2.7
	Excalibur	24.0 a	53.8 a
	Nitro	22.2 a	53.3 a
	Rangelander	23.6 a	50.8 a
	LSD (5%)	2.4	3.4
	Mean	23.2	52.6
Three	Well-watered	18.3 b	42.0 b
	Droughted	20.7 a	45.9 a
	LSD (5%)	1.2	2.4
	Alfagraze	18.6 bc	46.5 a
	Excalibur	21.6 a	47.3 a
	Nitro	18.8 bc	40.9 b
	Rangelander	20.5 ab	41.8 b
	South African	19.2 bc	46.7 a
	Wilson	18.2 c	40.5 b
	LSD (5%)	2.2	4.1
	Mean	19.5	43.9

Means within a continuate column followed by the same letter are not significantly different at alpha=0.05 (LSD).

'Excalibur' had the greatest shoot ETE in all three experiments, but was only significantly higher than the other cultivars in experiments one and three. It was suggested, based on observations in the field study, that 'Excalibur' may have an inferior ability for stomatal regulation. Although this may be a detrimental characteristic under prolonged drought conditions, it may serve as a basis for increased ETE in 'Excalibur' under short-term or moderate drought conditions. Evapotranspiration efficiency was also generally higher for 'Rangelander', although this was contrary to seasonal ETE results in the field study. One reason for the difference may be that root development was a limiting factor for 'Rangelander' in the field studies (as indicated with water extraction differences in Tables 4.7 and 4.8), which in turn caused greater plant stress and lower ETE. Because of the limited soil volume in the controlled water study, root production was not likely as limiting for soil water extraction. Therefore, unlike the more prolific root producing cultivars, 'Rangelander' did not waste resources producing unnecessary roots. 'Alfagraze' and 'Nitro' had the lowest shoot ETE in experiment one and were not significantly different from 'Wilson', which was lowest, in experiment three. 'Alfagraze' and 'Nitro' generally produced a larger root mass (Table 4.24), which may have been wasteful under the limits of a container-grown experiment. 'Wilson' simply had low shoot production in experiment three.

A large root system may an important drought tolerance characteristic for 'Alfagraze', 'Nitro' and the 'South African' cultivar. It may enable these cultivars to extract greater amounts of soil moisture to meet evaporative demand, thus reducing plant stress. However, if root growth is at the expense of shoot growth, aerial dry matter production of "root producers" (cultivars that produce relatively greater amounts of root mass) may not be as water-use-efficient under well-watered conditions (Passioura, 1983). For example, in the controlled water experiments, 'Alfagraze's greater root production may have been unnecessary and may have caused relatively lower shoot yields. Under field conditions, however, greater root production could increase soil moisture availability during critical periods and allow the maintenance of water relations favorable for growth.

When root production is included in ETE calculations, the true efficiency of carbon assimilation can be examined. In the controlled water experiments this calculation changed the previous ETE ranking of the cultivars. As a result, cultivars that produced a greater root mass, such as 'Alfagraze' and 'Nitro' in experiment one, and 'Alfagraze' and 'South African' in experiment three, had superior ETE values (Table 4.26).

4.2.4 Plant Water Relations: General Trends

Trends for water potential, osmotic potential, and the resulting turgor potential are presented by water treatment in Figures 4.19, 4.20 and 4.21 for experiments one, two and three, respectively. In Phase I, the well-watered treatment had nearly constant water, osmotic and turgor potentials. Over this same period, osmotic and water potentials for the droughted treatment generally decreased (but was variable), while turgor potential was variable. The consistency of Ψw values in the well-watered treatment reflects the non-limiting soil water conditions, while slight variations are likely due to differences in above-ground environmental conditions between dates. The variation in mean Ψw values for the droughted treatment is due to both differences in soil moisture and above-ground environmental conditions between dates.

Leaf RWC remained relatively constant for the well-watered treatment in Phase I. The consistency of RWC for the well-watered treatment during Phase I suggests that above-ground environmental conditions were reasonably similar on all four dates. This suggestion is based on the findings of Weatherly (1951), who reported that RWC fluctuations in cotton were affected solely by atmospheric conditions as long as soil moisture was above a critical value. Drought stressed plants, on the other hand, will show a response to both unfavorable atmospheric conditions, such as a high vapor pressure deficit and high temperatures, and to dry soil conditions. Without a controlled environment it is difficult to determine how much of the fluctuation in RWC for the droughted plants was due to air or soil effects. The differences observed between the two treatments in the present study isolate the soil water effect because both treatments were subject to the same above-ground conditions. However, over time, physiological changes within the plant also mask the above and below ground effects. This may be apparent at the end of Phase I in experiment one. The rise in RWC over the last two dates in the droughted treatment was likely due to plant adaptation for two reasons. First, it appears that above-ground conditions were less favorable due to direct (Appendix B; Table B1.1) and indirect evidence (i.e., RWC also decreased for the well-watered treatment during this period). Second, soil moisture in the droughted treatment was as low or lower on the fourth sampling date as compared with the third date.

For the droughted treatment, RWC trends through Phase I varied for each experiment (Figures 4.22, 4.23, and 4.24). In experiment one, the mean RWC decreased from the first date, then increased on the final date. The increase at the end of Phase I was likely due to OA, although differences in atmospheric conditions could have also played a role. In experiment two, RWC was stable over the first two dates, likely indicating that soil moisture was not below a critical level, then decreased gradually as soil moisture levels were decreased. Relative water content decreased rapidly by the second sampling date in experiment three, then stabilized. This observation was likely due to much lower soil moisture levels on the final two dates.

The ability of alfalfa to osmotically adjust is shown in Figures 4.22, 4.23, and 4.24. Adjusted osmotic potential decreased substantially for the droughted treatment in Phase I, while π_{100} for the well-watered treatment was stable or showed a very gradual decline. The gradual decline for the well-watered treatment is likely due to a decreasing growth rate as the plants began the transition from vegetative to reproductive growth stages, while the decline in the droughted treatment is a combination of the physiological response to drought stress and the maturation stage.

In Phase II, some differences resulting from pre-stress conditioning were observed. For example, a more rapid decline in all water relations variables was apparent for the previously well-watered treatment compared with the well-watered treatment in experiment one (Figure 4.19 and 4.22). This was likely due to the larger plant biomass (morphological difference), which reduced soil moisture content to critical levels more quickly. In both tests conducted in 1992, soil water content was maintained at similar levels between the water treatments during Phase II, thus revealing physiological differences due to previous drought conditioning. Although the values were sometimes dissimilar (data to be discussed later) the trends for the water relations parameters were similar for both the previously well-watered and droughted treatments.

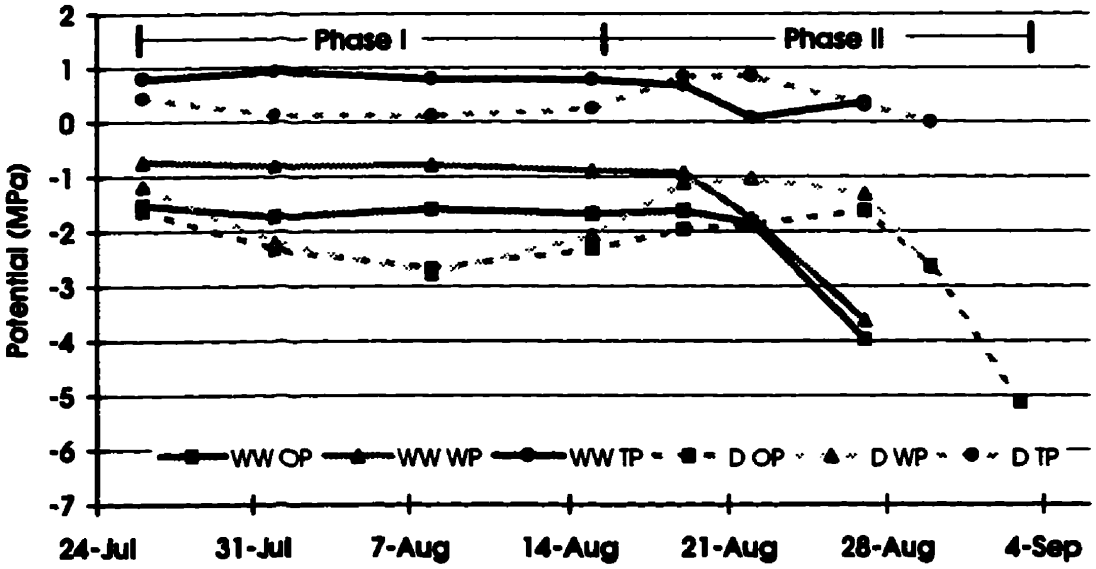

Figure 4.19 Trend of osmotic potential (OP), water potential (WP) and turgor potential (TP) over sampling dates for alfalfa grown under droughted (D) and well-watered (WW) water treatments at Winnipeg in experiment one. Average of all six alfalfa cultivars.

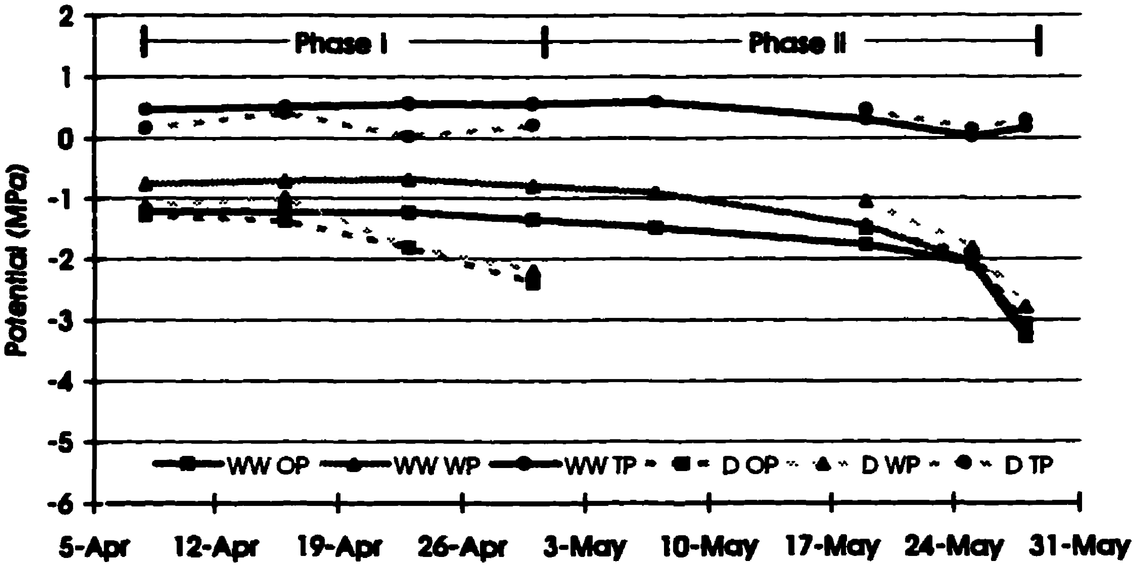

Figure 4.20 Trend of osmotic potential (OP), water potential (WP) and turgor potential (TP) over sampling dates for alfalfa grown under droughted (D) and well-watered (WW) water treatments at Winnipeg in experiment two. Average of all six alfalfa cultivars.

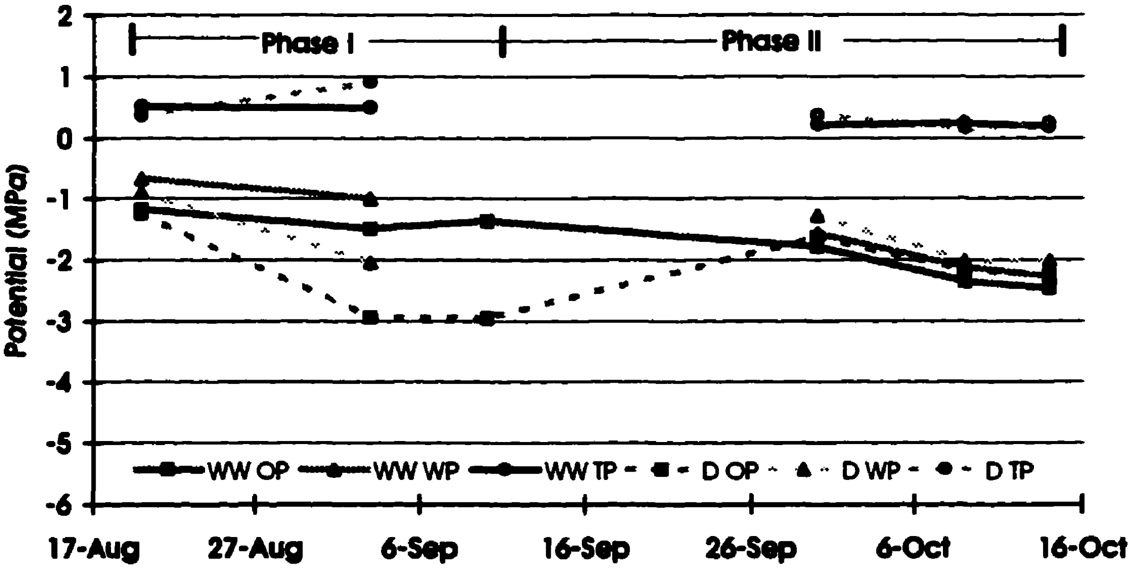

Figure 4.21 Trend of osmotic potential (OP), water potential (WP) and turgor potential (TP) over sampling dates for alfalfa grown under droughted (D) and well-watered (WW) water treatments at Winnipeg in experiment three. Average of all six alfalfa cultivars.

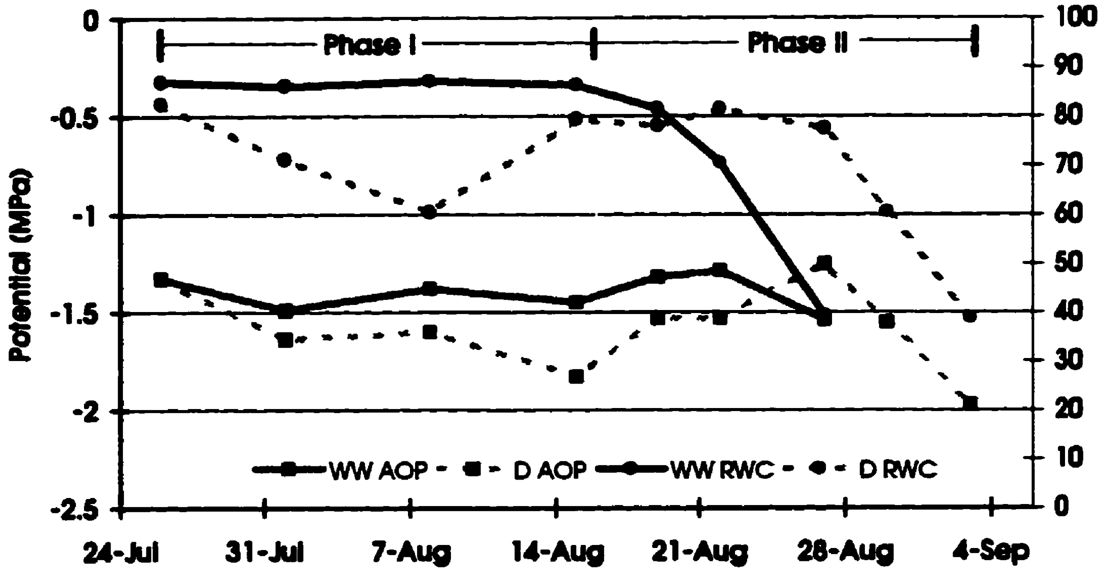

Figure 4.22 Trend of adjusted osmotic potential (AOP) and relative water content (RWC) over sampling dates for alfalfa grown under droughted (D) and well-watered (WW) water treatments at Winnipeg in experiment one. Average of all six alfalfa cultivars.

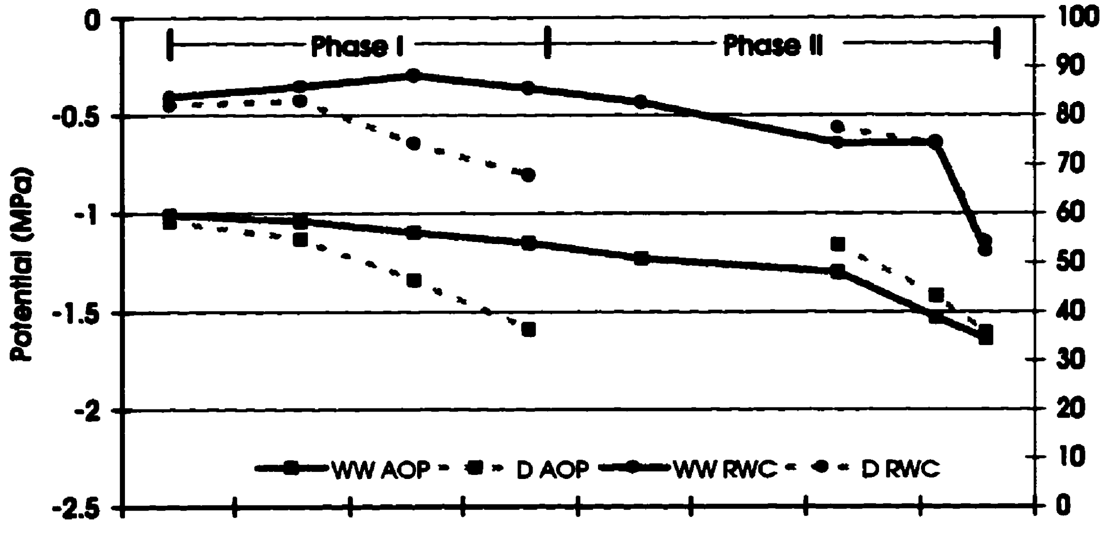

Figure 4.23 Trend of adjusted osmotic potential (AOP) and relative water content (RWC) over sampling dates for alfalfa grown under droughted (D) and well-watered (WW) water treatments at Winnipeg in experiment two. Average of all six alfalfa cultivars.

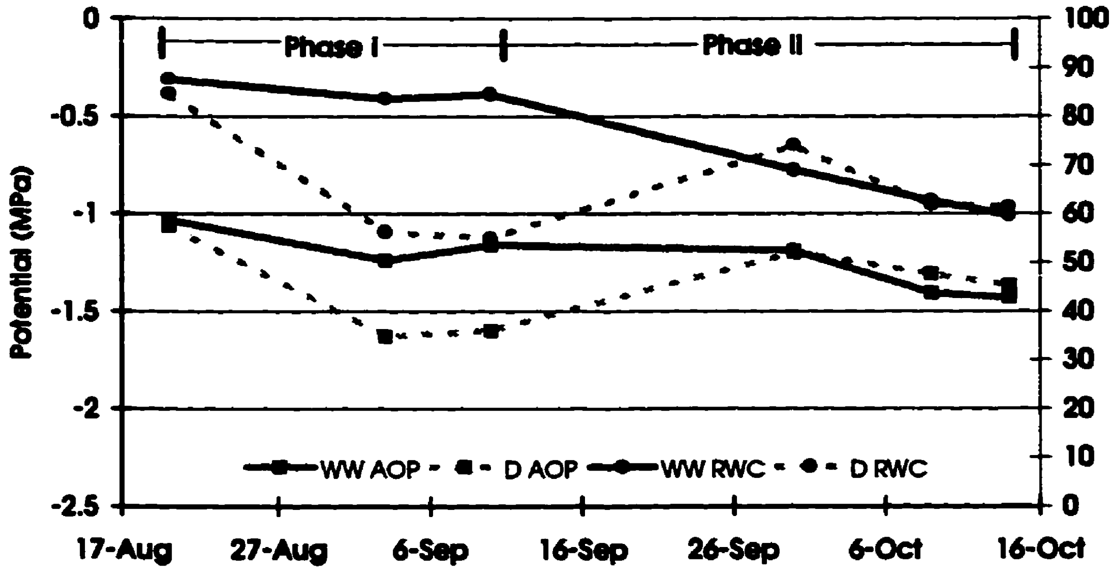

Figure 4.24 Trend of adjusted osmotic potential (AOP) and relative water content (RWC) over sampling dates for alfalfa grown under droughted (D) and well-watered (WW) water treatments at Winnipeg in experiment three. Average of all six alfalfa cultivars.

4.2.4.1 Effects of water treatments during Phase I

Differences in plant water relations between the water treatments were expected in Phase I because of different water applications. These differences were most apparent for Ψw. Water potential was significantly lower in the droughted treatment compared with the well-watered treatment on each sampling date of Phase I (Tables 4.27, 4.28 and 4.29). Relative water contents were also lower for the droughted treatment on all dates except for the first two dates in experiment two (Tables 4.30, 4.31 and 4.32). On these two dates, Ψw was -0.76, -0.71, -1.18 and -0.98 MPa for the well-watered and droughted treatments, respectively. The C.V. was < 5 for RWC compared with a C.V. < 23 for Ψw on these dates, which strengthens F-test results and leads to the conclusion that RWC is similar at low stress levels. However, RWC differences were detected for the water treatment effect at the beginning of Phase I in experiment three, when the mean Ψw for the well-watered and droughted treatments were only -0.67 and -0.89 MPa, respectively. Perhaps RWC differences under minor stress levels are only detectable under certain atmospheric conditions such as a high vapor pressure deficit.

Alfalfa plants in the droughted treatment responded to the imposed drought stress by adjusting osmotically. The difference in π_{100} between the treatments was not significant on the first date of Phase I, but was significant on each remaining date (Tables 4.33, 4.34, and 4.35). Droughted plants had only been stressed for a few days prior to the first sampling date, which was likely too short of a period to induce significant osmotic adjustment in the alfalfa plants. Osmotic adjustment (OA) was defined as the difference in π_{100} between subsequent sampling dates in this study. The droughted treatment showed a greater level of osmotic adjustment on several occasions during Phase I (Tables 4.36, 4.37 and 4.38). In cases where the water treatment effect was not significant for OA and showed little change from the previous date, it is possible that either the alfalfa had reached a physiological minimum for π_{100} or that stress levels since the prior sampling date were not great enough to induce further osmotic adjustment. Differences in π_{100} between the two water treatments were similar in magnitude at the end of Phase II in all three experiments (0.38, 0.44 and 0.44 for experiments one, two and three, respectively), which may indicate a maximum level of adjustment. In summary, these results demonstrate that alfalfa has the ability to osmotically adjust, via solute accumulation, when subjected to drought stress.

The alfalfa plants were not completely able to counteract the impact of the imposed stress, as P was generally lower for the droughted treatment (Tables 4.39, 4.40 and 4.41). However, two exceptions were noted. On the second sampling date in both experiments two and three, decreases in π (data not shown) were sufficient to maintain similar, or even higher P in the droughted treatment as compared with the well-watered treatment.

In summary, the impact of the drought treatment had the desired results, with Ψw, RWC, π_{100} and *P* often lower in the droughted treatment as compared with the well-watered treatment. Of the water relations parameters measured, Ψw appeared to be the most sensitive to the imposed stress, as all sampling times indicated significant differences. A longer period of time was required from the initiation of the droughted treatment, as compared with other measured parameters, before differences in π_{100} were detectable.

Table 4.27 Water treatment, cultivar and interaction means for water potential (MPa) in alfalfa grown under controlled watering at Winnipeg in experiment one.

Water		Sampling Date - Phase I				Sampling Date - Phase II			
Treatment	Cultivar	26 July	1 Aug.	8 Aug.	15 Aug.	19 Aug.	22 Aug.	27 Aug.	30 Aug.
Well-watered		-0.73 a	-0.81 a	-0.78 a	-0.90 a‡	-0.94 a	-1.76 b	-3.62 b	
Droughted		-1.18 b	-2.20 b	-2.77 b	-2.07 b	-1.12 b	-1.03 a	-1.32 a	-2.63
LSD (5%)		0.18	0.15	0.15	0.15	0.11	0.14	0.44	
	Alfagraze	-0.98 a	-1.51 b	-1.68 ab	-1.53 b	-1.05 a	-1.43 a	-2.61 a	-2.53 a
	Excalibur	-1.01 a	-1.54 b	-1.93 b	-1.63 b	-1.14 a	-1.50 a	-2.37 a	-2.39 a
	Nitro	-0.94 a	-1.61 b	-1.87 b	-1.52 ab	-0.96 a	-1.49 a	-2.74 a	-3.00 a
	Rangelander	-0.89 a	-1.22 a	-1.51 a	-1.27 a	-1.08 a	-1.28 a	-2.09 a	-2.51 a
	South African	-1.00 a	-1.67 b	-1.90 b	-1.58 b	-1.00 a	-1.39 a	-2.68 a	-2.77 a
	Wilson	-0.91 a	-1.48 ab	-1.79 b	-1.38 ab	-0.98 a	-1.30 a	-2.33 a	-2.60 a
LSD (5%)		0.31	0.26	0.27	0.25	0.19	0.23	0.76	0.65
Well-watered	Alfagraze	-0.69 a	-0.80 a	-0.75 a	-0.96 a	-1.02 a	-1.84 a	-3.93 a	
Well-watered	Excalibur	-0.82 a	-0.83 a	-0.85 a	-0.88 a	-0.93 a	-1.79 a	-3.35 a	
Well-watered	Nitro	-0.72 a	-0.86 a	-0.76 a	-0.79 a	-0.83 a	-1.92 a	-4.08 a	
Well-watered	Rangelander	-0.82 a	-0.77 a	-0.76 a	-1.01 a	-1.07 a	-1.48 a	-2.89 a	
Well-watered	South African	-0.60 a	-0.81 a	-0.89 a	-0.93 a	-0.88 a	-1.86 a	-3.99 a	
Well-watered	Wilson	-0.72 a	-0.77 a	-0.69 a	-0.84 a	-0.93 a	-1.67 a	-3.50 a	
LSD (5%)		0.27	0.15	0.23	0.23	0.17	0.33	1.48	
Droughted	Alfagraze	-1.27 a	-2.23 b	-2.60 ab	-2.11 bc	-1.08 a	-1.02 a	-1.29 a	-2.53 a
Droughted	Excalibur	-1.20 a	-2.25 b	-3.01 b	-2.38 c	-1.35 a	-1.20 a	-1.40 a	-2.39 a
Droughted	Nitro	-1.15 a	-2.35 b	-2.98 b	-2.25 bc	-1.08 a	-1.05 a	-1.41 a	-3.00 a
Droughted	Rangelander	-0.96 a	-1.67 a	-2.26 a	-1.53 a	-1.08 a	-1.07 a	-1.28 a	-2.51 a
Droughted	South African	-1.40 a	-2.53 b	-2.91 b	-2.22 bc	-1.12 a	-0.92 a	-1.36 a	-2.77 a
Droughted	Wilson	-1.10 a	-2.19 ab	-2.88 b	-1.92 ab	-1.03 a	-0.94 a	-1.17 a	-2.60 a
LSD (5%)		0.62	0.53	0.53	0.43	0.39	0.38	0.33	0.65

Means within a continuate column followed by the same letter are not significantly different at alpha=0.05 (LSD).

‡ - water treatment by cultivar interaction is significant at alpha=0.01.

Table 4.28 Water treatment, cultivar and interaction means for water potential (MPa) in alfalfa grown under controlled watering at Winnipeg in experiment two.

Water		Sampling Date - Phase I				Sampling Date - Phase II			
Treatment	Cultivar	8 April	16 April	23 April	30 April	7 May	19 May	25 May	28 May
Well-watered		-0.76 a	-0.71 a	-0.69 a	-0.79 a	-0.91	-1.46 b	-2.07 b†	-3.13 a
Droughted		-1.13 b	-0.98 b	-1.82 b	-2.19 b		-1.05 a	-1.81 a	-2.79 a
LSD (5%)		0.16	0.17	0.16	0.26		0.21	0.20	0.47
	Excalibur	-1.01 a	-0.99 b	-1.34 a	-1.60 a	-0.98 a	-1.45 b	-2.21 b	-3.27 a
	Nitro	-0.86 a	-0.75 a	-1.21 a	-1.39 a	-0.78 a	-1.19 ab	-1.79 a	-2.62 a
	Rangelander	-0.98 a	-0.80 ab	-1.21 a	-1.47 a	-0.96 a	-1.14 a	-1.84 a	-2.99 a
LSD (5%)		0.20	0.20	0.20	0.32	0.42	0.26	0.25	0.57
Well-watered	Excalibur	-0.75 a	-0.74 b	-0.71 a	-0.84 b	-0.98 a	-1.69 a	-2.14 a	-3.32 a
Well-watered	Nitro	-0.70 a	-0.63 a	-0.63 a	-0.66 a	-0.78 a	-1.40 a	-1.95 a	-2.94 a
Well-watered	Rangelander	-0.84 a	-0.76 b	-0.74 a	-0.88 b	-0.96 a	-1.29 a	-2.12 a	-3.12 a
LSD (5%)		0.16	0.09	0.37	0.16	0.42	0.52	0.39	0.98
Droughted	Excalibur	-1.26 b	-1.24 b	-1.97 a	-2.37 a		-1.20 a	-2.27 b	-3.22 b
Droughted	Nitro	-1.03 a	-0.86 a	-1.79 a	-2.12 a		-0.97 a	-1.62 a	-2.30 a
Droughted	Rangelander	-1.11 ab	-0.83 a	-1.68 a	-2.07 a		-0.99 a	-1.55 a	-2.85 b
LSD (5%)		0.19	0.33	0.31	0.53		0.39	0.25	0.50

Means within a continuate column followed by the same letter are not significantly different at alpha=0.05 (LSD).
† - water treatment by cultivar interaction is significant at alpha=0.05.

Table 4.29 Water treatment, cultivar and interaction means for water potential (MPa) in alfalfa grown under controlled watering at Winnipeg in experiment three.

Water		Sampling Date - Phase I		Sampling Date - Phase II		
Treatment	Cultivar	20 Aug.	3 Sept.	30 Sept.	9 Oct.	14 Oct.
Well-watered		-0.67 a	-1.00 a	-1.57 b	-2.11 a	-2.28 b
Droughted		-0.89 b	-2.04 b	-1.27 a	-2.02 a	-2.01 a
LSD (5%)		0.12	0.20	0.23	0.37	0.25
	Alfagraze	-0.81 a	-1.58 a	-1.32 a	-2.12 a	-2.28 a
	Excalibur	-0.87 a	-1.73 a	-1.60 a	-2.32 a	-2.32 a
	Nitro	-0.75 a	-1.21 a	-1.31 a	-2.00 a	-2.00 a
	Rangelander	-0.72 a	-1.54 a	-1.46 a	-2.12 a	-2.13 a
	South African	-0.72 a	-1.54 a	-1.39 a	-1.94 a	-1.98 a
	Wilson	-0.79 a	-1.52 a	-1.45 a	-1.89 a	-2.16 a
LSD (5%)		0.21	0.34	0.39	0.65	0.43
Well-watered	Alfagraze	-0.66 a	-1.06 a	-1.56 a	-2.20 a	-2.39 a
Well-watered	Excalibur	-0.77 a	-1.06 a	-1.77 a	-2.53 a	-2.65 a
Well-watered	Nitro	-0.64 a	-0.89 a	-1.54 a	-1.81 a	-2.16 a
Well-watered	Rangelander	-0.65 a	-1.00 a	-1.55 a	-2.10 a	-2.37 a
Well-watered	South African	-0.59 a	-1.00 a	-1.37 a	-1.92 a	-2.02 a
Well-watered	Wilson	-0.70 a	-0.99 a	-1.64 a	-2.08 a	-2.12 a
LSD (5%)		0.15	0.24	0.67	0.88	0.67
Droughted	Alfagraze	-0.95 a	-2.10 b	-1.09 a	-2.03 a	-2.17 a
Droughted	Excalibur	-0.97 a	-2.39 b	-1.42 a	-2.11 a	-1.98 a
Droughted	Nitro	-0.87 a	-1.54 a	-1.08 a	-2.18 a	-1.85 a
Droughted	Rangelander	-0.79 a	-2.08 b	-1.37 a	-2.13 a	-1.90 a
Droughted	South African	-0.86 a	-2.09 b	-1.41 a	-1.97 a	-1.94 a
Droughted	Wilson	-0.89 a	-2.06 b	-1.26 a	-1.70 a	-2.21 a
LSD (5%)		0.43	0.51	0.50	0.74	0.59

Means within a continuate column followed by the same letter are not significantly different at alpha=0.05 (LSD).

Table 4.30 Water treatment, cultivar and interaction means for relative water content (%) in alfalfa grown under controlled watering at Winnipeg in experiment one.

Water		Sampling Date - Phase I				Sampling Date - Phase II				
Treatment	Cultivar	26 July	1 Aug.	8 Aug.	15 Aug.	19 Aug.	22 Aug.	27 Aug.	30 Aug.	3 Sept.
Well-watered		87.0 a	86.1 a	87.0 a	86.3 a	81.5 a	70.5 b‡	39.4 b		
Droughted		82.4 b	71.1 b	60.2 b	79.3 b	77.9 b	81.3 a	77.5 a	60.5	38.8
LSD (5%)		2.9	3.9	4.1	1.9	1.7	2.3	2.5		
	Alfagraze	85.6 a	80.8 a	74.4 a	84.2 a	79.3 a	74.9 bc	59.5 a	66.9 a	43.9 a
	Excalibur	84.2 a	77.2 a	72.4 a	80.2 a	81.6 a	76.9 ab	59.7 a	67.9 a	42.1 a
	Nitro	83.1 a	75.7 a	73.6 a	82.9 a	79.7 a	71.9 b	51.8 b	48.3 b	34.3 c
	Rangelander	86.1 a	83.8 a	73.7 a	83.9 a	78.8 a	79.3 a	60.5 a	62.4 a	40.5 ab
	South African	83.5 a	77.4 a	75.6 a	82.1 a	79.5 a	74.9 bc	60.6 a	58.4 ab	36.2 bc
	Wilson	85.6 a	76.7 a	72.1 a	83.5 a	79.5 a	77.5 ab	58.8 a	59.1 ab	35.9 bc
LSD (5%)		5.0	6.8	7.1	3.4	3.0	4.1	4.4	12.1	5.8
Well-watered	Alfagraze	87.5 a	88.0 a	87.1 ab	88.7 a	80.5 a	70.1 abc	39.2 b		
Well-watered	Excalibur	88.5 a	87.0 a	88.9 a	84.8 a	82.5 a	70.6 ab	41.4 ab		
Well-watered	Nitro	84.0 b	84.8 a	88.4 a	86.6 a	81.4 a	63.2 c	30.2 c		
Well-watered	Rangelander	88.1 a	84.9 a	83.4 b	86.1 a	80.8 a	76.7 a	45.0 a		
Well-watered	South African	86.3 ab	86.9 a	87.5 a	86.4 a	82.1 a	66.5 bc	40.2 ab		
Well-watered	Wilson	87.2 a	85.1 a	86.9 ab	85.2 a	81.8 a	76.0 a	40.5 ab		
LSD (5%)		2.5	5.8	3.9	5.1	4.6	7.3	5.6		
Droughted	Alfagraze	83.8 a	73.6 a	61.8 a	79.8 a	78.0 a	79.8 a	79.7 a	66.9 a	43.9 a
Droughted	Excalibur	79.8 a	67.4 a	55.9 a	75.5 a	80.7 a	83.2 a	77.9 a	67.9 a	42.1 a
Droughted	Nitro	82.1 a	66.6 a	58.8 a	79.2 a	78.0 a	80.7 a	73.4 a	48.3 b	34.3 c
Droughted	Rangelander	84.1 a	82.6 a	63.9 a	81.8 a	76.8 a	81.9 a	76.0 a	62.4 a	40.5 ab
Droughted	South African	80.6 a	67.9 a	63.7 a	77.7 a	76.9 a	83.3 a	81.0 a	58.4 ab	36.2 bc
Droughted	Wilson	84.0 a	68.4 a	57.4 a	81.8 a	77.1 a	79.1 a	77.2 a	59.1 ab	35.9 bc
LSD (5%)		10.2	11.7	14.8	5.3	4.8	4.2	7.9	12.1	5.8

Means within a continuate column followed by the same letter are not significantly different at alpha=0.05 (LSD).

‡ - water treatment by cultivar interaction is significant at alpha=0.01.

Table 4.31 Water treatment, cultivar and interaction means for relative water content (%) in alfalfa grown under controlled watering at Winnipeg in experiment two.

Water		Sampling Date - Phase I				Sampling Date - Phase II			
Treatment	Cultivar	8 April	16 April	23 April	30 April	7 May	19 May	25 May	28 May
Well-watered		83.7 a	85.8 a	88.1 a	85.5 a	82.6	74.1 b†	74.4 a†	52.2 a
Droughted		82.1 a	83.0 a	74.2 b	67.8 b		77.4 a	74.0 a	54.1 a
LSD (5%)		3.0	3.4	4.0	5.8		2.7	4.0	5.5
	Excalibur	81.5 a	82.3 a	80.8 a	75.6 a	81.8 a	73.2 b	69.8 b	50.3 a
	Nitro	82.7 a	84.8 a	79.6 a	75.0 a	83.3 a	75.3 b	76.6 a	53.8 a
	Rangelander	84.4 a	86.0 a	83.1 a	79.3 a	82.7 a	78.8 a	76.2 a	55.3 a
LSD (5%)		3.7	4.2	4.9	7.1	4.81	3.30	4.9	6.8
Well-watered	Excalibur	84.4 a	86.6 a	88.4 a	86.1 a	81.8 a	73.6 a	73.6 a	51.4 a
Well-watered	Nitro	83.1 a	85.2 a	86.7 a	82.6 a	83.3 a	71.2 a	74.3 a	51.1 a
Well-watered	Rangelander	83.5 a	85.6 a	89.4 a	87.6 a	82.7 a	77.7 a	75.3 a	54.1 a
LSD (5%)		4.2	3.3	5.1	5.9	4.8	7.5	6.5	9.1
Droughted	Excalibur	78.6 a	78.0 a	73.1 a	65.1 a		72.8 b	66.0 b	49.2 a
Droughted	Nitro	82.3 a	84.5 a	72.7 a	67.4 a		79.5 a	79.0 a	56.5 a
Droughted	Rangelander	85.3 a	86.5 a	76.8 a	71.0 a		79.8 a	77.2 a	56.5 a
LSD (5%)		6.1	9.3	9.3	13.4		3.5	8.5	9.7

Means within a continuate column followed by the same letter are not significantly different at alpha=0.05 (LSD).
† - water treatment by cultivar interaction is significant at alpha=0.05.

Table 4.32 Water treatment, cultivar and interaction means for relative water content (%) in alfalfa grown under controlled watering at Winnipeg in experiment three.

Water		Sampling Date - Phase I			Sampling Date - Phase II		
Treatment	Cultivar	20 Aug.	3 Sept.	10 Sept.	30 Sept.	9 Oct.	14 Oct.
Well-watered		87.5 a	83.6 a	84.6 a	69.1 b	62.7 a	59.6 a
Droughted		84.7 b	56.3 b	54.9 b	74.0 a	61.8 a	61.4 a
LSD (5%)		2.3	2.8	4.1	5.1	6.1	5.2
	Alfagraze	86.9 a	69.2 a	71.1 a	70.9 a	63.7 a	61.4 a
	Excalibur	84.6 a	69.7 a	68.5 a	69.9 a	61.1 a	58.7 a
	Nitro	86.8 a	70.3 a	69.4 a	73.1 a	61.8 a	62.5 a
	Rangelander	87.0 a	73.7 a	72.1 a	73.2 a	60.7 a	61.5 a
	South African	86.4 a	67.6 a	68.8 a	71.5 a	63.9 a	59.5 a
	Wilson	85.0 a	69.2 a	68.5 a	70.7 a	62.2 a	59.2 a
LSD (5%)		4.0	4.9	7.0	8.8	10.5	9.0
Well-watered	Alfagraze	89.5 a	82.5 a	83.7 a	64.9 a	61.3 a	60.5 a
Well-watered	Excalibur	86.3 a	82.2 a	83.7 a	63.9 a	59.4 a	54.7 a
Well-watered	Nitro	87.6 a	85.0 a	85.5 a	71.6 a	66.6 a	61.5 a
Well-watered	Rangelander	87.5 a	84.7 a	83.6 a	72.2 a	63.2 a	60.0 a
Well-watered	South African	87.1 a	84.1 a	85.4 a	72.1 a	63.1 a	62.0 a
Well-watered	Wilson	87.2 a	82.9 a	85.5 a	69.6 a	62.6 a	58.6 a
LSD (5%)		4.0	5.1	6.3	12.4	18.4	14.5
Droughted	Alfagraze	84.3 a	55.8 a	58.5 a	77.0 a	66.1 a	62.3 a
Droughted	Excalibur	82.8 a	57.2 a	53.3 a	75.9 a	62.8 a	62.6 a
Droughted	Nitro	86.0 a	55.7 a	53.3 a	74.6 a	57.0 a	63.4 a
Droughted	Rangelander	86.6 a	62.7 a	60.7 a	74.3 a	58.2 a	63.0 a
Droughted	South African	85.7 a	51.0 a	52.2 a	70.8 a	64.8 a	57.1 a
Droughted	Wilson	82.7 a	55.4 a	51.5 a	71.7 a	61.8 a	59.8 a
LSD (5%)		7.5	8.4	13.8	12.5	12.4	11.6

Means within a continuate column followed by the same letter are not significantly different at alpha=0.05 (LSD).

Table 4.33 Water treatment, cultivar and interaction means for adjusted osmotic potential (MPa) in alfalfa grown under controlled watering at Winnipeg in experiment one.

Water		Sampling Date - Phase I				Sampling Date - Phase II				
Treatment	Cultivar	26 July	1 Aug.	8 Aug.	15 Aug.	19 Aug.	22 Aug.	27 Aug.	30 Aug.	3 Sept.
Well-watered		-1.33 a	-1.49 a	-1.38 a	-1.45 a	-1.32 a	-1.29 a	-1.54 b		
Droughted		-1.33 a	-1.64 b	-1.60 b	-1.83 b	-1.53 b	-1.53 b	-1.25 a	-1.55	-1.97
LSD (5%)		0.08	0.09	0.10	0.08	0.09	0.10	0.13		
	Alfagraze	-1.41 a	-1.66 a	-1.50 a	-1.72 a	-1.53 b	-1.48 a	-1.46 a	-1.69 a	-2.05 a
	Excalibur	-1.36 a	-1.57 a	-1.51 a	-1.71 a	-1.49 ab	-1.45 a	-1.41 a	-1.59 a	-1.98 a
	Nitro	-1.26 a	-1.49 a	-1.45 a	-1.61 a	-1.34 a	-1.36 a	-1.37 a	-1.49 a	-2.05 a
	Rangelander	-1.38 a	-1.62 a	-1.47 a	-1.55 a	-1.37 ab	-1.38 a	-1.31 a	-1.45 a	-1.81 a
	South African	-1.33 a	-1.56 a	-1.48 a	-1.65 a	-1.34 a	-1.43 a	-1.54 a	-1.62 a	-2.02 a
	Wilson	-1.25 a	-1.50 a	-1.52 a	-1.60 a	-1.47 ab	-1.39 a	-1.28 a	-1.50 a	-1.93 a
LSD (5%)		0.13	0.15	0.18	0.13	0.16	0.17	0.22	0.23	0.19
Well-watered	Alfagraze	-1.38 a	-1.55 bc	-1.42 a	-1.54 a	-1.43 a	-1.41 a	-1.59 a		
Well-watered	Excalibur	-1.37 a	-1.55 bc	-1.48 a	-1.51 a	-1.39 a	-1.30 a	-1.47 a		
Well-watered	Nitro	-1.25 a	-1.37 a	-1.37 a	-1.29 a	-1.22 a	-1.20 a	-1.56 a		
Well-watered	Rangelander	-1.37 a	-1.63 c	-1.35 a	-1.41 a	-1.26 a	-1.37 a	-1.52 a		
Well-watered	South African	-1.39 a	-1.41 ab	-1.27 a	-1.52 a	-1.25 a	-1.21 a	-1.68 a		
Well-watered	Wilson	-1.22 a	-1.43 ab	-1.39 a	-1.43 a	-1.37 a	-1.28 a	-1.41 a		
LSD (5%)		0.18	0.15	0.21	0.20	0.17	0.17	0.40		
Droughted	Alfagraze	-1.44 a	-1.76 a	-1.58 a	-1.90 a	-1.63 a	-1.56 a	-1.34 ab	-1.69 a	-2.05 a
Droughted	Excalibur	-1.35 a	-1.58 a	-1.54 a	-1.92 a	-1.58 a	-1.60 a	-1.35 ab	-1.59 a	-1.98 a
Droughted	Nitro	-1.26 a	-1.61 a	-1.54 a	-1.93 a	-1.46 a	-1.51 a	-1.18 ab	-1.49 a	-2.05 a
Droughted	Rangelander	-1.38 a	-1.60 a	-1.58 a	-1.68 a	-1.49 a	-1.38 a	-1.10 a	-1.45 a	-1.81 a
Droughted	South African	-1.28 a	-1.72 a	-1.69 a	-1.78 a	-1.43 a	-1.64 a	-1.39 b	-1.62 a	-2.02 a
Droughted	Wilson	-1.28 a	-1.58 a	-1.65 a	-1.78 a	-1.58 a	-1.51 a	-1.16 ab	-1.50 a	-1.93 a
LSD (5%)		0.19	0.25	0.33	0.21	0.29	0.28	0.27	0.23	0.19

Means within a continuate column followed by the same letter are not significantly different at alpha=0.05 (LSD).

Table 4.34 Water treatment, cultivar and interaction means for adjusted osmotic potential (MPa) in alfalfa grown under controlled watering at Winnipeg in experiment two.

Water		Sampling Date - Phase I				Sampling Date - Phase II			
Treatment	Cultivar	8 April	16 April	23 April	30 April	7 May	19 May	25 May	28 May
Well-watered		-1.01 a	-1.04 a	-1.10 a	-1.15 a	-1.23	-1.30 b†	-1.53 a	-1.64 a
Droughted		-1.04 a	-1.13 b	-1.34 b	-1.59 b		-1.16 a	-1.42 a	-1.61 a
LSD (5%)		0.07	0.06	0.08	0.09		0.08	0.17	0.18
	Excalibur	-1.07 b	-1.13 b	-1.27 b	-1.43 b	-1.27 a	-1.26 a	-1.59 a	-1.69 a
	Nitro	-0.97 a	-1.04 a	-1.17 a	-1.30 a	-1.22 a	-1.21 a	-1.44 a	-1.50 a
	Rangelander	-1.04 ab	-1.09 ab	-1.22 ab	-1.37 ab	-1.19 a	-1.21 a	-1.40 a	-1.69 a
LSD (5%)		0.09	0.07	0.09	0.11	0.13	0.09	0.20	0.22
Well-watered	Excalibur	-1.06 a	-1.10 b	-1.15 a	-1.21 b	-1.27 a	-1.30 a	-1.64 a	-1.73 a
Well-watered	Nitro	-0.95 a	-0.96 a	-1.05 a	-1.04 a	-1.22 a	-1.25 a	-1.49 a	-1.53 a
Well-watered	Rangelander	-1.01 a	-1.06 b	-1.09 a	-1.19 b	-1.19 a	-1.36 a	-1.47 a	-1.66 a
LSD (5%)		0.10	0.06	0.14	0.10	0.13	0.21	0.40	0.37
Droughted	Excalibur	-1.08 a	-1.16 a	-1.39 a	-1.65 a		-1.22 c	-1.53 a	-1.66 a
Droughted	Nitro	-0.98 a	-1.11 a	-1.28 a	-1.55 a		-1.17 b	-1.40 a	-1.46 a
Droughted	Rangelander	-1.06 a	-1.13 a	-1.36 a	-1.56 a		-1.07 a	-1.33 a	-1.72 a
LSD (5%)		0.17	0.15	0.17	0.22		0.02	0.25	0.32

Means within a continuate column followed by the same letter are not significantly different at alpha=0.05 (LSD).
† - water treatment by cultivar interaction is significant at alpha=0.05.

Table 4.35 Water treatment, cultivar and interaction means for adjusted osmotic potential (MPa) in alfalfa grown under controlled watering at Winnipeg in experiment three.

Water		Sampling Date - Phase I			Sampling Date - Phase II		
Treatment	Cultivar	20 Aug.	3 Sept.	10 Sept.	30 Sept.	9 Oct.	14 Oct.
Well-watered		-1.03 a	-1.24 a	-1.16 a	-1.19 a	-1.41 a	-1.43 a
Droughted		-1.06 a	-1.63 b	-1.60 b	-1.20 a	-1.31 a	-1.37 a
LSD (5%)		0.05	0.11	0.11	0.07	0.12	0.08
	Alfagraze	-1.09 a	-1.42 a	-1.46 a	-1.21 a	-1.47 a	-1.44 bc
	Excalibur	-1.07 a	-1.60 a	-1.32 a	-1.30 a	-1.36 a	-1.44 bc
	Nitro	-1.02 a	-1.37 a	-1.36 a	-1.15 a	-1.32 a	-1.39 abc
	Rangelander	-1.07 a	-1.45 a	-1.36 a	-1.19 a	-1.40 a	-1.51 c
	South African	-1.02 a	-1.40 a	-1.51 a	-1.20 a	-1.30 a	-1.37 ab
	Wilson	-1.00 a	-1.38 a	-1.27 a	-1.13 a	-1.31 a	-1.27 a
LSD (5%)		0.08	0.19	0.19	0.13	0.20	0.13
Well-watered	Alfagraze	-1.11 a	-1.25 a	-1.23 a	-1.25 a	-1.54 a	-1.41 abc
Well-watered	Excalibur	-1.05 a	-1.27 a	-1.11 a	-1.22 a	-1.46 a	-1.55 bc
Well-watered	Nitro	-1.03 a	-1.25 a	-1.13 a	-1.16 a	-1.35 a	-1.38 ab
Well-watered	Rangelander	-1.06 a	-1.24 a	-1.15 a	-1.22 a	-1.40 a	-1.64 c
Well-watered	South African	-0.97 a	-1.23 a	-1.25 a	-1.18 a	-1.30 a	-1.40 abc
Well-watered	Wilson	-0.97 a	-1.20 a	-1.08 a	-1.13 a	-1.41 a	-1.22 a
LSD (5%)		0.14	0.18	0.19	0.20	0.23	0.24
Droughted	Alfagraze	-1.07 a	-1.58 a	-1.68 a	-1.17 a	-1.39 a	-1.46 a
Droughted	Excalibur	-1.08 a	-1.93 a	-1.53 a	-1.38 a	-1.25 a	-1.33 a
Droughted	Nitro	-1.02 a	-1.49 a	-1.58 a	-1.14 a	-1.29 a	-1.40 a
Droughted	Rangelander	-1.08 a	-1.65 a	-1.56 a	-1.17 a	-1.40 a	-1.38 a
Droughted	South African	-1.06 a	-1.57 a	-1.76 a	-1.21 a	-1.29 a	-1.34 a
Droughted	Wilson	-1.03 a	-1.57 a	-1.45 a	-1.12 a	-1.22 a	-1.31 a
LSD (5%)		0.12	0.31	0.38	0.20	0.38	0.17

Means within a continuate column followed by the same letter are not significantly different at alpha=0.05 (LSD).

Table 4.36 Water treatment, cultivar and interaction means for osmotic adjustment (MPa) in alfalfa grown under controlled watering at Winnipeg in experiment one.

		-- Period of Adjustment - Phase I --			---------------- Period of Adjustment - Phase II ----------------				
Water Treatment	Cultivar	26 July-1 Aug.	1 Aug.-8 Aug.	8 Aug.-15 Aug.	15 Aug.-19 Aug.	19 Aug.-22 Aug.	22 Aug.-27 Aug.	27 Aug.-30 Aug.	30 Aug.-3 Sept.
Well-watered		-0.16 a	0.11 a	-0.07 a	0.13 b	0.03 a	-0.24 b		
Droughted		-0.31 b	0.05 a	-0.23 b	0.30 a	-0.01 a	0.28 a	-0.30 a	-0.42
LSD (5%)		0.12	0.09	0.12	0.11	0.12	0.16		
	Alfagraze	-0.25 a	0.16 a	-0.22 a	-0.19 a	0.04 a	0.02 a	-0.35 a	-0.36 a
	Excalibur	-0.21 a	0.05 a	-0.20 a	-0.23 a	0.04 a	0.04 a	-0.23 a	-0.39 a
	Nitro	-0.24 a	0.04 a	-0.15 a	-0.27 a	-0.02 a	-0.01 a	-0.31 a	-0.56 a
	Rangelander	-0.24 a	0.15 a	-0.08 a	-0.17 a	-0.00 a	0.07 a	-0.35 a	-0.36 a
	South African	-0.23 a	0.08 a	-0.17 a	-0.32 a	-0.09 a	-0.11 a	-0.23 a	-0.40 a
	Wilson	-0.25 a	-0.02 a	-0.08 a	-0.13 a	0.08 a	0.11 a	-0.34 a	-0.43 a
LSD (5%)		0.22	0.16	0.21	0.19	0.21	0.27	0.33	0.20
Well-watered	Alfagraze	-0.17 a	0.13 b	-0.12 a	0.11 a	0.03 a	-0.18 a		
Well-watered	Excalibur	-0.18 a	0.07 bc	-0.03 a	0.12 a	0.09 a	-0.17 a		
Well-watered	Nitro	-0.12 a	0.01 c	0.08 a	0.06 a	0.02 a	-0.35 a		
Well-watered	Rangelander	-0.26 a	0.28 a	-0.06 a	0.15 a	-0.11 a	-0.14 a		
Well-watered	South African	-0.02 a	0.13 b	-0.25 a	0.28 a	0.03 a	-0.47 a		
Well-watered	Wilson	-0.21 a	0.03 bc	-0.03 a	0.06 a	0.09 a	-0.13 a		
LSD (5%)		0.31	0.11	0.28	0.28	0.23	0.41		
Droughted	Alfagraze	-0.33 a	0.19 a	-0.32 a	0.27 a	0.06 a	0.22 a	-0.35 a	-0.36 a
Droughted	Excalibur	-0.23 a	0.04 a	-0.37 a	0.33 a	-0.02 a	0.25 a	-0.23 a	-0.39 a
Droughted	Nitro	-0.35 a	0.07 a	-0.39 a	0.47 a	-0.05 a	0.33 a	-0.31 a	-0.56 a
Droughted	Rangelander	-0.22 a	0.02 a	-0.10 a	0.19 a	0.11 a	0.28 a	-0.35 a	-0.36 a
Droughted	South African	-0.44 a	0.04 a	-0.10 a	0.35 a	-0.21 a	0.25 a	-0.23 a	-0.40 a
Droughted	Wilson	-0.30 a	-0.07 a	-0.13 a	0.20 a	0.06 a	0.35 a	-0.34 a	-0.43 a
LSD (5%)		0.28	0.32	0.36	0.32	0.38	0.35	0.33	0.20

Means within a continuate column followed by the same letter are not significantly different at alpha=0.05 (LSD).

Table 4.37 Water treatment, cultivar and interaction means for osmotic adjustment (MPa) in alfalfa grown under controlled watering at Winnipeg in experiment two.

		----- Period of Adjustment - Phase I -----			------------- Period of Adjustment - Phase II -------------			
Water Treatment	Cultivar	8 April- 16 April	16 April- 23 April	23 April- 30 April	30 April- 7 May	7 May- 19 May	19 May- 25 May	25 May- 28 May
Well-watered		-0.03 a	-0.05 a	-0.05 a	-0.08	-0.07	-0.23 a	-0.10 a
Droughted		-0.09 a	-0.21 b	-0.24 b			-0.27 a	-0.19 a
LSD (5%)		0.09	0.09	0.13			0.17	0.10
	Excalibur	-0.06 a	-0.14 a	-0.16 a	-0.06 a	-0.02 a	-0.33 a	-0.11 a
	Nitro	-0.07 a	-0.13 a	-0.13 a	-0.18 a	-0.03 a	-0.23 a	-0.05 a
	Rangelander	-0.06 a	-0.13 a	-0.15 a	-0.00 a	-0.16 a	-0.19 a	-0.29 b
LSD (5%)		0.11	0.11	0.16	0.19	0.13	0.21	0.12
Well-watered	Excalibur	-0.04 a	-0.05 a	-0.06 a	-0.06 a	-0.02 a	-0.35 a	-0.08 a
Well-watered	Nitro	-0.01 a	-0.09 a	0.01 a	-0.18 a	-0.03 a	-0.24 a	-0.04 a
Well-watered	Rangelander	-0.04 a	-0.03 a	-0.10 a	-0.00 a	-0.16 a	-0.12 a	-0.18 a
LSD (5%)		0.10	0.16	0.17	0.19	0.13	0.45	0.15
Droughted	Excalibur	-0.08 a	-0.23 a	-0.26 a			-0.31 a	-0.13 a
Droughted	Nitro	-0.13 a	-0.17 a	-0.27 a			-0.23 a	-0.06 a
Droughted	Rangelander	-0.07 a	-0.23 a	-0.20 a			-0.26 a	-0.39 b
LSD (5%)		0.24	0.16	0.28			0.25	0.15

Means within a continuate column followed by the same letter are not significantly different at alpha=0.05 (LSD).

Table 4.38 Water treatment, cultivar and interaction means for osmotic adjustment (MPa) in alfalfa grown under controlled watering at Winnipeg in experiment three.

		-- Period of Adjustment - Phase I --		---------- Period of Adjustment - Phase II ----------		
Water Treatment	Cultivar	20 Aug.- 3 Sept.	3 Sept.- 10 Sept.	10 Sept.- 30 Sept.	30 Sept.- 9 Oct.	9 Oct.- 14 Oct.
Well-watered		-0.21 a	0.08 a	-0.04 b	-0.22 a	-0.02 a
Droughted		-0.57 b	0.04 a	0.40 a	-0.11 a	-0.06 a
LSD (5%)		0.11	0.16	0.13	0.13	0.11
	Alfagraze	-0.33 a	-0.04 a	0.24 a	-0.25 a	-0.03 a
	Excalibur	-0.53 a	0.28 a	0.02 a	-0.06 a	-0.08 a
	Nitro	-0.35 a	0.02 a	0.20 a	-0.17 a	-0.07 a
	Rangelander	-0.38 a	0.09 a	0.16 a	-0.20 a	-0.11 a
	South African	-0.38 a	-0.11 a	0.31 a	-0.10 a	-0.07 a
	Wilson	-0.39 a	0.12 a	0.14 a	-0.19 a	-0.05 a
LSD (5%)		0.19	0.28	0.22	0.22	0.18
Well-watered	Alfagraze	-0.14 a	0.02 a	-0.02 a	-0.29 a	0.13 ab
Well-watered	Excalibur	-0.22 a	0.16 a	-0.11 a	-0.24 a	-0.09 bc
Well-watered	Nitro	-0.22 a	0.12 a	-0.03 a	-0.18 a	-0.03 abc
Well-watered	Rangelander	-0.18 a	0.10 a	-0.07 a	-0.18 a	-0.24 c
Well-watered	South African	-0.26 a	-0.02 a	0.07 a	-0.12 a	-0.10 bc
Well-watered	Wilson	-0.23 a	0.12 a	-0.05 a	-0.28 a	0.20 a
LSD (5%)		0.25	0.25	0.31	0.26	0.23
Droughted	Alfagraze	-0.51 a	-0.10 a	0.50 a	-0.22 a	-0.07 a
Droughted	Excalibur	-0.85 a	0.41 a	0.15 a	0.12 a	-0.08 a
Droughted	Nitro	-0.47 a	-0.09 a	0.44 a	-0.15 a	-0.10 a
Droughted	Rangelander	-0.57 a	0.09 a	0.39 a	-0.23 a	0.02 a
Droughted	South African	-0.51 a	-0.20 a	0.56 a	-0.08 a	-0.05 a
Droughted	Wilson	-0.54 a	0.11 a	0.33 a	-0.09 a	-0.10 a
LSD (5%)		0.26	0.51	0.39	0.42	0.34

Means within a continuate column followed by the same letter are not significantly different at alpha=0.05 (LSD).

Table 4.39 Water treatment, cultivar and interaction means for turgor potential (MPa) in alfalfa grown under controlled watering at Winnipeg in experiment one.

Water		Sampling Date - Phase I				Sampling Date - Phase II			
Treatment	Cultivar	26 July	1 Aug.	8 Aug.	15 Aug.	19 Aug.	22 Aug.	27 Aug.	30 Aug.
Well-watered		0.80 a	0.93 a	0.80 a	0.78 a	0.67 b	0.08 b	0.36 a	
Droughted		0.44 b	0.13 b	-0.11 b	0.25 b	0.84 a	0.85 a	0.31 a	0.00
LSD (5%)		0.15	0.09	0.12	0.14	0.17	0.17	0.36	
	Alfagraze	0.67 a	0.57 ab	0.42 abc	0.53 a	0.88 a	0.54 a	0.27 a	-0.01 a
	Excalibur	0.61 a	0.53 bc	0.29 bcd	0.53 a	0.68 a	0.38 a	0.28 a	-0.05 a
	Nitro	0.58 a	0.42 bc	0.23 cd	0.45 a	0.73 a	0.40 a	0.64 a	0.20 a
	Rangelander	0.71 a	0.71 a	0.54 a	0.58 a	0.67 a	0.46 a	0.34 a	-0.13 a
	South African	0.60 a	0.41 c	0.15 d	0.45 a	0.69 a	0.51 a	0.26 a	0.02 a
	Wilson	0.55 a	0.52 bc	0.47 ab	0.54 a	0.88 a	0.49 a	0.19 a	-0.06 a
LSD (5%)		0.26	0.16	0.20	0.24	0.29	0.29	0.62	0.47
Well-watered	Alfagraze	0.89 a	0.96 b	0.88 a	0.78 a	0.75 a	0.16 a	0.16 a	
Well-watered	Excalibur	0.72 a	0.96 b	0.82 a	0.90 a	0.75 a	0.04 a	0.21 a	
Well-watered	Nitro	0.76 a	0.76 d	0.78 a	0.70 a	0.67 a	-0.01 a	1.07 a	
Well-watered	Rangelander	0.74 a	1.15 a	0.86 a	0.63 a	0.49 a	0.31 a	0.51 a	
Well-watered	South African	1.00 a	0.81 cd	0.57 a	0.84 a	0.64 a	-0.03 a	0.17 a	
Well-watered	Wilson	0.67 a	0.91 bc	0.91 a	0.84 a	0.75 a	0.01 a	0.02 a	
LSD (5%)		0.24	0.15	0.30	0.37	0.22	0.30	1.31	
Droughted	Alfagraze	0.45 a	0.18 a	-0.05 abc	0.28 a	1.01 a	0.93 a	0.39 a	-0.01 a
Droughted	Excalibur	0.49 a	0.11 a	-0.24 bc	0.16 a	0.62 a	0.72 a	0.35 a	-0.05 a
Droughted	Nitro	0.40 a	0.08 a	-0.32 c	0.20 a	0.79 a	0.82 a	0.21 a	0.20 a
Droughted	Rangelander	0.68 a	0.27 a	0.22 a	0.53 a	0.85 a	0.61 a	0.18 a	-0.13 a
Droughted	South African	0.19 a	0.01 a	-0.26 bc	0.07 a	0.74 a	1.05 a	0.36 a	0.02 a
Droughted	Wilson	0.42 a	0.12 a	0.02 ab	0.25 a	1.01 a	0.98 a	0.35 a	-0.06 a
LSD (5%)		0.46	0.33	0.34	0.35	0.57	0.54	0.33	0.47

Means within a continuate column followed by the same letter are not significantly different at alpha=0.05 (LSD).

Table 4.40 Water treatment, cultivar and interaction means for turgor potential (MPa) in alfalfa grown under controlled watering at Winnipeg in experiment two.

Water		Sampling Date - Phase I				Sampling Date - Phase II			
Treatment	Cultivar	8 April	16 April	23 April	30 April	7 May	19 May	25 May	28 May
Well-watered		0.45 a	0.50 a	0.55 a	0.55 a	0.58	0.30 a	0.01 a	0.16 a
Droughted		0.15 b	0.40 a	0.01 b	0.20 b		0.45 a	0.13 a	0.28 a
LSD (5%)		0.17	0.15	0.13	0.14		0.24	0.19	0.40
	Excalibur	0.33 a	0.40 a	0.28 a	0.38 a	0.58 a	0.28 a	0.08 a	0.16 a
	Nitro	0.31 a	0.48 a	0.28 a	0.43 a	0.68 a	0.44 a	0.11 a	0.29 a
	Rangelander	0.25 a	0.48 a	0.28 a	0.32 a	0.48 a	0.41 a	0.02 a	0.20 a
LSD (5%)		0.21	0.18	0.16	0.17	0.30	0.30	0.24	0.49
Well-watered	Excalibur	0.51 a	0.54 a	0.60 a	0.57 a	0.58 a	0.08 a	0.11 a	0.15 a
Well-watered	Nitro	0.45 ab	0.50 a	0.58 a	0.61 a	0.68 a	0.37 a	0.06 a	0.16 a
Well-watered	Rangelander	0.38 b	0.48 a	0.47 a	0.48 b	0.48 a	0.46 a	-0.14 a	0.16 a
LSD (5%)		0.11	0.12	0.24	0.08	0.30	0.63	0.53	0.60
Droughted	Excalibur	0.14 a	0.26 a	-0.04 a	0.18 a		0.48 a	0.06 a	0.16 a
Droughted	Nitro	0.17 a	0.46 a	-0.03 a	0.26 a		0.51 a	0.16 a	0.43 a
Droughted	Rangelander	0.13 a	0.48 a	0.09 a	0.17 a		0.36 a	0.17 a	0.24 a
LSD (5%)		0.40	0.22	0.25	0.39		0.35	0.30	1.04

Means within a continuate column followed by the same letter are not significantly different at alpha=0.05 (LSD).

Table 4.41 Water treatment, cultivar and interaction means for turgor potential (MPa) in alfalfa grown under controlled watering at Winnipeg in experiment three.

Water		------ Sampling Date - Phase I ------		------------------ Sampling Date - Phase II ---------------		
Treatment	Cultivar	20 Aug.	3 Sept.	30 Sept.	9 Oct.	14 Oct.
Well-watered		0.51 a	0.49 b	0.20 b	0.24 a	0.18 a
Droughted		0.36 b	0.90 a	0.36 a	0.14 a	0.24 a
LSD (5%)		0.09	0.22	0.12	0.21	0.19
	Alfagraze	0.45 a	0.62 a	0.46 a	0.25 a	0.10 a
	Excalibur	0.39 a	0.75 a	0.29 a	0.00 a	0.16 a
	Nitro	0.43 a	0.89 a	0.28 a	0.19 a	0.26 a
	Rangelander	0.51 a	0.53 a	0.21 a	0.27 a	0.38 a
	South African	0.46 a	0.75 a	0.29 a	0.13 a	0.36 a
	Wilson	0.38 a	0.64 a	0.15 a	0.30 a	0.02 a
LSD (5%)		0.15	0.37	0.21	0.36	0.33
Well-watered	Alfagraze	0.58 a	0.46 a	0.48 a	0.39 a	0.00 a
Well-watered	Excalibur	0.45 a	0.48 a	0.17 a	0.00 a	0.17 a
Well-watered	Nitro	0.53 a	0.58 a	0.11 a	0.27 a	0.16 a
Well-watered	Rangelander	0.57 a	0.47 a	0.20 a	0.27 a	0.45 a
Well-watered	South African	0.53 a	0.47 a	0.28 a	0.23 a	0.30 a
Well-watered	Wilson	0.41 a	0.46 a	-0.01 a	0.27 a	0.00 a
LSD (5%)		0.19	0.19	0.31	0.52	0.52
Droughted	Alfagraze	0.32 a	0.78 a	0.44 a	0.11 a	0.19 a
Droughted	Excalibur	0.33 a	1.02 a	0.41 a	0.00 a	0.15 a
Droughted	Nitro	0.33 a	1.19 a	0.45 a	0.10 a	0.36 a
Droughted	Rangelander	0.46 a	0.58 a	0.22 a	0.28 a	0.31 a
Droughted	South African	0.39 a	1.02 a	0.30 a	0.02 a	0.43 a
Droughted	Wilson	0.35 a	0.82 a	0.32 a	0.32 a	0.03 a
LSD (5%)		0.27	0.79	0.33	0.43	0.51

Means within a continuate column followed by the same letter are not significantly different at alpha=0.05 (LSD).

4.2.4.2 Cultivar differences during Phase I

On two occasions during Phase I, experiment one, 'Rangelander' had a higher Ψw than the other cultivars, although values were similar for 'Wilson' on 1 August and 'Alfagraze' on 8 August (Table 4.27). Although the water treatment × cultivar interaction was not significant on these dates, separate analyses by water treatment indicated that there were no significant differences in the well-watered treatment, while differences were apparent at $\alpha<0.07$ in the droughted treatment. On the final sampling date of Phase I, there was a significant water treatment × cultivar interaction. In the droughted treatment, 'Rangelander' had the highest Ψw (similar to dates 1 and 8 August), while there were no significant cultivar differences in the well-watered treatment. This difference in ranking likely contributed to the significant interaction. The higher Ψw for 'Rangelander' in the droughted treatment was likely due to its lower water use. Soil moisture contents were brought to equilibrium at least two days before measurements were taken in experiment one. Because 'Rangelander' used less total water (Table 4.25), soil moisture could have been higher at the time of sampling than for the other cultivars, which would confound the results.

No significant cultivar differences were observed in Phase I of experiment two; however, other cases were significant at $\alpha<0.06$ and warrant discussion. At the beginning of Phase I, the Ψw for 'Nitro' was higher than for 'Excalibur' in the droughted treatment ($P=0.0562$) (Table 4.28). A similar ranking was observed on 16 April for the combined water treatments ($P=0.0507$). Although neither the cultivar nor interaction effects were significant, a separate analysis by water treatment indicated that 'Nitro' had a higher Ψw in the well-watered treatment on this and the final date of Phase I. The capability of 'Nitro' to maintain a higher Ψw was similar to field study results, where 'Nitro' had a higher overall Ψw compared with the other cultivars. A larger root mass, as noted in Table 4.24, is one possible morphological feature that would allow 'Nitro' to maintain sufficient water flow to meet transpirative demand and reduce its relative stress level. On 16 April, a lower Ψw for 'Excalibur' in the droughted treatment indicated its inferior ability to cope with the drought stress.

None of the ANOVA analyses were significant at alpha=0.05 in experiment three for the cultivar or water treatment × cultivar interaction effects. Similarly, there were no significant effects for cultivar in separate analyses by water treatment. However, cultivar differences were significant at P=0.0690 for the stressed treatment on 3 September (Table 4.29). In this case, the LSD test indicated that 'Nitro' had a higher Ψw than any of the other cultivars.

In experiment one, no cultivar differences were detected for RWC in the combined water treatment analysis (Table 4.30). However, contrasting results were obtained in separate analysis of the well-watered treatment. On 26 July, 'Rangelander' had the highest RWC and 'Nitro' the lowest, while on 8 August, 'Nitro' had the highest RWC and 'Rangelander' the lowest. No cultivar differences in RWC were found in experiments two or three during Phase I (Tables 4.29 and 4.30).

'Rangelander' had a lower π_{100} compared to 'Nitro', 'South African' and 'Wilson' in the well-watered treatment on 1 August 1991 (Table 4.36). In experiment two, 'Excalibur' had lower π_{100} than 'Nitro' through Phase I (P=0.0622, 0.0374, 0.0850 and 0.0630 for 8, 16, 23 and 30 April, respectively), but it was not significantly different than 'Rangelander' (Table 4.37). In separate water treatment analysis, 'Excalibur' and 'Rangelander' had lower π_{100} than 'Nitro' on 16 and 30 April in the well-watered treatment, while no differences were found in the droughted treatment. No cultivar differences in π_{100} were observed in Phase I of experiment three (Table 4.38).

Cultivar differences in P were rare. Again, the combined errors in Ψw and π measurements (Turner, 1981) likely made P differences difficult to detect. On 1 and 8 August 1991, 'Rangelander' had the highest P, while 'South African' had the lowest (Table

4.39). In this test, there were possible differences in soil water content (as previously noted in the Ψw section), which would confound these results. At the beginning of Phase I in experiment two, 'Rangelander' had a lower *P* than 'Excalibur', while at the end of the Phase, 'Rangelander' had a lower *P* than both 'Excalibur' and 'Nitro' in the well-watered treatment (Table 4.40). No cultivar differences for *P* were detected in experiment three (Table 4.41).

In summary, Ψw was generally higher for 'Rangelander' in Phase I during experiment one (although the results may be confounded), while in experiments two and three, Ψw was generally higher for 'Nitro', where differences were significant. Cultivar differences for other water relations parameters were rare and inconclusive.

4.2.4.3 Effects of previous water treatments during Phase II

As stated earlier, the purpose of Phase II was to investigate the impact of pre-conditioning on water relations of alfalfa cultivars during a subsequent drought.

In experiment one, Ψw was unexpectedly higher (less negative) for the previously well-watered treatment at the beginning of Phase II (Table 4.27). Although root length or mass were not measured at this time, differences in root mass between the two water treatments at the end of the experiment implied that roots were not as prolific in the previously droughted treatment compared to the well-watered treatment. The less developed root system may have been insufficient to maintain adequate water uptake to meet the demands of the warm, dry atmospheric conditions. At the same time, soil moisture may not have been below a critical level required to impose significant stress on the unconditioned plants.

Further into Phase II of experiment one, Ψw was significantly lower for the previously well-watered treatment. It is important to remember that in experiment one, water was completely withheld from both treatments in Phase II. The previously well-watered treatment depleted its water supply more rapidly because it was not previously conditioned and had greater above-ground biomass to support. The differences detected here illustrate the benefit of pre-stress conditioning (and subsequent lower water use) mainly due to a reduced plant biomass. The previously well-watered treatment depleted its soil moisture to a lethal level before 30 August; therefore, only plants from the previously droughted treatment could be measured beyond this point.

On the second and third sampling dates of Phase II in experiment two, Ψw was significantly higher for the previously droughted treatment (Table 4.28). Because soil moisture was maintained at similar levels between water treatments in Phase II of the 1992 tests, the treatment difference indicates pre-stress conditioning effects, at least in part, due to physiological modifications (such as solute accumulation). No treatment difference was detected on the final sampling date. Two possible reasons can explain the lack of a significant difference on this occasion. One, the previously well-watered treatment may have been subjected to enough stress by this stage, that it had acquired drought resistance properties similar to the previously droughted treatment. Two, because plant stress increases exponentially as soil moisture falls below a critical level (Carter and Sheaffer, 1983a), small differences in soil moisture at this point would increase within-treatment variability. The soil moisture content on the final sampling date may have been so low that this made treatment differences difficult to detect.

Water potential for the previously droughted treatment was significantly lower on 30 September and 14 October 1992 in experiment three (Table 4.29). On 30 September, the treatment means were -1.27 MPa for the previously droughted treatment and -1.57 MPa for the previously well-watered treatment. The implication of this difference is impressive. Based on mathematical relationships between plant stress and growth in alfalfa (Carter and Sheaffer, 1983a), the relative growth rate is predicted to be 5.4 kg kg^{-1} $d^{-1} \times 100$ for the previously droughted treatment versus 3.1 kg kg^{-1} $d^{-1} \times 100$

for the previously well-watered treatment. In other words, the growth rate is estimated to be 74% higher for the previously droughted treatment. Although the present study did not examine relative growth rates following the initial stress in Phase I, others have suggested that compensatory growth (increased growth relative to normal) occurs in alfalfa released from drought stress (Hall, 1993). Hall found that dry matter yield increased 88 to 91% in previously drought-stressed alfalfa compared with well-watered alfalfa when growth was measured under subsequent well-watered conditions. Consequently, compensatory growth observed in previously-stressed alfalfa may be partly explained because conditioned plants can have a higher Ψw than non-conditioned plants under subsequent drought conditions.

Relative water content of the previously droughted treatment remained below that of the previously well-watered treatment at the beginning of Phase II in experiment one (Table 4.30); however, values were closer than at the end of Phase I. Pan evaporation was greatest on this date (Table B1.1), due to a combination of high solar radiation and a high wind run. These above-ground stresses, combined with the theory of a less developed root system (as previously discussed), may help explain the lag in RWC recovery. Over the next two dates, RWC of the previously well-watered treatment dropped well below that of the previously droughted treatment, as soil moisture was depleted more rapidly in the unconditioned treatment.

In experiment two, benefits of pre-conditioning were evident on 19 May, as the RWC of the previously well-watered treatment was lower than the previously droughted treatment (Table 4.31). However, this benefit was quickly lost, as the water treatment differences were not significant on the following two dates. This observation also occurred in experiment three, where the RWC was 5% lower ($P=0.0535$) for the previously well-watered treatment compared to the previously droughted treatment on 30 September, but not significantly different on the following two dates (Table 4.32). The apparent loss of the pre-stress conditioning benefit on the final two dates was likely due to the considerable length of time between the start of Phase II and the sampling date. Benefits of pre-stress conditioning for RWC abated between 19 and 24 days and 20 and 28 days after the drought stress was relieved in experiments two and three, respectively, suggesting that benefits of pre-stress conditioning may last about three weeks.

In experiment one, π_{100} values for the previously droughted treatment remained lower for the first two sampling dates in Phase II (Table 4.33). These dates were only 2 and 5 days after water was withheld from the treatments, which was not likely long enough for osmotic adjustment to occur in the previously well-watered treatment. Similarly, the π_{100} remained lower for the previously droughted treatment because little growth and translocation of the solute pool had occurred. The period of time to the following sampling date was of sufficient length to allow the π_{100} of the previously well-watered treatment to fall below that of the previously droughted treatment. This series of data also provides other interesting observations. First, it shows how quickly and dramatically π_{100} can increase when stress is removed (i.e., mean π_{100} for the previously droughted treatment increased from -1.83 to -1.25 MPa in 12 days from the end of Phase I to 27 August). Secondly, it shows the spectacular response of a well-watered plant reacting to a recently initiated stress (i.e., the π_{100} of the previously well-watered plant fell by 0.25 MPa in just 5 days from 22 to 27 August).

Both of the experiments conducted in 1992 failed to prove a relationship between π_{100} and pre-stress conditioning. Again, this was likely due to the significant time lag from the initiation of Phase II and the date the first comparative measurements were taken (unfortunately, on 7 May 1992, only the previously well-watered treatment was measured and no comparison could be made between the treatments). On 19 May 1992, the previously well-watered treatment had a lower mean π_{100} value, likely because it was under greater stress (i.e., the Ψw were -1.46 and -1.05 MPa for the previously well-watered and previously droughted treatments respectively).

Significant changes in π_{100} between sampling dates (OA) occurred at the beginning of Phase II in experiment one and three. Where differences between water treatments were significant, the direction of OA was opposite. For example, during the third period of adjustment in Phase II of experiment one, the π_{100} increased by 0.28 MPa in the previously droughted treatment, but decreased by 0.24 MPa in the previously well-watered treatment. The opposing change in osmotic adjustment resulted from the previously well-watered treatment reacting to the imposed stress, while the solute pool was being reduced in the previously droughted treatment, due to increased growth following watering.

These results do not prove a role for osmotic adjustment in pre-stress conditioning, they simply show that if π_{100} is a factor, the role is short lived (i.e., less than 20 days). However, stress-induced solute accumulation may provide an explanation for the occurrence of compensatory growth in alfalfa. The "pool" of solutes built up during pre-stress conditioning may be readily translocated to growing cells, thus helping to produce the "burst" of growth exhibited upon re-watering. In addition, it was found that previously well-watered plants adapt as quickly to stress as previously drought-hardened plants, demonstrating that alfalfa plants have the ability to quickly adapt to stress.

Samples of taproot taken at the end of the experiment indicated that π_{r100} differed between the two water treatments at two points along the taproot in experiment two. Root osmotic potential adjusted to full turgor was -1.72 MPa and -1.49 MPa in samples 2-5 cm below the crown and -1.57 and -1.49 MPa in samples 25-28 cm below the crown in the previously well-watered and previously droughted treatments, respectively. Similar results occurred for the root segment sampled in experiment three. Here, π_{r100} was -1.48 and -1.29 MPa for the previously well-watered and previously droughted treatments, respectively, for taproot samples 2-5 cm below the crown. These results are not unexpected, since Ψw was lower in the previously well-watered treatment at, or near the end of Phase II (Tables 4.28 and 4.29), which likely induced greater solute accumulation. In addition, the previously droughted treatment had resumed growth in Phase II, which would reduce its π_{100}, while growth was likely waning in the previously well-watered treatment.

Although the previously described pre-stress conditioning effects are important, the most significant benefit of pre-stress conditioning would be the maintenance of leaf turgor under subsequent stress conditions. This would allow conditioned plants to maintain superior growth rates in subsequent droughts. On the first two dates of Phase II in experiment one (P=0.0580 on 19 August) and on the first date of Phase II in experiment three, this benefit was realized, as the previously droughted treatment had higher P in all cases. Water potential was also higher for the previously droughted treatment on each of these dates and likely played the greatest role in the P difference. However, π_{100} was also an important factor in experiment one.

Turgor potential data for the previously well-watered treatment on 27 August 1991 is suspect. Given that the mean Ψw was -3.62 and the mean RWC was 39.4, the P would be expected to be 0. The C.V. was also very high for this set of data (>200). Similar to exponential changes in Ψw as soil water falls below a critical level, differences between plants likely becomes larger as turgor potential approaches 0. Because Ψw and π were measured on different samples, this likely exaggerated the error in calculating P.

In summary, benefits of pre-stress conditioning, as demonstrated by superior water relations parameters in the previously droughted treatment compared with the previously well-watered treatment, were apparent for all parameters. Osmotic adjustment likely played an important, but short-lived role in the phenomena of pre-stress conditioning, and in turn, influenced other water relations variables, such as P. Sometimes the benefits were considered minor, either because the benefits acquired by the previous drought treatment were quickly lost, or because the previously well-watered treatment acquired similar benefits during the subsequent drought in Phase II. Because a rapid osmotic response was demonstrated in the previously well-watered treatment, the involvement of other factors in the benefit of pre-stress conditioning was implicated.

4.2.4.4 Cultivar differences during Phase II

Cultivar differences in plant water relations during Phase II showed genetic variation under an imposed drought stress, while significant water treatment × cultivar interactions indicated that some cultivars benefited from pre-stress conditioning and others did not.

No cultivar differences were detected for Ψw in Phase II of experiments one and three (Tables 4.27 and 4.29). However, in experiment two, Ψw was lower for 'Excalibur' than for 'Rangelander' on the first day of significant stress (19 May; Table 4.28). On the following date, the water treatment × cultivar interaction was significant. On this date, the Ψw was lower for 'Excalibur' compared with the other two cultivars in the previously droughted treatment, while no differences were observed in the previously well-watered treatment. Because the previously droughted treatment had a higher Ψw, this implies that either 'Excalibur' does not acquire similar pre-stress conditioning benefits as compared with the other cultivars, or that it loses those benefits more rapidly. At the end of Phase II, 'Nitro' still maintained a higher Ψw than the other two cultivars in the previously stressed treatment. These results are not what one would intuitively expect. Given that 'Nitro' is highly non-dormant, 'Nitro' would be expected to resume growth quickly upon rewatering (the beginning of Phase II), which should reduce the benefits of pre-stress conditioning. These results imply that previously conditioned 'Nitro' can withstand subsequent drought conditions better than 'Excalibur' or 'Rangelander' when exposed to the same pre-stress conditioning.

It was noteworthy that more significant differences between cultivars were detected for Ψw in experiment two, than in the other experiments. Two explanations may account for this observation. First, experiment two had one additional replicate, which increased the degrees of freedom. Second, and likely more important, fewer treatments allowed samples within a replicate to be taken during a shorter time frame. Because of the diurnal water relations response in alfalfa, as shown earlier in the field experiment section 4.1.8, a shorter sampling period within a replicate would reduce the sampling error.

On the second sampling date of Phase II in experiment one, a significant water treatment × cultivar interaction for RWC likely resulted from the contrasting cultivar significance within the two water treatments. In the previously well-watered treatment, 'Nitro' had a lower RWC than most of the other cultivars, while no differences were observed in the previously droughted treatment. Similar results occurred on the following sampling date, although the interaction was not significant. On the final two dates, when only the previously droughted treatment was measured, 'Nitro' has the lowest RWC, while the RWC of 'Alfagraze', 'Excalibur' and 'Rangelander' was generally higher.

The water treatment × cultivar interaction for RWC was significant 18 and 24 days into Phase II in experiment two. On both of these dates, the previously well-watered treatment did not show any cultivar differences, while 'Excalibur' had a significantly lower RWC compared to 'Rangelander' and 'Nitro' in the previously droughted treatment. A pre-stress conditioning benefit, which would allow the previously droughted plants to maintain a higher RWC (as shown on 19 May) may not occur in 'Excalibur', or if it does, that benefit is lost more quickly than with the other cultivars.

'Alfagraze' had a lower π_{100} (P=0.0688) than 'Nitro' or 'South African' at the beginning of Phase II in experiment one. A separate analysis by water treatment, however, failed to show any significant differences. This makes it impossible to conclude whether the difference cited above arose because 'Alfagraze' has a superior ability to osmotically adjust or whether it retains that benefit after released from drought stress better than other cultivars. In the middle of Phase II, 'South African' had a lower π_{100} than 'Rangelander' in the previously droughted treatment. Water potentials were similar between the cultivars on this and the previous sampling date (Table 4.27), so the difference in π_{100} between the cultivars was not due to a stress response. Therefore, this indicates that 'South African' has a superior ability to maintain π_{100} as compared with 'Rangelander'.

In experiment two, the water treatment × cultivar interaction for π_{100} on 19 May was likely significant because cultivar differences were only evident in the previously droughted treatment. A higher π_{100} for 'Rangelander' indicated that its cell solute concentration decreased the most from the end of Phase I, while 'Excalibur' retained a greater portion of its solutes accumulated during the drought phase. Again, Ψw values were similar (Table 4.28), therefore the maintenance of π_{100} in 'Excalibur' was not due to greater water stress.

At the end of Phase II in experiment three, 'Rangelander' had lower π_{100} levels in both the overall analysis and in a separate analysis of the previously well-watered treatment data, while 'Wilson' had higher π_{100} values compared with many of the other cultivars. Because Ψw for 'Rangelander' and 'Wilson' was similar at this time, the differences in π_{100} imply a differential ability to osmotically adjust.

Cultivar differences in OA between sampling dates were only significant at the end of Phase II in experiment two and in the middle of Phase II in experiment three for the previously well-watered treatment. In experiment two, 'Rangelander' osmotically adjusted to a greater extent than the other two cultivars. 'Excalibur' had already adjusted to a greater extent over the previous two sampling dates, as π_{100} was the same for

'Rangelander' and 'Excalibur' on this date (which probably limited 'Excalibur's potential for additional osmotic regulation), while 'Nitro' was under less stress (i.e., 'Nitro' had a higher Ψw, Table 4.28) and did not need to osmotically adjust to the same extent to maintain its turgor potential. In experiment three, 'Rangelander' osmotically adjusted to the greatest extent, while 'Alfagraze' and 'Wilson' lost osmotic potential in the middle of Phase II.

Cultivar differences for π_{r100} were significant in experiment two, but not in experiment three. In experiment two, 'Nitro' had lower π_{r100} than either 'Excalibur' or 'Rangelander' for taproot segments at both 2-5 cm and 25-28 cm below the crown. Mean π_{r100} in experiment two were -1.77, -1.56 and -1.49 MPa (LSD = 0.08) and -1.64, -1.48, and -1.47 MPa (LSD = 0.08) for 'Nitro', 'Excalibur' and 'Rangelander' for the 2-5 cm and 25-28 cm taproot segments, respectively. Water potential was lower for 'Excalibur' (Table 4.28) during Phase II, therefore, a lower π_{r100} was not expected for 'Nitro'. However, 'Nitro' was selected for greater root nitrogen concentration (Barnes et al., 1988b), which could explain the lower π_{r100} observed in the present study.

No P differences between cultivars were detected in Phase II (Tables 4.39, 4.40, and 4.41).

In summary, few cultivar differences in water relations, as a result of pre-stress conditioning, were noted. Of these, the most noteworthy was in experiment two, where Ψw and RWC data showed that 'Excalibur' had an inferior ability to withstand subsequent drought stress, either because it does not acquire similar benefits of pre-stress conditioning, or because it loses those benefits more quickly compared with 'Nitro' and 'Rangelander'. All cultivars showed the ability to osmotically adjust and any differences in OA or π_{100} were generally due to differences in apparent stress level, as indicated by a lower Ψw. However, 'Nitro' was shown to have lower π_{r100} even though it generally had higher Ψw during experiment two.

4.2.5 Turgid Leaf Weight:Dry Leaf Weight Ratio

Differences in TW:DW ratio were rare between the two water treatments (Tables 4.42, 4.43 and 4.44). Because the mass of an individual alfalfa leaf is so small (typically 0.5 to 1.5 mg), it was thought that this would increase the variability and thus be largely responsible for the scarcity of significant differences. However, this was not the case, as C.V. were generally between 10 and 20%.

The TW:DW ratio was higher for the droughted treatment on 8 August 1991, but was similar on other dates in Phase I. Generally, one would expect the TW:DW ratio to decrease with increased drought stress (Turner et al., 1987), because cells produced under drought stress should be smaller and less elastic (Cutler et al., 1977). In addition, the accumulation of solutes and other osmotically inactive matter would also decrease the TW:DW ratio (Turner et al., 1987). However, Turner et al. (1987) found that the TW:DW ratio of stress-droughted lupin leaves decreased and then recovered, while the ratio for well-watered plants increased over time. One possible explanation for a higher TW:DW ratio in the droughted treatment on 8 August, is that because drought was imposed so quickly, growth essentially ceased in the previously well-watered plants. Therefore, leaf material in the previously well-watered plants may have been from older tissue, which was produced before the stress was imposed. The increase in the TW:DW ratio for both treatments at the end of Phase I in experiment one may be due to additional factors that mask the drought effect. For example, cell size may vary with the stage of growth in alfalfa. In Phase I of experiment two, a significant difference in water treatment TW:DW means was as expected, with a lower TW:DW ratio for the droughted treatment. No significant water treatment differences for TW:DW were found in experiment three (Table 4.44).

Cultivar differences for TW:DW during Phase I were also inconsistent between experiments. The TW:DW ratio was highest for 'Wilson' and lowest for 'Rangelander' in experiment one, whereas at the beginning and midway through Phase I of experiment two, 'Nitro' had the lowest TW:DW ratio. In experiment three, 'Rangelander' had a higher TW:DW ratio than all the other cultivars, except 'South African', on 10 September.

The only significant difference in TW:DW ratios between the water treatments in Phase II occurred on the first date that both treatments were sampled in experiment two. Here, the previously well-watered plants had a lower TW:DW ratio than the previously stressed treatment.

Through the middle of Phase II in experiment one, TW:DW ratios were generally higher for 'Nitro' and generally lower for 'Alfagraze' and 'Excalibur'. On 19 May 1992 there was a significant water treatment × cultivar interaction. This was due to a non-significant cultivar effect in the previously well-watered treatment, while 'Rangelander' had a higher TW:DW ratio than 'Nitro' in the previously droughted treatment.

In summary, although some cultivar and water treatment differences in TW:DW were detected, results were inconsistent. This was true even between water treatments during Phase I, which showed significant differences for other parameters. Therefore, TW:DW, as measured in the present study, is deemed to be a poor indicator of drought stress and response in alfalfa. The reason for this may be that cell size, and thus TW:DW ratio, is dependent on conditions when the leaf develops. Because conditions during the leaf's development could be different (possibly less stressful) than at the time of measurement, results could generate inconsistent, and sometimes unexpected, results. Using leaves which developed during stress, rather than using the most recent, fully expanded leaf, may yield more consistent differences.

Table 4.42 Water treatment, cultivar and interaction means for turgid weight:dry weight ratios of alfalfa leaves grown under controlled watering at Winnipeg in experiment one.

Water		Sampling Date - Phase I				Sampling Date - Phase II				
Treatment	Cultivar	26 July	1 Aug.	8 Aug.	15 Aug.	19 Aug.	22 Aug.	27 Aug.	30 Aug.	3 Sept.
Well-watered		4.38 a	4.77 a	4.22 b	6.37 a	4.47 a	4.38 a	5.60 a		
Droughted		4.77 a	5.38 a	4.68 a	6.20 a	4.41 a	4.41 a	5.76 a	5.33	6.01
LSD (5%)		0.65	0.70	0.44	1.21	0.48	0.32	0.61		
	Alfagraze	3.90 a	4.31 c	4.49 a	7.31 a	4.24 a	4.10 b	5.34 b	4.99 abc	5.60 a
	Excalibur	5.19 a	4.77 bc	4.58 a	5.76 a	3.99 a	3.97 b	5.15 b	4.75 bc	5.61 a
	Nitro	4.98 a	5.60 ab	4.51 a	6.72 a	5.04 a	4.69 a	6.75 a	6.08 a	6.70 a
	Rangelander	4.65 a	4.04 c	4.11 a	6.18 a	4.33 a	4.41 ab	5.59 b	4.53 c	5.58 a
	South African	4.23 a	5.25 b	4.44 a	6.34 a	4.55 a	4.81 a	5.65 b	5.87 ab	6.38 a
	Wilson	4.50 a	6.72 a	4.58 a	5.29 a	4.50 a	4.41 ab	5.48 b	5.77 ab	6.27 a
LSD (5%)		1.13	na	0.76	na	0.83	0.56	na	1.24	na
Well-watered	Alfagraze	3.94 a	3.42 c	4.12 a	7.44 a	4.52 a	3.97 a	5.33 a		
Well-watered	Excalibur	4.32 a	4.32 bc	4.13 a	5.43 a	4.01 a	4.09 a	5.25 a		
Well-watered	Nitro	4.70 a	5.24 ab	4.66 a	6.72 a	4.85 a	4.91 a	6.36 a		
Well-watered	Rangelander	4.51 a	4.50 bc	3.89 a	7.96 a	5.04 a	4.59 a	6.14 a		
Well-watered	South African	4.37 a	4.66 bc	4.18 a	6.70 a	4.15 a	4.40 a	5.41 a		
Well-watered	Wilson	4.42 a	6.51 a	4.35 a	4.61 a	4.27 a	4.34 a	5.13 a		
LSD (5%)		1.15	1.78	1.51	na	1.20	0.69	1.00		
Droughted	Alfagraze	3.86 a	5.20 b	4.86 a	7.17 a	3.95 bc	4.23 a	5.37 a	4.99 abc	5.60 a
Droughted	Excalibur	6.06 a	5.22 b	5.04 a	6.25 a	3.98 bc	3.84 a	5.05 a	4.75 bc	5.61 a
Droughted	Nitro	5.26 a	5.96 ab	4.36 a	6.72 a	5.24 a	4.47 a	7.14 a	6.08 a	6.70 a
Droughted	Rangelander	4.80 a	3.58 c	4.33 a	4.99 a	3.62 c	4.22 a	5.03 a	4.53 c	5.58 a
Droughted	South African	4.09 a	5.84 ab	4.71 a	6.10 a	4.95 ab	5.22 a	6.01 a	5.87 ab	6.38 a
Droughted	Wilson	4.58 a	7.03 a	4.81 a	5.97 a	4.73 ab	4.48 a	6.01 a	5.77 ab	6.27 a
LSD (5%)		2.16	na	0.80	na	1.06	1.03	na	1.24	na

Means within a continuate column followed by the same letter are not significantly different at alpha=0.05 (LSD).
na - not available due to missing values.

Table 4.43 Water treatment, cultivar and interaction means for turgid weight:dry weight ratios of alfalfa leaves grown under controlled watering at Winnipeg in experiment two.

Water		Sampling Date - Phase I				Sampling Date - Phase II			
Treatment	Cultivar	8 April	16 April	23 April	30 April	7 May	19 May	25 May	28 May
Well-watered		8.66 a	7.87 a	8.61 a	7.95 a	8.60	8.40 b†	5.72 a	5.86 a
Droughted		9.21 a	7.15 b	9.84 a	9.06 a		9.49 a	5.80 a	6.45 a
LSD (5%)		0.92	0.44	1.52	1.19		0.90	0.76	0.77
	Excalibur	9.22 a	7.39 a	9.09 ab	8.28 a	8.81 a	9.03 ab	5.86 a	6.09 a
	Nitro	7.95 b	7.38 a	7.94 b	8.36 a	8.29 a	8.13 b	5.69 a	6.07 a
	Rangelander	9.65 a	7.75 a	10.65 a	8.86 a	8.69 a	9.68 a	5.73 a	6.31 a
LSD (5%)		1.13	0.53	1.86	1.46	1.74	1.10	0.93	0.95
Well-watered	Excalibur	9.37 a	7.62 a	8.05 a	8.44 a		8.56 a	5.67 a	5.87 a
Well-watered	Nitro	7.79 a	7.98 a	8.18 a	7.47 a		8.27 a	5.51 a	5.70 a
Well-watered	Rangelander	8.83 a	7.99 a	9.61 a	7.93 a		8.38 a	5.99 a	6.01 a
LSD (5%)		2.42	0.60	2.14	1.47		1.88	0.95	1.28
Droughted	Excalibur	9.06 b	7.16 a	10.13 a	8.13 a		9.50 ab	6.04 a	6.32 a
Droughted	Nitro	8.11 b	6.78 a	7.69 a	9.24 a		7.99 b	5.87 a	6.43 a
Droughted	Rangelander	10.47 a	7.52 a	11.69 a	9.80 a		10.98 a	5.48 a	6.61 a
LSD (5%)		1.37	0.80	3.59	3.41		1.94	1.88	1.77

Means within a continuate column followed by the same letter are not significantly different at alpha=0.05 (LSD).

† - water treatment by cultivar interaction is significant at alpha=0.05.

Table 4.44 Water treatment, cultivar and interaction means for turgid weight:dry weight ratios of alfalfa leaves grown under controlled watering at Winnipeg in experiment three.

Water		Sampling Date - Phase I			Sampling Date - Phase II		
Treatment	Cultivar	20 Aug.	3 Sept.	10 Sept.	30 Sept.	9 Oct.	14 Oct.
Well-watered		7.60 a	7.75 a	7.99 a	7.78 a	7.45 a	7.37 a
Droughted		7.26 a	8.57 a	7.84 a	8.11 a	7.64 a	7.34 a
LSD (5%)		0.81	1.27	0.81	1.07	1.08	0.50
	Alfagraze	7.41 a	8.22 a	7.36 b	7.87 a	6.35 a	7.68 a
	Excalibur	8.19 a	7.45 a	7.14 b	7.05 a	8.15 a	6.73 a
	Nitro	7.08 a	8.75 a	7.87 b	7.67 a	6.93 a	7.03 a
	Rangelander	7.26 a	9.01 a	9.54 a	9.26 a	8.16 a	7.53 a
	South African	7.06 a	8.28 a	8.46 ab	8.65 a	7.70 a	7.77 a
	Wilson	7.61 a	7.22 a	7.21 b	7.14 a	7.78 a	7.40 a
LSD (5%)		1.40	2.20	na	1.85	na	0.87
Well-watered	Alfagraze	7.74 a	7.76 a	7.93 a	7.52 a	6.57 a	7.78 a
Well-watered	Excalibur	9.11 a	7.40 a	7.14 a	6.32 a	8.18 a	6.83 a
Well-watered	Nitro	6.82 a	8.17 a	7.64 a	7.19 a	7.20 a	6.75 a
Well-watered	Rangelander	8.04 a	9.00 a	9.28 a	9.37 a	7.47 a	7.39 a
Well-watered	South African	7.20 a	7.22 a	8.71 a	9.56 a	7.12 a	7.53 a
Well-watered	Wilson	6.70 a	6.92 a	7.21 a	6.68 a	7.87 a	7.94 a
LSD (5%)		2.55	2.07	2.25	3.22	na	0.97
Droughted	Alfagraze	7.07 a	8.67 a	6.79 a	8.22 a	6.21 a	7.57 a
Droughted	Excalibur	7.28 a	7.50 a	7.14 a	7.78 a	8.12 a	6.62 a
Droughted	Nitro	7.33 a	9.34 a	8.10 a	8.15 a	6.66 a	7.31 a
Droughted	Rangelander	6.47 a	9.02 a	9.80 a	9.16 a	8.84 a	7.68 a
Droughted	South African	6.91 a	9.34 a	8.07 a	7.73 a	8.29 a	8.01 a
Droughted	Wilson	8.52 a	7.52 a	7.20 a	7.60 a	7.70 a	6.85 a
LSD (5%)		1.84	3.40	na	2.50	3.57	1.50

Means within a continuate column followed by the same letter are not significantly different at alpha=0.05 (LSD).
na - not available due to missing values.

4.2.6 Water Loss from Detached Leaves

Plants with higher detached leaf water retention ability are thought to be more drought resistant (Dedio, 1975; Clarke and McCaig, 1982). It has not been determined whether this conclusion is valid for alfalfa, although Jefferson et al. (1989) found that there was no consistent relationship between leaf water loss rates and epicuticular wax production in alfalfa. In the present study, water loss from leaves sampled during Phase I of experiment three was measured to determine if leaf water loss rates differed among the cultivars and if so, how that might relate to other characteristics. In addition, the impact of drought stress on leaf water loss was evaluated.

Water loss from detached alfalfa leaves was greatest during the first hour (Figures 4.25, 4.26 and 4.27). The water loss rate, although slightly greater from hour 1 to hour 2 on August 25, was generally linear from hour 1 to hour 8 for all three dates. The stabilization of the water loss rate beyond hour 1-2 in the current study, suggests that the stomata have essentially closed and that the remaining water loss is via cuticular conductance or through leaky stomata.

Carter and Sheaffer (1983b) found that g_l stabilized in alfalfa at a Ψw below -2.5 MPa when measured with a porometer. They concluded that this was evidence for either cuticular conductance or incomplete stomatal closure. Although it is impossible to be certain when the stomata closed in this study, they appear to have closed much sooner than Carter and Sheaffer (1983b) determined. This conclusion is based on the estimated π values as given in Table 4.45 at the approximate point where the dehydration curve became linear. On 25 August this occurred between hour 1 and 2, which would imply a π of between 1.35 and 1.51 MPa for both the non-droughted and droughted alfalfa. On the remaining two dates, the stomata closed prior to the π reaching -1.8 MPa for the well-watered plants and prior to -2.0 MPa for the droughted plants. Because Ψw would have to be equal or greater than the π to leave the plant with a positive P, the stomata closed prior to the Ψw dropping into the -1.8 to -2.0 MPa range. Under field conditions, however, the water loss rate would be more gradual and the stomata may not react as quickly. This may explain the difference in findings between Carter and Sheaffer (1983b) and the present study.

Table 4.45 Estimated osmotic potential (MPa) of alfalfa leaves by water treatment, based on mean π_{100} values at the closest sampling date and the relative water content of the dehydrated leaves, at Winnipeg, in experiment three.

Date	Water Treatment	Hours of Leaf Dehydration 1	2	4	6	8
25 August	Well-watered	-1.35	-1.51	-1.84	-2.34	-3.01
	Droughted	-1.35	-1.48	-1.71	-2.04	-2.44
1 September	Well-watered	-1.84	-2.03	-2.36	-2.80	-3.37
	Droughted	-2.36	-2.56	-2.91	-3.36	-3.95
11 September	Well-watered	-1.75	-1.92	-2.27	-2.71	-3.35
	Droughted	-2.01	-2.15	-2.47	-2.83	-3.31

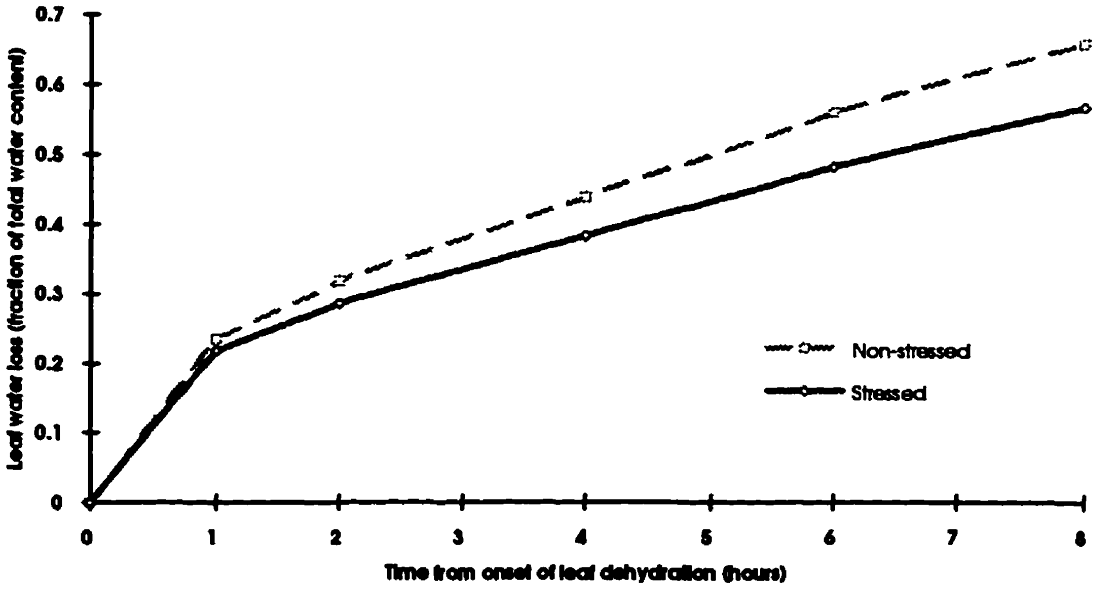

Figure 4.25 Dehydration of detached alfalfa leaves grown at Winnipeg under droughted and well-watered water treatments. Sampled on 25 August 1992.

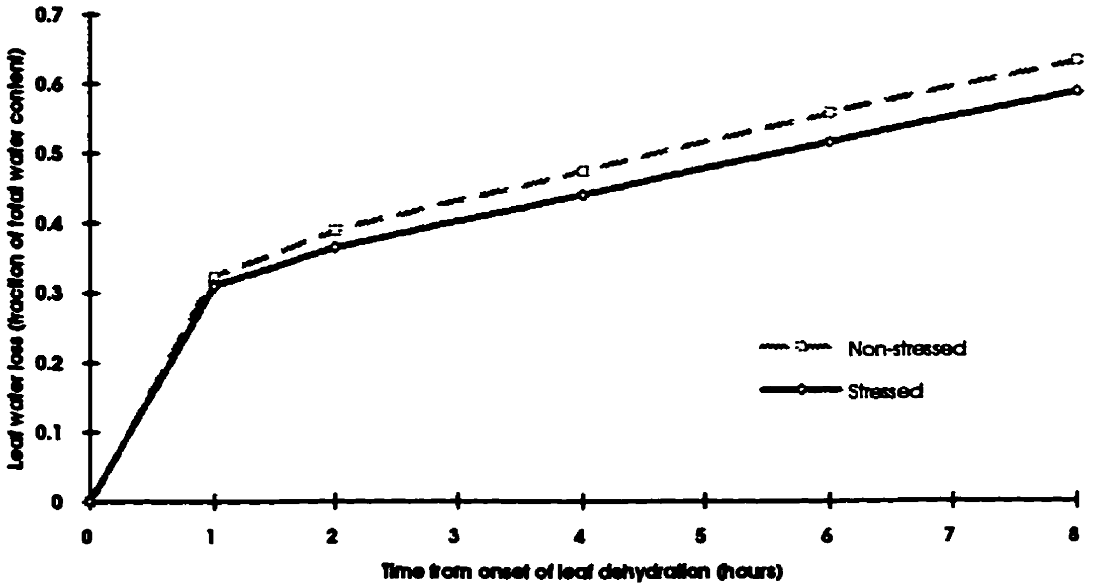

Figure 4.26 Dehydration of detached alfalfa leaves grown at Winnipeg under droughted and well-watered water treatments. Sampled on 1 September 1992.

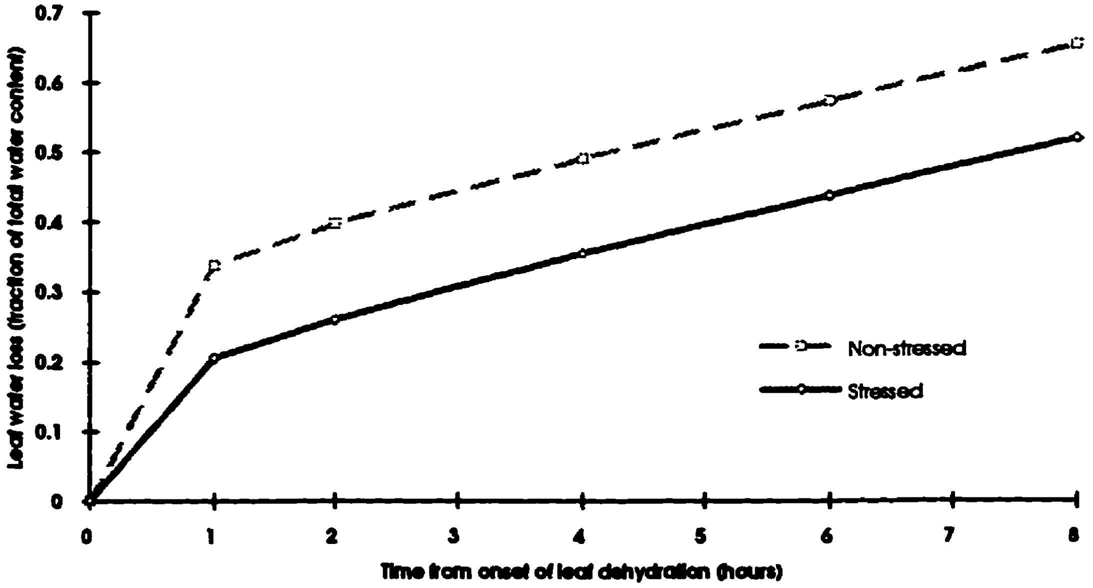

Figure 4.27 Dehydration of detached alfalfa leaves grown at Winnipeg under droughted and well-watered water treatments. Sampled on 11 September 1992.

Chi-square test analysis for homogeneity of error variances found that results of leaf dehydration for the three sampling dates could be combined. However, a significant date by water treatment interaction required that the dates be presented separately. The interactions for cultivar × date or cultivar × water treatment were not significant and will be presented as combined analysis.

The well-watered alfalfa always lost a greater portion of its total leaf water content as compared with the droughted alfalfa after all sampling times on all three dates. The differences in leaf water loss between the two water treatments were significant after 4 hours on 25 August and 1 September, but were significant after the first hour on 11 September (Table 4.46). The observation of significant differences after a shorter dehydration period on 11 September may be due to pre-stress conditioning effects. More responsive stomata are a logical explanation because the difference occurs within the first hour when the stomates likely closed. The parameter involved in the stomatal response could not be determined; however, π does not appear to be involved. This conclusion is based on the estimated π values given in Table 4.45. The difference in π between the two water treatments was greater on 1 September than on 11 September. In addition, the well-watered treatment dehydrated more than the droughted treatment after 4 hours of dehydration on August 25, even though the estimated π was higher for the well-watered treatment.

In the combined date analysis, the cultivar effect was highly significant ($P<0.01$) after 4, 6 and 8 hours of dehydration (Table 4.46). However, cultivar differences were greatest after 8 hours and only results for this time period will be discussed. Proportional leaf water loss was greatest for 'Nitro', 'Rangelander' and 'Wilson', while 'Alfagraze' lost the least water. Leaf water loss for 'Excalibur' was not significantly different than for 'Alfagraze'. Although the ability of detached alfalfa leaves to retain a greater portion of water could be a drought tolerant trait, it can be concluded from the results in the current study that it is not a predominant characteristic in some cultivars that are considered drought tolerant (i.e., 'Rangelander' and 'Wilson').

Table 4.46 Fraction of total leaf water content lost in detached alfalfa leaves over time for water treatment and cultivar effects in experiment three.

		Hours of dehydration				
Date	Water/Cultivar	1	2	4	6	8
25 Aug.	Well-watered	0.23 a	0.32 a	0.44 a	0.56 a	0.66 a
	Droughted	0.22 a	0.29 a	0.38 b	0.48 b	0.57 b
	LSD (5%)	0.03	0.03	0.04	0.05	0.06
1 Sept.	Well-watered	0.32 a	0.39 a	0.47 a	0.56 a	0.63 a
	Droughted	0.31 a	0.36 a	0.44 b	0.51 b	0.59 b
	LSD (5%)	0.02	0.03	0.03	0.03	0.04
11 Sept.	Well-watered	0.34 a	0.40 a	0.49 a	0.57 a	0.65 a
	Droughted	0.21 b	0.26 b	0.35 b	0.44 b	0.52 b
	LSD (5%)	0.03	0.03	0.04	0.04	0.04
Combined Dates	Alfagraze	0.26 a	0.31 b	0.39 c	0.47 c	0.54 c
	Excalibur	0.28 a	0.33 ab	0.42 bc	0.50 bc	0.57 bc
	Nitro	0.28 a	0.35 a	0.46 a	0.56 a	0.65 a
	Rangelander	0.28 a	0.35 a	0.44 ab	0.54 ab	0.62 a
	South African	0.27 a	0.34 ab	0.43 ab	0.52 ab	0.61 ab
	Wilson	0.26 a	0.33 ab	0.44 ab	0.54 ab	0.63 a
	LSD (5%)	0.03	0.03	0.04	0.04	0.04

Means within a continuate column followed by the same letter are not significantly different at alpha=0.05 (LSD).

Because cultivar differences became more apparent after several hours as compared with 1 hour of dehydration, when stomates were likely partly open, it appears that much of the cultivar variation for detached leaf dehydration is linked to differences in cuticular conductance. This beneficial characteristic would only be important under field growing conditions where soil water deficits are sufficient to induce stomatal closure.

In summary, detached leaf dehydration indicated that droughted plants lost less water via cuticular conductance or leaky stomates on the first two sampling dates, while on the third date, after a longer conditioning period, leaves from droughted plants lost less water mainly because of more responsive stomata. Although the factor or factors involved in this stomatal response were not determined, π was ruled out. Among the cultivars studied, 'Nitro' and 'Rangelander' dehydrated the most, while 'Alfagraze' and 'Excalibur' dehydrated the least. Further study to determine if there is a linkage between detached leaf water loss and drought tolerance in alfalfa is warranted.

4.2.7 Leaf Conductance and Temperature

As expected, measurements of conductance rates indicated that well-watered alfalfa plants had higher conductance rates than droughted plants. Homogeneity of variances allowed the data from both sampling dates, 1 and 11 September, to be combined. Mean rates of conductance for the well-watered treatment were 1.17 cm s^{-1} versus 0.14 cm s^{-1} for the droughted treatment.

No cultivar differences for conductance were detected in either sampling date or in the combined analysis (data not shown). The coefficient of variation was less than 25 in the individual analysis and 35.5 in the combined analysis. Although this is fairly high, it is not likely the reason for the inability to detect cultivar differences given the P value of 0.9807.

Calculating the difference between leaf and cuvette temperatures is another indirect method of measuring conductance, because leaf temperature is lowered in the process of transpiration. Similar to conductance measurements, $T_l - T_a$ data indicated that the droughted plants were transpiring at lower rates than the well-watered plants. Transpiration rates were so low in the droughted plants that leaf temperatures were actually 0.23°C higher than the cuvette (ambient) temperature. In the well-watered treatment, transpiration rates lowered leaf temperatures 0.89°C below that of the droughted plants (mean leaf minus cuvette temperature for the well-watered treatment was -0.66°C).

4.2.8 Relationships between Water Relations Variables

The measurement of several water relations variables over a wide range of stress levels in the controlled water experiments enabled the general characterization of alfalfa water relations as soil water became limiting. Because not all cultivars were measured on each date (i.e., only 3 cultivars were used in Experiment 2) and because the range of values differed between cultivars, regression analysis was conducted on the dataset as a whole. Although this approach does not separate cultivar effects, it does teach us more about general plant-water relationships in alfalfa.

Water relations variables were compared to Ψw using regression analysis, as Ψw was assumed to be the primary indicator of plant stress (due to both soil water deficiency and atmospheric stresses). The relationships between the various variables and Ψw are shown in Figure 4.28, while the equations for these and other relationships are given in Table 4.47.

Relative water content decreased as Ψw fell (Figure 4.28). Although the quadratic equation was significant, the relationship was largely linear as R^2 values were similar (i.e., 0.77 and 0.77 for the linear and quadratic equations, respectively). It was questioned whether the environment influences the relationship between the two variables. However, predicted values from separate regressions on data from the outdoor (under the "rainout" shelter) and indoor studies (data not shown) were surprisingly similar. For example, both quadratic equations (not given) estimated that RWC would drop to 40% at a Ψw of -4.2 MPa.

Osmotic potential also decreased quadratically with Ψw. This non-linear response was primarily due to the decrease in RWC, however, osmotic adjustment was also involved. Calculating π_{100} from the π and RWC equations, the amount of OA that occurred decreased as stress level increased. For example, the amount of OA that occurred as Ψw decreased from -0.5 to -1.5 MPa was estimated at 0.23 MPa, whereas, the OA that occurred from -1.5 to -2.5 and -2.5 to -3.5 MPa was estimated at 0.19 and 0.10 MPa, respectively. This is expected because greater stress levels would increase the duration of stomatal closure and therefore, would limit the amount of photosynthesis that could occur. This in turn, would reduce the plant's ability to produce osmotically active solutes. Osmotic potential at full turgor reached a minimum at a midday Ψw level of -3.9 MPa. Alfalfa likely loses its ability to sufficiently recover during less stressful parts of the day to increase its solute concentration beyond this level.

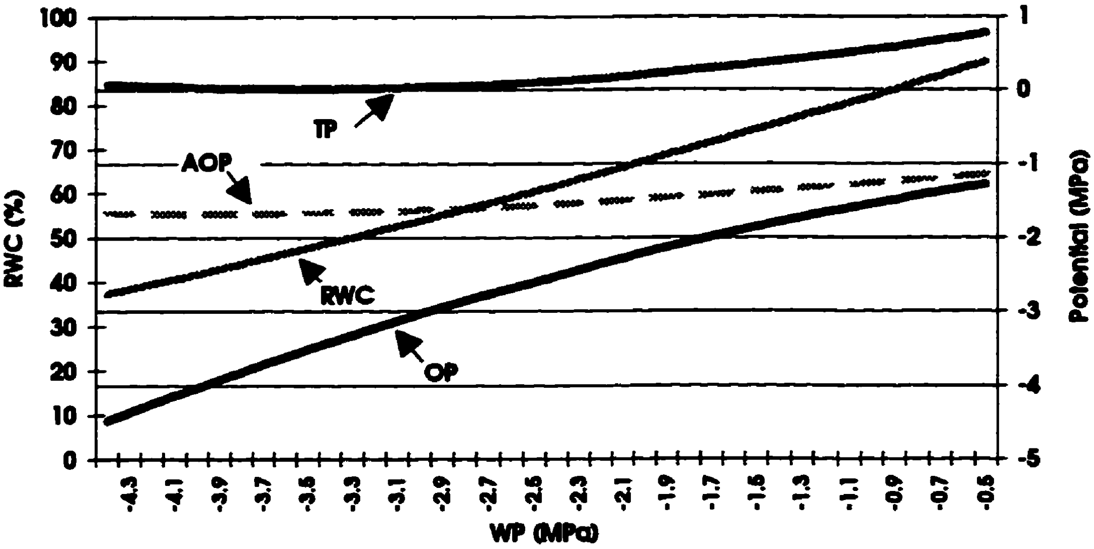

Figure 4.28 Trend of relative water content (RWC), osmotic potential (OP), osmotic potential adjusted to 100% RWC (AOP), and turgor potential (TP) versus water potential (WP) for alfalfa grown in containers under various soil moisture conditions at Winnipeg in experiments one, two and three.

Brown and Tanner (1983a) determined that little stem and leaf growth occurs in alfalfa below a Ψw of -1.0 MPa. Based on this finding, they estimated that P was 0.3 ± 0.07 MPa (using a pressure-volume relationship) when leaf expansion in alfalfa ceased. In the present study, it was estimated that P was 0.55 MPa at a Ψw of -1.0 MPa. In addition, it was estimated that Ψw would be -1.7 MPa at a P of 0.3 MPa. Brown and Tanner (1983b) criticized the freeze-thaw method of π determination, as they found it overstated π, compared with the water-release method, by about 0.2 to 0.6 MPa. If the freeze-thaw method is inferior, then the results between the two studies would be closer. However, because the P equation in the present study approached 0 and reached a minimum of 0.016 MPa at a Ψw of -3.5 MPa, the π and derived P values seem realistic. If the freeze-thaw method was understating π by the amount suggested by Brown and Tanner (1983b), then P values would become negative somewhere between a Ψw of -1.0 and -2.0 MPa.

Table 4.47 Linear and quadratic regression equations for various water relations relationships in alfalfa. Alfalfa plants were grown under a wide range of soil moisture levels under controlled watering in experiments one, two and three.

	Equation	n	P > F	R^2
$\pi =$	$-1.055 + 0.410 (\Psi w) - 0.084 (\Psi w)^2$	630	0.0001	0.81
$\pi =$	$116.595 + 26.211 (RWC) + 2.006 (RWC)^2$	684	0.0001	0.80
RWC =	$95.849 + 14.035 (\Psi w)$	630	0.0001	0.77
RWC =	$97.976 + 16.755 (\Psi w) + 0.675 (\Psi w)^2$	630	0.0001	0.77
$\pi_{100} =$	$-1.039 + 0.253 (\Psi w) + 0.022 (\Psi w)^2$	630	0.0051	0.33
$P =$	$1.055 + 0.590 (\Psi w) + 0.084 (\Psi w)^2$	630	0.0001	0.36
$P =$	$2.649 - 0.082 (RWC) + 0.001 (RWC)^2$	630	0.0001	0.24
$P =$	$1.005 + 0.504 (\pi) + 0.087 (\pi)^2$	630	0.0001	0.05

4.2.9 Pressure-Volume Curves

Numerous variations of the pressure-volume curve have been used by researchers (Melkonian et al., 1982; Richter, 1978; Cheung et al., 1975) as a tool to describe plant water relations. They are useful because they allow several parameters to be derived from two simple measurements: balancing pressure and the fraction of expressed sap. However, their utility is limited because measurements are very time consuming. In the current study, a reliable technique was developed for alfalfa after several attempts. Only results from the final set of measurements, for droughted 'Nitro' and 'Rangelander' treatments in experiment two, will be presented in detail.

The fraction of expressed sap for droughted 'Nitro' and 'Rangelander' treatments, plotted against a transformed (inverse balance pressure) pressure scale, is shown in Figure 4.29. Estimates of the slope and intercept, determined from regression analysis on the linear portion of the curve (i.e., measurements from 1.6 to 2.8 MPa), were used to calculate the portion of apoplastic water and π_{100}. The equations were determined as:

'Nitro': $1\text{-RWC} = -1.105(\text{Balancing Pressure}^{-1}) + 0.907$; $R^2=0.92$

'Rangelander': $1\text{-RWC} = -1.154(\text{Balancing Pressure}^{-1}) + 0.935$; $R^2=0.93$

Apoplastic water content was estimated at 9.3 and 6.5% for 'Nitro' and 'Rangelander', respectively, although analysis proved the two lines and intercepts to be similar. These values are comparable or lower than those found in some other species. For example, Cheung et al. (1975) determined that the portion of apoplastic water in various species of tree leaves ranged from 5 to 30%, while Campbell et al. (1979) found that this portion averaged 30, 5 and 17% in wheat, potato and wheatgrass, respectively.

Bittman and Simpson (1989) determined that the apoplastic portion of smooth bromegrass and crested wheatgrass was 28.2 and 22.8%, respectively.

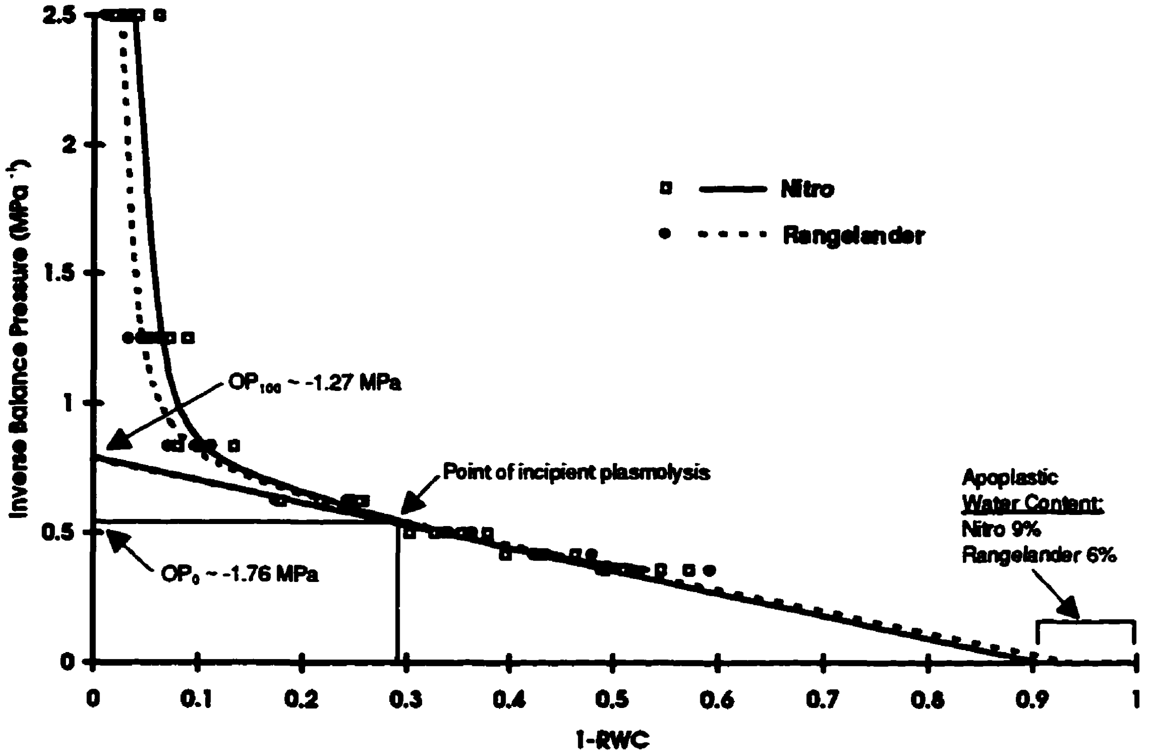

Figure 4.29 Pressure-volume curves for droughted 'Nitro' and 'Rangelander' (mean of four and three replications, respectively) stems taken from experiment two at Winnipeg, 1992.

Osmotic potential at full turgor was estimated at -1.26 and -1.27 MPa for 'Nitro' and 'Rangelander', respectively, from the regression equations. These values are slightly higher than those determined by osmotic and RWC measurements, which averaged -1.40 and -1.33 MPa for droughted 'Nitro' and 'Rangelander' treatments, respectively, on the closest sampling date, 25 May 1992 (Table 4.34). Brown and Tanner (1983b) also found lower values for freeze-thaw determined osmotic potential compared with pressure-volume methodology despite the fact that, theoretically, the freeze-thaw methodology should be higher due to dilution of the symplast (Wenkert, 1980). A comparison of the two methods in the current study shows a difference in π_{100} of about 5-10%. Turner et al., (1978) found that π_{100} decreased about 10 and 30% from sunflower and sorghum respectively, after thawing was increased from 1 to 24 hours, attributing the decrease to starch hydrolysis. Given that thawing was only about 30 minutes in the current study, the magnitude of difference here is relatively high. However, alfalfa leaves contain large concentrations of starch and sugars (Brown and Tanner 1983b), and if enzymatic hydrolysis is occurring, this would help explain a relatively greater π_{100} increase in alfalfa. In addition, alfalfa contains a smaller portion of apoplastic water relative to wheat, for example, reducing the dilution effect.

Osmotic potential at zero turgor (π_0) was estimated at -1.76 MPa for both 'Nitro' and 'Rangelander', which implies that turgor was lost at a Ψw of -1.76 MPa. This is much higher than shown in Figures 4.12 or 4.28; however, if a 5-10% adjustment from the analysis above is taken into account, the values would be similar. Brown and Tanner (1983b) reported that the point of zero turgor was reached between -1.2 and -1.6 MPa, but generally about -1.5 MPa, in their pressure-volume analysis on alfalfa leaves.

The point of zero turgor was estimated to be at about 72% RWC, which is significantly lower than 89 to 95% as reported for single alfalfa leaves by Brown and Tanner (1983b), but higher than estimated values calculated in sections 4.1.6 and 4.2.8. A lower RWC at π_0 would be expected in their study because leaf cells would likely be more elastic than the combination of leaf and stem cells in the present study. The differences cannot be logically explained, because Brown and Tanner's plants were also stressed. Their methodology, however, was slightly different, as they allowed the leaves to dehydrate between the determination of successive balancing pressures during the phase of positive turgor pressure, which may explain the difference.

The pressure-volume relationship in the current section is generally consistent with those established in the field and controlled study sections, 4.1.6 and 4.2.8, respectively.

For example, predicted values of Ψw at a RWC of 70% are -1.7, -1.8 and -1.8 MPa for the field study, controlled study and pressure-volume relationship, respectively. This consistency, however, does not prove if the pressure-volume relationship changes significantly with stress (as all plants had some stress) or with age. These questions would require further investigation.

Bulk modulus of elasticity (ε) was found to be greater in plant material from droughted 'Rangelander' compared with droughted 'Nitro' at 21.7 and 12.6 MPa, respectively. These results indicate that droughted 'Rangelander' has less elastic cells than droughted 'Nitro'. Because sampling periods in the current study for well-watered and droughted treatments were different, a statistical comparison is not valid. However, mean ε results of 12.5 and 10.9 MPa for previously well-watered 'Rangelander' and 'Nitro' treatments, respectively, imply that cell elasticity increases in droughted 'Rangelander' to a greater extent than for 'Nitro' and could be an important drought tolerance mechanism. An increase in ε from 22.3 to 27.9 MPa as a result of drought stress was also found in wheat (Melkonian et al., 1982). Comparing these results with the current study, it is apparent that even cell walls of droughted alfalfa are more elastic than cell walls of well-watered wheat. The conflicting reports of drought conditioning effects on ε, as noted by Melkonian et al. (1982) and Bittman and Simpson (1989), may be due to differential species or even cultivar effects. Possible genetic differences in cell wall elasticity changes as a result of drought stress in alfalfa deserves further study.

5.0 Summary and Conclusions

Little information is available in the literature comparing water relations between alfalfa cultivars. Most studies limited their comparison to a couple of cultivars and only considered either aspects of water relations, productivity or water use (e.g., Hattendorf et al., 1990). Our study took a "shot gun" approach as a first step in combining these areas with the hope of identifying potential differences between a divergent group of alfalfa cultivars. To achieve this, productivity, water use and water relations among several alfalfa cultivars grown under various soil water conditions were examined. In addition, the effects of a previous drought cycle on water relations during a subsequent drought were investigated. Relationships between water relations variables were analyzed to yield a better understanding of general alfalfa water relations.

From results obtained in this study, it was apparent that relationships between plant water relations, water use and productivity in alfalfa are complex. For example, cultivars that exhibited superior water relations did not necessarily have greater productivity or higher water use efficiencies. This was shown in the field study, where 'Nitro' generally had a higher Ψw, but its total and first harvest aerial dry matter production were average or lower than other cultivars. Results for 'Excalibur', on the other hand, indicated that it was able to produce average or higher yields despite having a lower RWC and *P* on some occasions. Based on this information, it can be concluded that using water relations information as criteria for drought resistance selection in alfalfa may result in yield potential limitations if droughts are periodic or short-lived.

Comparing water relations and productivity of alfalfa cultivars during the year of establishment with adequate soil moisture levels at planting and above normal rainfall during the growing season (i.e., total June through September rainfall measured at the plot site was 141 and 143% above the 30 year average for the Winnipeg International Airport in 1991 and 1992, respectively) was a limitation of the field study. Because alfalfa is a perennial plant, which is usually continuously cropped for a period of 6 years in the eastern prairies (Katepa-Mupondwa et al., 1993), drought resistance characteristics would likely be more important for productivity and survival of the stand in subsequent seasons. With 'Rangelander', lower water use may have limited its production capability relative to the other cultivars in the present study, but potentially higher water reserves in subsequent seasons may allow greater production or better survival rates under more arid growing conditions. The low stress levels that prevailed through the majority of the growing season, especially in the field tests, favored productivity in cultivars that were selected for yield rather than drought resistance. Superior yields for the hay- or dual-purpose-type alfalfa cultivars in the present study, over 'Wilson' or 'Rangelander', demonstrates this conclusion. However, 'Alfagraze', which was selected for persistence under grazing, also yielded well. Any drought stress incurred during either season was simply insufficient in duration or severity to elucidate the benefits of drought resistance characteristics. Water relation measurements were generally conducted in later plant growth stages and during mid-day, which may not be indicative of conditions through the plant's growth cycle. Including measurements during other parts of the day or season, may have helped explain some of the productivity differences. Yield results in the controlled experiment were not entirely consistent with field results, however, hay-type or dual purpose cultivars (i.e., 'Alfagraze', 'Excalibur' and 'Nitro') still generally produced more dry matter than drought tolerant types (i.e., 'Rangelander', 'South African' and 'Wilson').

Root production was generally highest for 'Alfagraze', 'Nitro' and 'South African'. A more extensive root system may allow these cultivars to extract more water during stressful parts of the day and may partly explain the higher Ψw observed for 'Nitro'.

Evapotranspiration differences between the cultivars were rare, but generally indicated lower water usage for 'Rangelander'. This was likely due to its slower rate of growth, especially in the latter part of the year. However, 'Rangelander' also used less water in the controlled water study where dormancy was less of a factor. Lower water use could allow 'Rangelander' to survive longer periods of time without rainfall and may be an important mechanism for its persistence. 'Nitro' extracted the most water in the controlled water study even though the impact of a larger root system was not likely fully realized in a limited soil volume.

Evapotranspiration efficiency was generally greatest for hay-type or dual purpose cultivars. Under light to moderate drought stress conditions differences in alfalfa ETE can be generally attributed to differences in herbage production; therefore, selection for yield without regard for water use or extraction capabilities is likely valid in similar environments.

The detached leaf water loss method determined that the alfalfa cultivars used in this study dehydrated at different rates. The mechanisms for this difference are not understood, but nonetheless, this screening technique may be a useful tool in discerning genetic differences in an alfalfa breeding program. Although several samples over several dates may be required, the method is relatively quick compared with other water relations measurements previously discussed. This trait has been shown to be associated with drought hardiness in winter wheat (Clarke and McCaig, 1982). The link between lower leaf water loss and drought hardiness in alfalfa has not been proven here, but it warrants further investigation. However, unlike wheat, alfalfa is a perennial plant, and depending on its growing environment, other characteristics, such as slower growth to avoid drought, may be more useful for improving performance in droughty conditions.

As noted above, cultivar differences for the selected parameters were not always clear or consistent. The length of time required to sample a replication, as concluded from the diurnal sampling, may have increased the sampling error and limited the significant differences. However, some general tendencies for each of the cultivars were identified. These characteristics are summarized in Table 4.48.

Table 4.48 General water relations, water use or productivity characteristics of alfalfa cultivars included in this study.

Cultivar	Characteristic
Alfagraze	• high yields in the field, moderate yields in the containers • high root yields • good P maintainer • superior ability to maintain water content in detached leaves
Excalibur	• moderate to high yields • generally lower Ψw • generally lower RWC • poor osmotic adjuster • poor P maintainer • does not acquire pre-stress conditioning benefits to the same extent or loses those benefits more quickly
Legend	• high yields, highest ETE
Nitro	• moderate above-ground yields • high root yields • average water use in the field, highest water use in the containers • generally higher Ψw
Rangelander	• low yields, especially from the second-cut • low root yields • lower water use in both the field and controlled water studies • generally lower Ψw under field conditions, average Ψw when root depth/density were less of a factor (i.e., when grown in containers) • generally higher RWC • good osmotic adjuster • generally had inferior maintenance of P • generally higher π_{r100} • less elastic cell walls than 'Nitro'
South African	• moderate to low yields • lower ETE
Wilson	• moderate to low yields • lower ETE in both field and controlled water studies

In addition to observations noted for each cultivar, several findings and conclusions for alfalfa in general can be made. Effective rooting depth under field conditions was similar among cultivars, although some differences in total water use were noted. This depth was determined to be about 80 to 120 cm at the time of first cut, but extended to 140 to 180 cm by the end of the season. This extensive rooting capability would likely allow alfalfa to be beneficial in phytoremediation of soils (e.g., deep-leached nitrogen extraction), even in the establishment year. Soil water only accounted for about 22 to 28% of seasonal ET despite the extensive rooting, likely because precipitation was above average in both 1991 and 1992.

Alfalfa has the ability to osmotically adjust. The degree of adjustment, about 0.4 to 0.45 MPa (calculated from the difference in well-watered and droughted treatments at the end of Phase I) is similar to that reported in wheat (up to 0.44 MPa; Johnson et al., 1984), barley (up to 0.46 MPa, Blum, 1989) and lupins (0.5 MPa; Turner et al., 1987). Most of the osmotic adjustment was due to stress (both drought and heat), with little effect from plant age over the stages of maturity included in this study. This osmotic adjustment was also shown to play a short-lived role in subsequent drought conditions. Because other parameters (e.g., Ψw, RWC) continued to be superior in the previously droughted treatment after the benefits of OA had waned, other factors were implicated.

From the relationships established from water relations parameters in the field study and the pressure-volume curve, it was estimated that the point of turgor loss occurred at a Ψw of -1.95 and -1.76 MPa and a RWC of about 63 and 72%, respectively. Due to the nature of the relationships, a quadratic curve comparing these relationships in the controlled water study never crossed zero, but reached minima at -3.5 MPa and 41%, respectively. These results compare with a Ψw of about -1.2 to -1.6 MPa and a RWC of 89 to 95% as reported by Brown and Tanner (1983b). Part of these differences could be accounted for if starch hydrolysis occurred when using the freeze-thaw methodology, which would result in erroneously low π_{100} measurements.

Alfalfa was found to have more elastic cell walls compared with observations and values reported in the literature (i.e., wheatgrass; Frank et al., 1984; and wheat Melkonian et al., 1982). Although osmotic adjustment plays a part in the maintenance of turgor pressure as water potential decreases, cell wall elasticity is thought to be important for alfalfa because it facilitates the maintenance of turgor pressure through tissue dehydration. However, the maintenance of RWC as Ψw is reduced, which implies rigid cell walls, has been associated with drought tolerance (Noy-Meir and Ginzburg, 1969; Ihe and Thurtell, 1981). Droughted 'Rangelander' was shown to have more rigid cell walls than 'Nitro' in pressure-volume analysis. This characteristic, combined with its superior ability to osmotically adjust, may form the basis for drought tolerance in 'Rangelander'.

Of the water relations variables measured, Ψw and RWC were more responsive to changes in environmental conditions, while OA occurred more slowly. Maximum daily air temperature had the most affect on Ψw and RWC under field conditions.

In summary, differences in water relations parameters, productivity and water use between the alfalfa cultivars examined this study were revealed. There appeared to be a tradeoff between productivity and drought tolerance, as cultivars previously identified as drought tolerant, generally produced less dry matter. However, under different circumstances, i.e., longer, more severe droughts or in years subsequent to the establishment year, this tradeoff may be more balanced. Most of the cultivars had at least one trait, such as a larger roots, better osmotic adjustment, lower leaf dehydration or lower water use, which could allow it to tolerate or avoid droughts. The combination and interaction of these traits will likely produce distinct results in different environments. Therefore, it would be difficult to identify and incorporate a specific trait into a breeding program and achieve predictable results. Because environmental conditions were different within and between tests, the relative importance of each trait noted above would have varying impacts and likely contributed to some of the inconsistencies noted in this study.

6.0 Future Research

This study was only a first step in the investigation of differences in water relations among divergent alfalfa cultivars. Suggestions for further research are listed below.

1) Conduct similar measurements on established alfalfa.
2) Contrast the diurnal water relations and growth rates of 'Nitro' with other cultivars like 'Rangelander' and 'Excalibur'. The Ψw measurements (and P calculations) were done at midday in the current study. Perhaps under drought conditions this time period is less important to overall productivity rates than the rest of the daily cycle. For example, some cultivars may recover and grow faster late in the day, while others may maintain growth later in the morning. Additional research in comparative diurnal responses among cultivars is warranted, which may better explain higher rates of productivity in some cultivars despite inferior midday plant water status.
3) Determine whether cultivar differences in leaf water loss rates are correlated with drought tolerance or leaf conductance during drought.
4) Establish if hydrolysis occurs in the freeze-thaw method for determining osmotic potential in alfalfa and derive a correction factor.
5) Further investigate possible genetic differences in cell wall elasticity as a result of drought stress.
6) Use even more divergent alfalfa germplasm (e.g., non-dormant genotypes from Saudi Arabia that would be rated at FD=12 and *M. falcata* spp. from Siberia that would possess extreme stress avoidance mechanisms)
7) Because there is a lot of genetic diversity within an alfalfa cultivar, similarities between randomly selected plants from different cultivars may have biased the results. Working with genotypes (individual plants) would reduce this problem.

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APPENDIX A

Table A1.1 Guidelines to ensure accurate pressure bomb measurements†

1) Water loss from time of sampling to time of measurement must be prevented by such means as enclosing the tissue with a plastic bag, to avoid large errors (Turner and Long, 1980).

2) Only make one clean cut on the leaf or petiole.

3) Millar and Hansen (1975) suggest that only the minimum length of petiole required for sample reading should protrude out of the pressure chamber; this will minimize exclusion errors. The amount of stem inside the chamber is not critical (Waring and Cleary, 1967), however it should be considerably larger than the amount protruding.

4) Pressurization of the chamber should be slow. Brown and Tanner (1981) used a rate of about 0.006 MPa sec^{-1} on alfalfa, with a lower rate near the endpoint.

5) Gas leakage from the chamber during measurements should be prevented.

6) False endpoints can arise from gas forcing water from outside of the xylem through intercellular spaces to the cut surface. Drying the cut surface during measurement or constricting the stem (McCown and Wall, 1979) can help to distinguish the correct endpoint.

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† - Adapted from Turner (1981) and others as noted.

APPENDIX B

Table B1.1 General characteristics of alfalfa cultivars.

	Main Use(s)		Dormancy	Tolerates
Cultivar	Grazing	Hay	Rating†	Stress‡
Alfagraze	✓	✓	2	✓
Excalibur		✓	4	
Legend		✓	4	
Nitro		✓	8	
Rangelander	✓		1	✓
South African			7-8^{z}	✓
Wilson		✓	6	✓

† - Scale 1-9; 1 indicates a fall dormant cultivar that would produce minimal fall growth in Manitoba if cut in early September and 9 indicates a non-dormant cultivar that continues to grow in the fall until a killing frost.

‡ - Selected under grazing or drought stress conditions.

z - Has not been tested, but it is believed to be near the upper end of the scale.

APPENDIX C

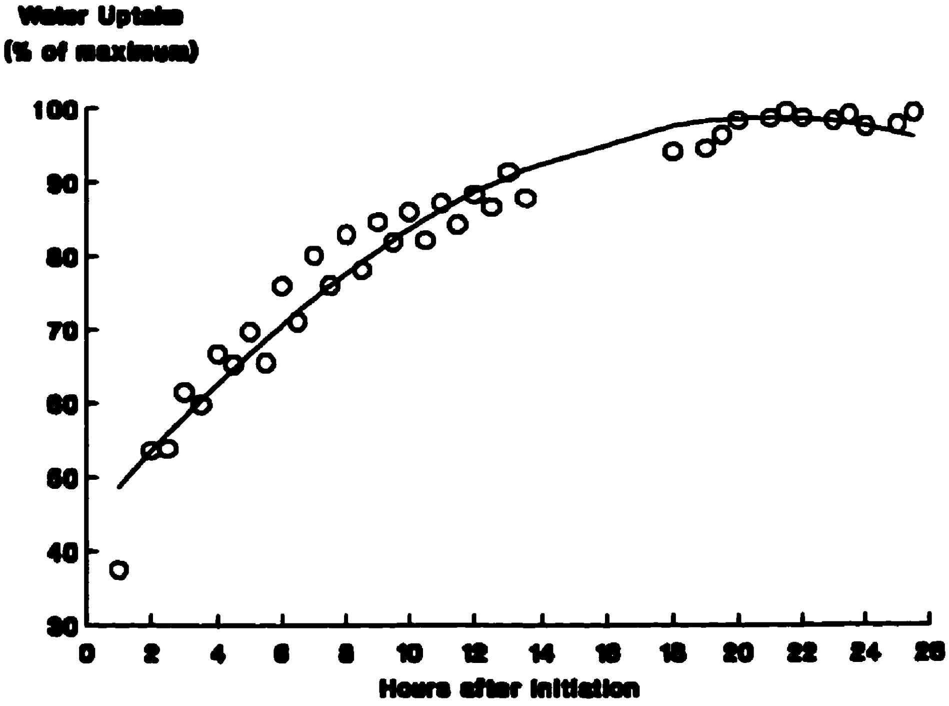

Figure C1.1 Mean water uptake (% of maximum uptake) of root segments.

APPENDIX D

Table D1.1 Summary of ANOVA between water treatments, alfalfa cultivars, and their interaction, for components of plant water relations under controlled watering at Winnipeg, in experiment one.

Date	Treatment	Water Relations Parameter					
		π_{100}	RWC	π	Ψw	P	TW:DW
26 July	Water	ns	**	ns	**	**	ns
	Cultivar	ns	ns	ns	ns	ns	ns
	W×C	ns	ns	ns	ns	ns	ns
1 Aug.	Water	**	**	**	**	**	ns
	Cultivar	ns	ns	ns	*	**	**
	W×C	ns	ns	**	ns	ns	ns
8 Aug.	Water	**	**	**	**	**	*
	Cultivar	ns	ns	ns	*	**	ns
	W×C	ns	ns	ns	ns	ns	ns
15 Aug.	Water	**	**	**	**	**	ns
	Cultivar	ns	ns	*	ns	ns	ns
	W×C	ns	ns	*	**	ns	ns
19 Aug.	Water	**	**	**	**	ns	ns
	Cultivar	ns	ns	*	ns	ns	ns
	W×C	ns	ns	ns	ns	ns	ns
22 Aug.	Water	**	**	ns	**	**	ns
	Cultivar	ns	*	ns	ns	ns	*
	W×C	ns	**	ns	ns	ns	ns
27 Aug.	Water	**	**	**	**	ns	ns
	Cultivar	ns	**	**	ns	ns	ns
	W×C	ns	ns	*	ns	ns	ns
30 Aug.	Cultivar	ns	*	ns	ns	ns	ns
3 Sept.	Cultivar	ns	*	ns	NA	NA	ns

*,** - F-test significant at alpha=0.05 and 0.01 levels, respectively.
ns - no significant difference.
NA - not applicable.

Table D1.2 Summary of ANOVA between water treatments, alfalfa cultivars, and their interaction, for components of plant water relations under controlled watering at Winnipeg, in experiment two.

		Water Relations Parameter					
Date	Treatment	π_{100}	RWC	π	Ψw	P	TW:DW
8 April	Water	ns	ns	ns	**	**	ns
	Cultivar	ns	ns	ns	ns	ns	*
	W×C	ns	ns	ns	ns	ns	ns
16 April	Water	**	ns	**	**	ns	**
	Cultivar	*	ns	*	ns	ns	ns
	W×C	ns	ns	ns	ns	ns	ns
23 April	Water	**	**	**	**	**	ns
	Cultivar	ns	ns	ns	ns	ns	*
	W×C	ns	ns	ns	ns	ns	ns
30 April	Water	**	**	**	**	**	ns
	Cultivar	ns	ns	ns	ns	ns	ns
	W×C	ns	ns	ns	ns	ns	ns
7 May	Cultivar	ns	ns	ns	ns	ns	ns
19 May	Water	**	*	**	**	ns	*
	Cultivar	ns	**	ns	ns	ns	*
	W×C	*	*	ns	ns	ns	*
25 May	Water	ns	ns	ns	*	ns	ns
	Cultivar	ns	*	*	**	ns	ns
	W×C	ns	*	ns	*	ns	ns
28 May	Water	ns	ns	ns	ns	ns	ns
	Cultivar	ns	ns	ns	ns	ns	ns
	W×C	ns	ns	ns	ns	ns	ns

*,** - F-test significant at alpha=0.05 and 0.01 levels, respectively.
ns - no significant difference.

Table D1.3 Summary of ANOVA between water treatments, alfalfa cultivars, and their interaction, for components of plant water relations under controlled watering at Winnipeg, in experiment three.

		Water Relations Parameter					
Date	Treatment	π_{100}	RWC	π	Ψw	P	TW:DW
20 Aug.	Water	ns	*	*	**	**	ns
	Cultivar	ns	ns	ns	ns	ns	ns
	W×C	ns	ns	ns	ns	ns	ns
3 Sept.	Water	**	**	**	**	**	ns
	Cultivar	ns	ns	ns	ns	ns	**
	W×C	ns	ns	ns	ns	ns	ns
10 Sept.	Water	**	**	**	NA	NA	ns
	Cultivar	ns	ns	ns	NA	NA	**
	W×C	ns	ns	ns	NA	NA	ns
30 Sept.	Water	ns	ns	ns	*	*	ns
	Cultivar	ns	ns	ns	ns	ns	ns
	W×C	ns	ns	ns	ns	ns	ns
9 Oct.	Water	ns	ns	ns	ns	ns	ns
	Cultivar	ns	ns	ns	ns	ns	ns
	W×C	ns	ns	ns	ns	ns	ns
14 Oct.	Water	ns	ns	ns	*	ns	ns
	Cultivar	*	ns	ns	ns	ns	ns
	W×C	ns	ns	ns	ns	ns	ns

*,** - F-test significant at alpha=0.05 and 0.01 levels, respectively.

ns - no significant difference.

NA - not applicable.

APPENDIX E

Table E1.1 Water treatment, cultivar and interaction means for osmotic potential (MPa) in alfalfa grown under controlled watering at Winnipeg in experiment one.

Water		Sampling Date - Phase I				Sampling Date - Phase II				
Treatment	Cultivar	26 July	1 Aug.	8 Aug.	15 Aug.	19 Aug.	22 Aug.	27 Aug.	30 Aug.	3 Sept.
Well-watered		-1.53 a	-1.73 a‡	-1.59 a	-1.68 a†	-1.62 a	-1.84 a	-3.98 b†		
Droughted		-1.62 a	-2.33 b	-2.67 b	-2.32 b	-1.96 b	-1.88 a	-1.62 a	-2.63	-5.13
LSD (5%)		0.09	0.10	0.12	0.10	0.10	0.11	0.29		
	Alfagraze	-1.65 a	-2.08 a	-2.09 a	-2.06 bc	-1.93 c	-1.98 a	-2.89 abc	-2.52 a	-4.68 a
	Excalibur	-1.62 a	-2.07 a	-2.22 a	-2.16 c	-1.82 abc	-1.88 a	-2.65 ab	-2.35 a	-4.76 a
	Nitro	-1.52 a	-2.03 a	-2.11 a	-1.97 ab	-1.69 a	-1.89 a	-3.39 c	-3.20 a	-6.04 a
	Rangelander	-1.60 a	-1.93 a	-2.05 a	-1.85 a	-1.74 ab	-1.74 a	-2.43 a	-2.38 a	-4.53 a
	South African	-1.60 a	-2.08 a	-2.05 a	-2.03 bc	-1.69 ab	-1.90 a	-2.94 bc	-2.79 a	-5.36 a
	Wilson	-1.46 a	-2.00 a	-2.25 a	-1.92 ab	-1.86 bc	-1.80 a	-2.52 ab	-2.55 a	-5.39 a
LSD (5%)		0.16	0.18	0.21	0.17	0.17	0.19	0.51	0.29	1.19
Well-watered	Alfagraze	-1.58 a	-1.76 ab	-1.64 a	-1.74 a	-1.77 d	-2.00 a	-4.09 a		
Well-watered	Excalibur	-1.54 a	-1.78 ab	-1.67 a	-1.78 a	-1.68 cd	-1.84 a	-3.56 a		
Well-watered	Nitro	-1.49 a	-1.62 a	-1.55 a	-1.49 a	-1.51 a	-1.91 a	-5.15 b		
Well-watered	Rangelander	-1.56 a	-1.92 b	-1.62 a	-1.64 a	-1.56 abc	-1.79 a	-3.40 a		
Well-watered	South African	-1.61 a	-1.62 a	-1.46 a	-1.76 a	-1.52 ab	-1.83 a	-4.16 a		
Well-watered	Wilson	-1.40 a	-1.68 a	-1.60 a	-1.68 a	-1.68 bcd	-1.68 a	-3.53 a		
LSD (5%)		0.20	0.18	0.22	0.26	0.16	0.25	0.98		
Droughted	Alfagraze	-1.72 a	-2.41 b	-2.55 a	-2.38 bc	-2.09 a	-1.95 a	-1.68 a	-2.52 a	-4.68 a
Droughted	Excalibur	-1.69 a	-2.36 b	-2.77 a	-2.54 c	-1.96 a	-1.93 a	-1.74 a	-2.35 a	-4.76 a
Droughted	Nitro	-1.55 a	-2.43 b	-2.67 a	-2.44 c	-1.87 a	-1.87 a	-1.62 a	-3.20 a	-6.04 a
Droughted	Rangelander	-1.64 a	-1.94 a	-2.48 a	-2.06 a	-1.93 a	-1.69 a	-1.46 a	-2.38 a	-4.53 a
Droughted	South African	-1.59 a	-2.54 b	-2.64 a	-2.30 abc	-1.86 a	-1.97 a	-1.72 a	-2.79 a	-5.36 a
Droughted	Wilson	-1.52 a	-2.32 b	-2.90 a	-2.17 ab	-2.04 a	-1.91 a	-1.51 a	-2.55 a	-5.39 a
LSD (5%)		0.29	0.30	0.38	0.26	0.32	0.30	0.37	0.29	1.19

Means within a continuate column followed by the same letter are not significantly different at alpha=0.05 (LSD).
†, ‡ - water treatment by cultivar interaction is significant at alpha=0.05 and 0.01, respectively.

Table E1.2 Water treatment, cultivar and interaction means for osmotic potential (MPa) in alfalfa grown under controlled watering at Winnipeg in experiment two.

Water		Sampling Date - Phase I				Sampling Date - Phase II			
Treatment	Cultivar	8 April	16 April	23 April	30 April	7 May	19 May	25 May	28 May
Well-watered		-1.21 a	-1.22 a	-1.24 a	-1.35 a	-1.49	-1.77 b	-2.08 a	-3.28 a
Droughted		-1.28 a	-1.38 b	-1.82 b	-2.39 b		-1.51 a	-1.94 a	-3.07 a
LSD (5%)		0.13	0.09	0.11	0.24		0.13	0.29	0.59
	Excalibur	-1.33 a	-1.39 b	-1.62 a	-1.98 a	-1.56 a	-1.73 a	-2.29 b	-3.43 a
	Nitro	-1.18 a	-1.22 a	-1.49 a	-1.82 a	-1.46 a	-1.63 a	-1.90 a	-2.91 a
	Rangelander	-1.23 a	-1.27 a	-1.49 a	-1.80 a	-1.44 a	-1.55 a	-1.85 a	-3.19 a
LSD (5%)		0.16	0.11	0.14	0.29	0.23	0.16	0.36	0.72
Well-watered	Excalibur	-1.26 b	-1.27 b	-1.30 a	-1.41 a	-1.56 a	-1.77 a	-2.25 a	-3.48 a
Well-watered	Nitro	-1.15 a	-1.13 a	-1.21 a	-1.27 a	-1.46 a	-1.78 a	-2.01 a	-3.09 a
Well-watered	Rangelander	-1.22 ab	-1.24 b	-1.21 a	-1.36 a	-1.44 a	-1.75 a	-1.99 a	-3.28 a
LSD (5%)		0.08	0.07	0.14	0.15		0.35	0.69	1.34
Droughted	Excalibur	-1.41 a	-1.51 a	-1.93 a	-2.54 a		-1.68 c	-2.33 b	-3.38 a
Droughted	Nitro	-1.20 a	-1.32 a	-1.76 a	-2.38 a		-1.49 b	-1.78 a	-2.73 a
Droughted	Rangelander	-1.24 a	-1.31 a	-1.77 a	-2.24 a		-1.35 a	-1.72 a	-3.09 a
LSD (5%)		0.30	0.26	0.27	0.61		0.08	0.37	0.82

Means within a continuate column followed by the same letter are not significantly different at alpha=0.05 (LSD).

Table E1.3 Water treatment, cultivar and interaction means for osmotic potential (MPa) in alfalfa grown under controlled watering at Winnipeg in experiment three.

Water		----------- Sampling Date - Phase I -----------			------------- Sampling Date - Phase II ----------		
Treatment	Cultivar	20 Aug.	3 Sept.	10 Sept.	30 Sept.	9 Oct.	14 Oct.
Well-watered		-1.18 a	-1.49 a	-1.37 a	-1.78 a	-2.35 a	-2.47 a
Droughted		-1.25 b	-2.94 b	-2.96 b	-1.63 a	-2.16 a	-2.25 a
LSD (5%)		0.06	0.27	0.27	0.20	0.31	0.27
	Alfagraze	-1.26 a	-2.20 a	-2.19 a	-1.78 a	-2.36 a	-2.38 a
	Excalibur	-1.26 a	-2.48 a	-2.10 a	-1.89 a	-2.32 a	-2.48 a
	Nitro	-1.18 a	-2.10 a	-2.17 a	-1.59 a	-2.18 a	-2.26 a
	Rangelander	-1.23 a	-2.06 a	-1.97 a	-1.67 a	-2.39 a	-2.51 a
	South African	-1.18 a	-2.29 a	-2.45 a	-1.69 a	-2.07 a	-2.34 a
	Wilson	-1.18 a	-2.16 a	-2.10 a	-1.61 a	-2.19 a	-2.18 a
LSD (5%)		0.11	0.47	0.47	0.34	0.54	0.47
Well-watered	Alfagraze	-1.24 a	-1.52 a	-1.47 a	-2.04 a	-2.59 a	-2.39 a
Well-watered	Excalibur	-1.22 a	-1.54 a	-1.33 a	-1.95 a	-2.53 a	-2.83 a
Well-watered	Nitro	-1.17 a	-1.47 a	-1.32 a	-1.65 a	-2.08 a	-2.32 a
Well-watered	Rangelander	-1.22 a	-1.47 a	-1.37 a	-1.75 a	-2.37 a	-2.82 a
Well-watered	South African	-1.12 a	-1.47 a	-1.46 a	-1.65 a	-2.15 a	-2.32 a
Well-watered	Wilson	-1.11 a	-1.45 a	-1.26 a	-1.63 a	-2.35 a	-2.12 a
LSD (5%)		0.12	0.21	0.22	0.65	0.87	0.81
Droughted	Alfagraze	-1.27 a	-2.87 a	-2.91 a	-1.53 a	-2.13 a	-2.36 a
Droughted	Excalibur	-1.31 a	-3.41 a	-2.86 a	-1.83 a	-2.11 a	-2.13 a
Droughted	Nitro	-1.20 a	-2.73 a	-3.01 a	-1.53 a	-2.28 a	-2.20 a
Droughted	Rangelander	-1.25 a	-2.66 a	-2.57 a	-1.59 a	-2.41 a	-2.21 a
Droughted	South African	-1.24 a	-3.11 a	-3.44 a	-1.72 a	-1.99 a	-2.37 a
Droughted	Wilson	-1.25 a	-2.88 a	-2.93 a	-1.58 a	-2.02 a	-2.24 a
LSD (5%)		0.21	0.76	0.99	0.34	0.72	0.56

Means within a continuate column followed by the same letter are not significantly different at alpha=0.05 (LSD).