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**ENVIRONMENTAL AND PHYSIOLOGICAL CONTROLS ON WATER
SOURCE USE BY SEMI-ARID RIPARIAN TREE SPECIES**

by

Keirith Ann Snyder

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A Dissertation Submitted to the Faculty of the
SCHOOL OF RENEWABLE NATURAL RESOURCES

In Partial Fulfillment of the Requirements
For the Degree of

DOCTOR OF PHILOSOPHY
WITH A MAJOR IN RENEWABLE NATURAL RESOURCES STUDIES

In the Graduate College

THE UNIVERSITY OF ARIZONA

2001

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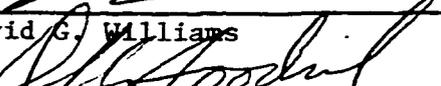
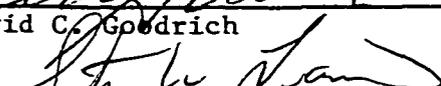
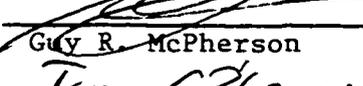
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As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Keirith Ann Snyder entitled Environmental and Physiological Controls on Water Source Use by Semi-Arid Riparian Tree Species

and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy

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ACKNOWLEDGEMENTS

I am very grateful to my advisor David Williams for introducing me to the field of ecophysiology. I was a newcomer to this field and I appreciate his willingness to take me into the lab and give me the opportunity and encouragement to pursue this research. I thank him for all his help and advice with this research. I am indebted to Guy McPherson for seeing me down this long road and being an exceptional mentor. I thank my other committee members David Goodrich, Steve Leavitt and Jim O'Leary for their help and input on this project and their willingness to review manuscripts, write letters and provide assistance.

I thank Bob Steidl for assistance during statistical analyses, David Dettman for assistance with the mass spectrometer, and Cecily McCleave for her help with managing fellowship funds.

I am also extremely grateful to all the people in the lab group that provided invaluable help in the field, laboratory and greenhouse. These intensive tasks were accomplished with the help of Dennis Casper, Alessandra Fravolini, Katy Gonzales, Kevin Hultine, Matt Iles, Dan Koepke, Gretchen Lopez, Matt Ross, Sean Schaeffer, Alea Trafton, and Jeff Wingfield. Thank you all very much for your assistance, patience and input. Many thanks to Vicki Gempko for her conscientious help in every arena.

I'd also like to thank the entire Guy McPherson/David Williams discussion group for the interesting exchange of ideas and excellent input on presentations that I have benefited from over the years.

I'd like to thank Jake Weltzin, Erika Geiger, Laurie Abbott, Libby O'Shea, Ginie Webber, and Mike McLeroy for their insight, friendship and willingness to listening. I am grateful to Sheila Snyder and Marty Martineau for all their support, encouragement and love.

The Sierra Vista District of the Bureau of Land Management provided access to the study sites, which were located with the San Pedro National Conservation Area. Financial support from the USDA National Research Initiative Grant Program and USDA-ARS Global Change Research Program, NASA grant W-18,997 is gratefully acknowledged. I am appreciative of the supportive and stimulating inter-disciplinary framework provided by the SALSA (Semi-Arid Land-Surface-Atmosphere) Program within which the field component of this research was carried out. Additional support to K.A. Snyder is also gratefully acknowledged and was provided by an EPA STAR Fellowship, an American Association of University Women Fellowship, a UA/NASA Space Grant Graduate Fellowship, a William G. McGinnies Scholarship in Arid Lands studies, and a Graduate College Fellowship and Travel Grants.

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ABSTRACT

A general paradigm in semi-arid and arid systems is that woody plants with dimorphic root systems will exhibit preferential use of deeper soil water because it represents a more stable source of water than short duration pulses of shallow soil moisture derived from summer rainfall. However, whether this holds across all woody species is undetermined. Whether use of deeper soil water interacts with use of shallow soil water also is not determined for many species in different ecosystems. Understanding the amount of water plants derive from groundwater and shallow soil water is critically important to accurate calculations of local and regional water balance. The focus of this research was to determine if dominant woody species in semi-arid riparian ecosystems used shallow soil water and how depth to groundwater and defoliation might affect root proliferation and water uptake patterns. This research has shown that the functional grouping “phreatophytes” encompasses a wide variety of responses to environmental variation. Stable isotopic analyses determined that *Prosopis velutina* Woot. (Velvet mesquite) and *Populus fremontii* Wats. (Frémont cottonwood) used shallow soil water derived from summer rainfall, and proportion of shallow soil water was higher at sites with greater depth to groundwater. In contrast *Salix gooddingii* Ball (Goodding willow) did not appear to use shallow soil water at any location regardless of depth to groundwater. Field experiments using defoliation treatments, to limit carbon assimilation and reduce plant photosynthate pools, at sites with differing groundwater availability confirmed that *Prosopis velutina* exhibited flexible response in

water uptake patterns in response to environmental variation and defoliation. Greater depth to groundwater promoted use of shallow soil water for this species. Defoliation, which presumably reduced available photosynthate, increased the reliance of this species on shallow soil water; contrary to predictions that woody species should maintain extensive deep root systems to buffer themselves from seasonal drought. Greenhouse experiments with *Populus fremontii* and *Prosopis velutina* also indicated changes in belowground biomass of fine roots which were associated with changes in water-source use for *Populus fremontii*, but not for *Prosopis velutina*. These results imply that species-specific responses to changes in environmental conditions need to be considered when assessing the effect of plant water acquisition on the hydrologic cycle. Additionally, certain species exhibited flexibility of root response to changes in environmental conditions and defoliation on short time-scales, while other species were relatively inflexible. In terms of predicting plant response to changes in future climates, or modeling fluxes of water from the soil to the atmosphere that are largely controlled by plant transpiration, intra- and interspecific variability will need to be considered.

CHAPTER 1

INTRODUCTION

Spatial and temporal differences in water availability are believed to play a critical role in the coexistence of plant species in aridland ecosystems (Ehleringer 1993). Effective natural resource management and conservation in these sensitive regions demands that we understand how ecosystems will respond to natural and anthropogenic perturbations to water availability. The effects of human-induced alterations in hydrology and climate on terrestrial vegetation are of great concern. In semi-arid and arid regions of the world, groundwater pumping and surface water diversions have produced dramatic changes in stand structure and species composition of riparian ecosystems (Fenner *et al.* 1985; Bradley and Smith 1986; Rood and Mahoney 1990; Stromberg and Patten 1990). As well, patterns of regional precipitation may be altered by changes in global temperature that are predicted to occur as a result of increasing global carbon dioxide concentrations (Houghton *et al.* 1996). It has been proposed that large C₃ woody species are dependent on winter rains that percolate deep into the soil profile and are accessed by deeper root systems of woody plants (Archer 1990; Ehleringer *et al.* 1991). If plant roots exhibit limited ability to respond to changes in soil moisture patterns, then changes in the amount of seasonal precipitation, streamflow regime, or groundwater depth may substantially alter species distributions. Therefore, understanding inter-specific and intra-specific variations in water-source use and

mechanisms that determine patterns of root proliferation and water uptake is critical to establishing predictive models of vegetation response and for calculations of local and regional water balances.

Riparian ecosystems are typically a narrow corridor bordering creeks or rivers and generally dominated by deciduous tree species in semi-arid and arid regions. They are the interface between aquatic environments and surrounding uplands. Vegetation in these areas is usually adapted to shallow groundwater levels and periodic flooding. These ecosystems are spatially limited and frequently threatened by human activity. In North America and Europe, an estimated 80% of riparian areas have disappeared in the last two hundred years (Naiman *et al.* 1993). Aridland riparian ecosystems are especially in jeopardy, due to increasing demand for water in arid regions. These riparian areas support a unique assemblage of forested vegetation in contrast to surrounding uplands (Reichenbacher 1984). The dynamics between groundwater and surface water in these systems create a heterogeneous water environment, with spatial and temporal variation in water availability within which I investigated vertical patterns of soil water extraction and root function. The main objectives of this research were to understand the environmental and physiological controls on water use and root allocation behavior of tree species.

Plants represent a dominant pathway for water transport from soil to atmosphere. Importantly, plants determine the exact subsurface location of water lost to transpiration by having roots accessing water stored in various soil layers. However, plant variations have been explicitly considered in catchment level hydrologic models only recently (*see review by Dawson and Ehleringer 1998*). Most groundwater models in riparian

ecosystems have assumed that all plant water originates from the saturated zone, which is a oversimplification. Riparian ecosystems in the southwestern United States are characterized by a mixture of obligate (plants that send their roots into or below the capillary fringe to use ground water) and facultative phreatophytes (plants that can use groundwater but are not restricted to areas with groundwater). It seems likely that there may be substantial differences in water sources (groundwater, stream water, and rain-derived soil moisture) used by these species. Furthermore, within a given species there may be significant interactions with the environment that influence the amount of water taken up from these different sources.

Stable isotopes provide a means of nondestructively assessing patterns of root water uptake. The natural abundance ratios of hydrogen and oxygen stable isotopes vary considerably among different source waters (precipitation, soil moisture, stream water, groundwater). Different source waters have isotopic values derived primarily physical processes such as evaporation, seasonality of rainfall, and condensation (Dansgaard 1964). There is no isotopic fractionation during plant water uptake from the soil (White *et al.* 1985; Ehleringer and Dawson 1992; Brunel *et al.* 1995). Consequently, stable isotope assessment represents a valuable tool for determining plant water source. Isotope values of xylem sap are an integrated measure of water sources of the plant (Ehleringer and Dawson 1992). Isotopic data from plant xylem water in combination with various linear mixing models can be used to quantify the proportion of plant water derived from various sources (Dawson 1993).

The stable isotope ratio of hydrogen or oxygen in water is expressed using delta notation (δ) in parts per thousand (‰) as:

$$\delta D = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000$$

where R_{sample} and R_{standard} are the molar ratios of D/H or $^{18}\text{O}/^{16}\text{O}$ of the sample and standard water, respectively. Molar ratios are determined by analysis with a mass spectrometer. The international standard for hydrogen and oxygen is V-SMOW (Vienna Standard Mean Ocean Water) (Craig 1957). Standard lab waters are calibrated against V-SMOW and are used in linear corrections of raw values obtained from the mass spectrometer.

Semi-arid ecosystems with bi-modal precipitation regimes are characterized by summer precipitation that is substantially enriched in heavy isotopes of oxygen and hydrogen relative to winter precipitation. Summer precipitation generally is effective in wetting only the surface layers of soil (Caldwell 1985), thus significant gradients may be present in the natural abundance ratios of water isotopes throughout the soil profile. Winter precipitation in mountain front regions penetrates to deeper soil layers and recharges deep groundwater with water substantially depleted in abundance ratios relative to other source water. Therefore, the use of different potential water sources by riparian tree species can be assessed by sampling xylem water.

Plants may not be using water from all potential sources (Dawson and Ehleringer 1991; Lin *et al.* 1996). Depth of water extraction has been found to vary among species (Ehleringer and Dawson 1992; Flanagan *et al.* 1992) and within a species (Donovan and Ehleringer 1994; Williams and Ehleringer 2000). Some isotopic analyses of water

sources have found that obligate phreatophytes do not use surface soil moisture (Dawson and Ehleringer 1991, Busch 1992). However, other research indicates phreatophytes used rainfall-derived shallow soil water (Smith *et al.* 1991; Dawson and Pate 1996; Kolb *et al.* 1997). In pinyon-juniper woodlands, there is substantial variation between populations of the same species for use of rainfall derived soil water (Williams and Ehleringer 2000). Why do some populations and species use shallow soil water and others do not? It seems plausible that trees with access to a stable water source, such as groundwater or perennial streamwater, would be less likely to expend carbon to grow lateral surface roots to acquire sporadic precipitation. However in marginal sites, such as along an ephemeral stream reach, the reduced accessibility of groundwater may promote increased use of shallow soil water.

A plant's ability to respond to the external availability of water resources may be modified by internal physiological processes. I looked specifically at the role of limitations on carbon assimilation, imposed by defoliation, on plant water-source use. If a plant is carbon limited, it may face allocation tradeoffs between various plant structures. The trade-off between production of an extensive or deep root system or maintenance of small lateral roots in the upper soil layers is not well-quantified (Dawson and Pate 1996). However, plants function as balanced systems with communication between carbon-gaining shoots and nutrient- and water-gaining roots (Bazzaz 1997). Therefore, one objective was to determine if there were tradeoffs associated with water-source use in different parts of the rhizosphere.

The goal of this research was to understand how water-use patterns of dominant riparian tree species varied spatially and temporally, and to understand how root proliferation and root water uptake were affected by the belowground availability of water resources and aboveground perturbations to carbon assimilation at the leaf. My research consisted of 2 descriptive surveys of plant physiological response, a detailed greenhouse experiment, and an experimental field manipulation. This research is presented in article format in accordance with the *Renewable Natural Resources Studies* graduate program guidelines, and contains as four manuscripts submitted or intended for submission to peer-reviewed journals. All manuscripts are co-authored with Dr. David G. Williams.

A pilot study to determine if diurnal variation in plant water use and the choice of plant tissue had an effect on the isotopic values of extracted xylem sap is discussed in chapter 2. The pilot study was done along upper Rincon Creek. The remainder of the field work was done along the San Pedro river in southeastern Arizona (Fig 1-1). Water-source use was determined throughout the growing season for the obligate phreatophytes *Populus fremontii* Wats. (Frémont cottonwood) and *Salix goodingii* Ball (Goodding willow) and for the facultative phreatophyte *Prosopis velutina* Woot. (Velvet mesquite). This was done at riparian sites with different flow regimes (perennial, intermittent, ephemeral) and groundwater depths to determine if spatial and temporal water availability affected the dynamics of water uptake. The perennial reach of the San Pedro was located near Lewis Springs, the ephemeral tributary was Escapule Wash near the confluence with the San Pedro, and the intermittent reach of the San Pedro was located

near Boquillas Ranch (Fig 1-1). Results are presented in chapter 3, which is included as it appears in published form in *Agricultural and Forest Meteorology*. A field experiment determined the role of groundwater availability and defoliation on the response of mesquite to summer precipitation. Results are presented in chapter 4, which will be submitted to *Functional Ecology*. A greenhouse experiment with *Populus fremontii* and *Prosopis velutina* determined if carbon allocation to fine and coarse roots, and water uptake from hydraulically separated upper and lower soil compartments, were altered by available soil moisture and defoliation. This work is presented in chapter 5 and will be submitted to *Oecologia*. Thus, this research represents an integrated approach to understanding the environmental and physiological mechanisms underlying patterns of water-source use in trees.

Upper San Pedro River Basin

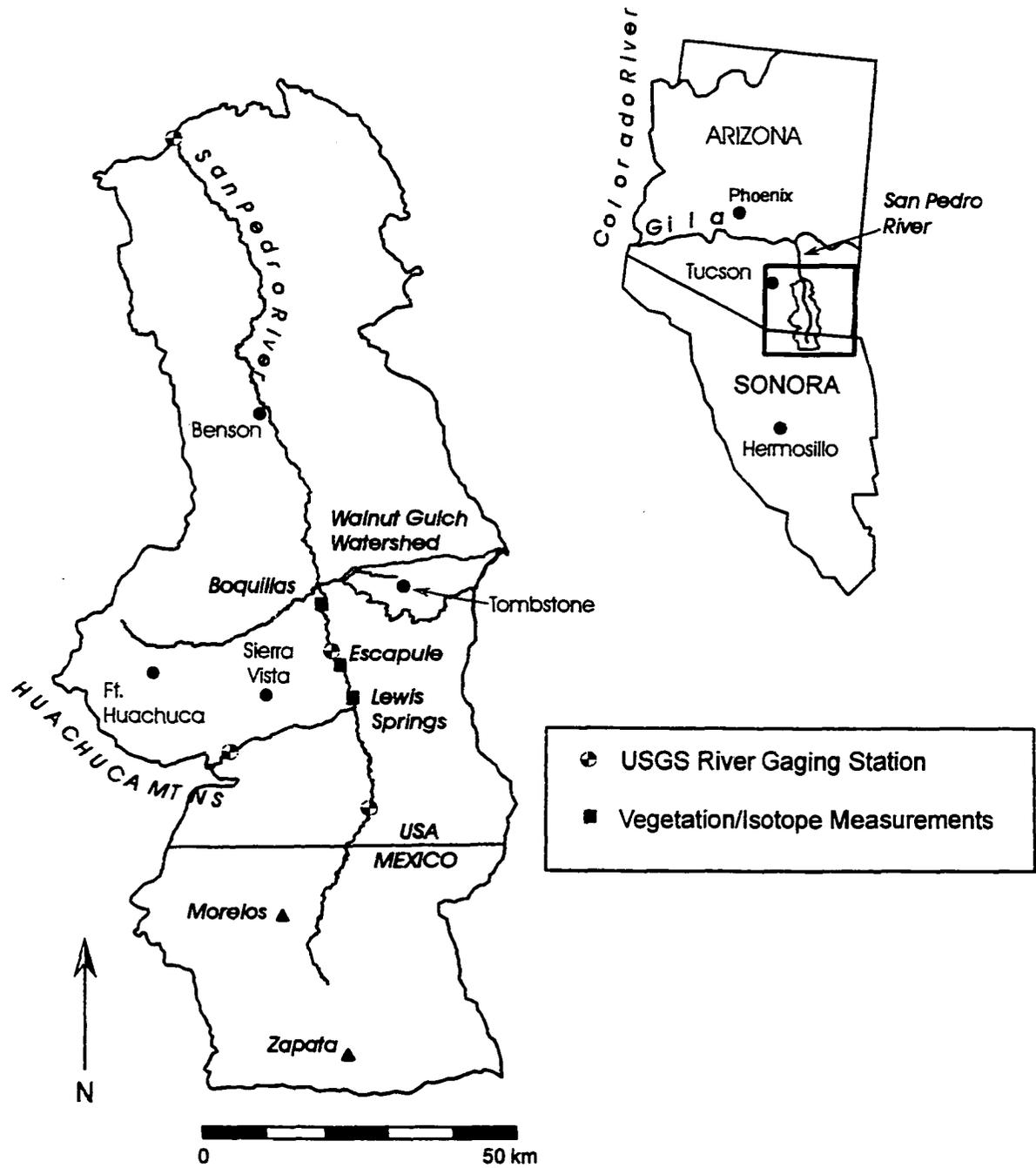


Figure 1- 1 Location map of study sites along the San Pedro River, Arizona.

CHAPTER 2

POTENTIAL VARIATIONS IN THE ISOTOPIC COMPOSITION OF TREE XYLEM SAP AS A RESULT OF SAMPLING PROTOCOL

INTRODUCTION

Isotopic analyses used to determine plant water sources rely on four basic assumptions. First, plants do not fractionate water during uptake by roots and transport in xylem. Second, evaporative enrichment from twigs is minimal and phloem water from leaves is insignificant and does not cause waters extracted from suberized twigs (branches) to differ significantly from actively transpiring sapwood. Third, diurnal variability in water uptake is negligible, therefore sampling time is not critical. Fourth, water stored in heartwood is not a significant source of water for transpiring plants.

That there is no fractionation of the stable isotopes of soil water during uptake by plants has been verified in a variety of field and glasshouse experiments (Walker and Richardson 1991; Thorburn *et al.* 1993 and Brunel *et al.* 1995, but see Lin *et al.* 1993). Validation studies on woody species found that branch (twig) water did not differ significantly from sapwood water (Busch *et al.* 1992, Brunel *et al.* 1995). Busch *et al.* (1992) studied two of the species used in the current study, *Populus fremontii* Wats. and *Salix gooddingii* Ball, and found no variation between heartwood, sapwood or branch water in δD , thus verifying assumption two.

Little has been done to test assumption three, so the role of diurnal variability is not well understood. There is a discrepancy in validation studies testing assumption four. White et al (1985) found that heartwood water in *Pinus strobus* substantially contributed to sapwood water during times of drought-stress, as evidenced by the convergence of the isotopic composition of water from heartwood and sapwood. However, Busch *et al.* (1992) found no differences in heartwood and sapwood isotope composition, yet concluded that heartwood water was not contributing to xylem water in transpiring trees. This study tests whether diurnal variation is negligible and whether heartwood water does not contribute to xylem water for the tree species *Populus fremontii* Wats., *Prosopis velutina* Woot., and *Salix goodingii* Ball. These species are found in riparian areas of the semi-arid southwestern United States. They are generally believed to have roots that access groundwater or capillary fringe water, and are classified as phreatophytes. However, they have extensive root systems that may be able to access water stored in other soil layers.

There are several potential mechanisms that cast doubt on the last two assumptions. If plant roots occupy various soil horizons, daytime transpiration demands that change water potential gradients from soil to leaf may change patterns of water-source use. During daylight hours, high vapor pressure deficits may create conditions in which water uptake from unsaturated soil layers is then physically possible due to reduced midday leaf water potentials. However, at night when stomata are closed and the plant xylem is refilling, leaf water potentials are reduced. This could produce a situation in which unsaturated soil water is no longer taken up by the plant, and nighttime refilling

is from wetter soil layers which would cause differences in the isotopic composition of xylem sap to fluctuate diurnally. Conversely, diurnal variation in isotopic values of xylem water could reflect the isotopic values of shallow soil during cooler periods (night) when surface soil temperatures decrease and transpiration flux is reduced. During warmer times of the day and peak transpiration, it is possible that isotope values of water from the unsaturated soil profile may be masked by the volume of water derived from groundwater. Alternative hypotheses are that shallow root cavitation (Sperry *et al.* 1998) or gaps at the soil water interface due to soil drying (Passioura 1988; Nobel and Cui 1992) during the day may limit uptake from shallow soil layers during sunlit hours. Increased humidity at night and upward flux of water through the soil column at night may facilitate refilling of soil air gaps or partially cavitared root tissue.

Heartwood exchange with active sapwood is a poorly understood potential contributor to isotopic variation in tree xylem sap. The importance of heartwood water storage is generally assumed to be negligible because the magnitude of transpiration flux is much greater than that which could be stored in heartwood tissue. Therefore, it has been assumed to be a small potential contributor to the isotopic composition of xylem sap. However, if heartwood water, which may be stored in previous seasons or under varying environmental conditions, does contribute to xylem water it may contribute to isotopic variation in xylem water that confounds water source interpretation, especially if isotopic values of heartwood deviate substantially from active xylem water.

Understanding the potential exchange between heartwood and sapwood may be critical to unraveling temporal trends in water-source use.

METHODS

This study was conducted in 1996 along an intermittent stream reach, Rincon Creek (ca. 32° 07' N; 110°39' W), near Tucson, Arizona at an elevation of 950 m. Four individuals each of cottonwood, willow, and mesquite were selected and sampled after a summer rainfall event. An increment borer was used to sample tree trunk cores at breast height three times: just after midday (13:00 to 16:00 h) on July 23rd, predawn (02:30 to 04:30 h) on July 24th, and again after midday on July 24th. Bark including phloem was discarded and cores were separated into heartwood and sapwood increments and stored in airtight vials for later analysis. There was very little active sapwood relative to the heartwood in mesquite; therefore heartwood and sapwood samples were pooled to provide enough material for water extraction. Twigs were sampled from trees at the same time. Soil was sampled at three locations from depths of 15- and 30-cm. Groundwater was sampled from an existing well located near the sampled trees.

Plant and soil water were extracted using cryogenic vacuum distillation (Ehleringer and Osmond 1989; Smith *et al.* 1991). Plant water, soil water, and groundwater samples were analyzed for hydrogen isotope ratios (δD) using a dual inlet isotope ratio mass spectrometer (Delta-S, Finnigan –MA, Bremen, Germany). A chromium reduction furnace attached on-line to the mass spectrometer was used to convert liquid water to hydrogen gas (HD-Device, Finnigan-MAT, Bremen, Germany).

RESULTS AND DISCUSSION

Soil δD values in the upper 30 cm of soil averaged -27 ± 8 (1 SE) ‰ and were more positive than δD values of groundwater (-58 ‰). The isotopic composition of water extracted from the sapwood of *Populus* and from the heartwood and sapwood of *Prosopis* exhibited temporal trends. However, these trends were not due to diurnal oscillations, but shifted in one direction only over the sampling periods (Fig. 2-1). Sapwood δD of *Populus* became increasingly more positive throughout the sampling periods, while *Prosopis* sapwood and heartwood water exhibited decreased δD values through time. *Salix* did not show any variations in the δD values of sapwood through time

δD values of water from the heartwood and sapwood of *Populus* exhibited the same increasing trend through time, and had similar δD values (Fig. 2-2). δD values of *Salix* heartwood water had less negative δD values than sapwood by at least 2 ‰.

We sampled just after a summer rainfall event and water in the upper 30 cm of soil had more positive δD values than groundwater. In the absence of diurnal variation, trees that were exploiting shallow soil water should show a steady decrease in δD values through time as the shallow pulse of moisture was depleted and these trees returned to using groundwater. This was the case only for *Prosopis*. The combined heartwood and sapwood δD values showed a decrease through time that may reflect the drying down of

the shallow soil layers. However, because we pooled heartwood and sapwood we cannot rule out the potential hypothesis that exchange with heartwood may have been responsible for the decrease in δD values. Twig values of *Prosopis*, sampled at the same times as heartwood and sapwood, were more positive ($\delta D = -34 \text{ ‰}$) than the combined heartwood and sapwood value. This suggests that the water stored in heartwood, which made up the majority of the sampled cores, had a very different isotopic composition than active sapwood and twig water. Therefore, during times of soil drought, if there is exchange with the large volume of heartwood found in mature mesquite trees, then heartwood water may need to be considered as a source of water in isotopic analyses of water-source use of this species. Alternatively, less negative twig water may indicate that twig water was affected by evaporation at the leaves or within the twig.

Interestingly, *Populus* showed the opposite pattern from *Prosopis* with an increase in δD values through time, and did not show any changes in δD that can be attributed specifically to diurnal variation. The increase in δD values was likely produced by a movement of shallow soil water to layers where *Populus* had active roots. As to the degree of connectivity between heartwood and sapwood it is difficult to assess from this single data set. It appears that heartwood water of *Populus* was closely linked to sapwood, and heartwood water was variable through time. This suggests that there is likely a great deal of connectivity and water turnover in heartwood, in contrast to the other two species. Water extracted from several twig samples of *Populus* closely matched the δD values of sapwood. δD values of twig water were -65 ‰ at predawn and -

58 ± 0.05 ‰ at the second midday sampling, supporting the results of Busch et al (1992), that twig water is a good indicator of sapwood water.

Salix heartwood and sapwood δD values were constant through time and differed from each other. This suggests that heartwood water was more static and likely there was reduced connectivity between heartwood and sapwood. However, limited analyses of δD of twig water show that twig water was intermediate between δD values of sapwood and heartwood water for this species. Twig water was -57 ± 7 ‰ at midday and -62 ‰ at the first midday sampling. This suggests that some exchange with heartwood may occur, or that there is a very slight effect of evaporation on twig water, though the differences are so slight they fall within the range of error involved with sampling, extraction and analysis which has been estimated at 5 ‰ (Brunel *et al* 1995).

The role of diurnal variation on isotopic composition of xylem sap appears negligible for these species in this riparian ecosystem. It is likely that due to the mesic nature of this system and stomatal regulation of these species, that tends to maintain midday leaf water potentials at constant levels (Snyder and Williams 2000) that the proposed mechanisms are relatively unimportant. In more arid environments the role of changing vapor pressure gradients from soil-to-leaf during sunlit hours may be more important for explaining variation in the isotopic composition of xylem. However, the role of heartwood cannot be completely discounted as a source of potential water to plants. More research to determine the degree of connectivity to sapwood and the turnover time of heartwood water is warranted.

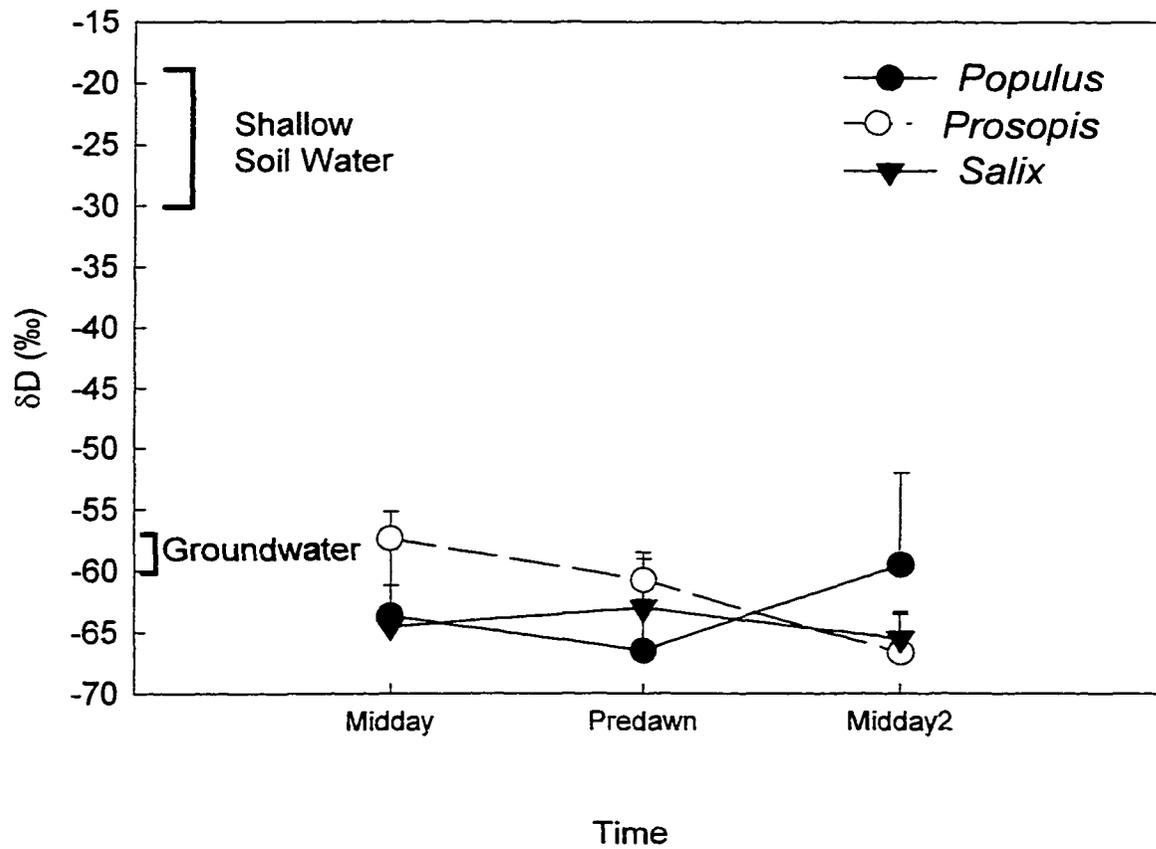


Figure 2-1 Mean tree δD values (1 SE) of bole sapwood of *Populus fremontii* and *Salix gooddingii* and pooled bole heartwood and sapwood values of *Prosopis velutina*, during successive sampling periods from trees along Rincon Creek, Arizona. δD values of shallow soil water (15-30 cm) and groundwater are also shown.

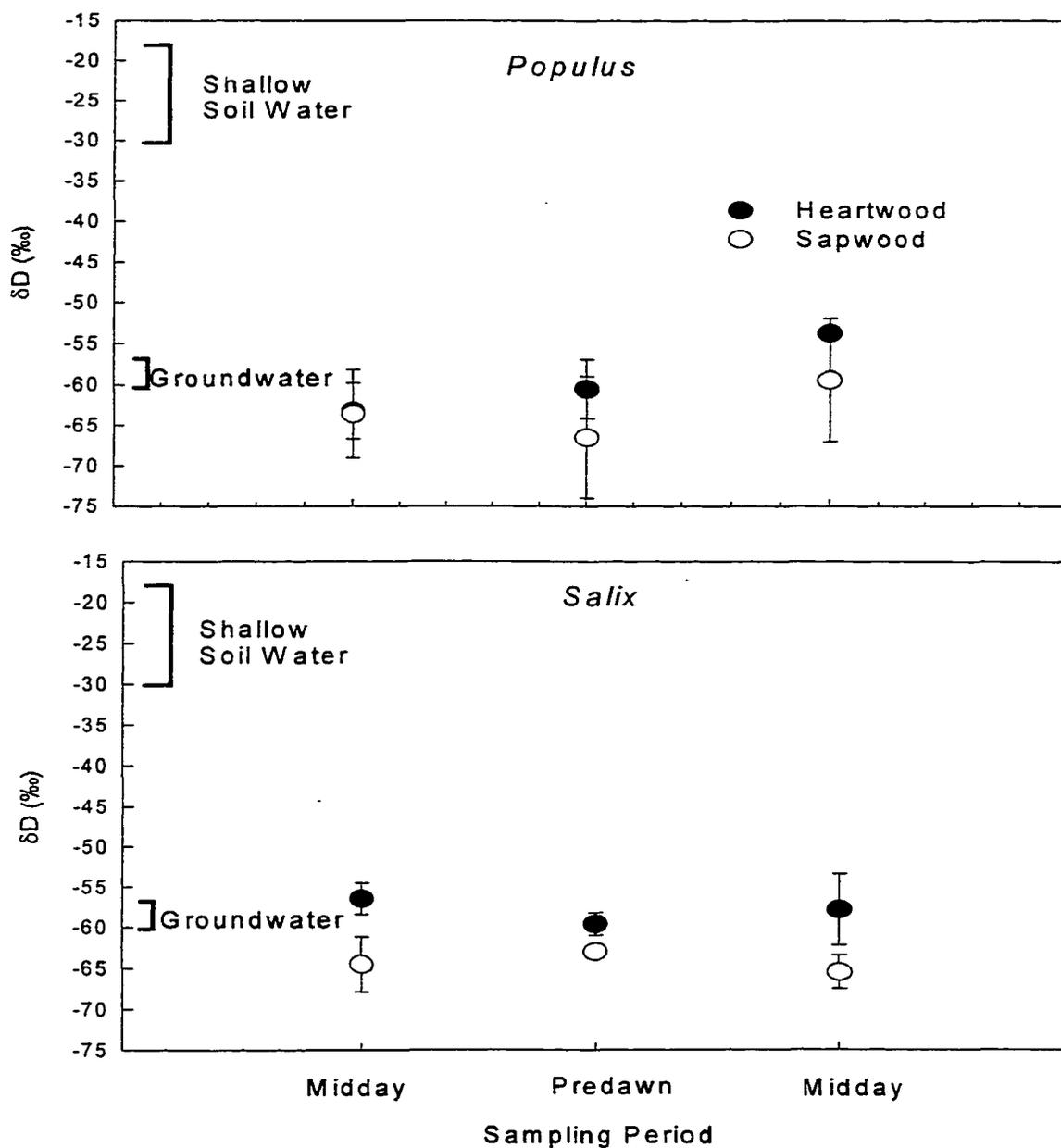


Figure 2-2 Mean tree δD values (1 SE) of bole heartwood and sapwood of *Populus fremontii* and *Salix gooddingii* sampled at different time periods on successive days. δD ranges of shallow soil water (15-30 cm) and groundwater are also shown.

CHAPTER 3

WATER SOURCES USED BY RIPARIAN TREES VARIES AMONG STREAM TYPES ON THE SAN PEDRO RIVER, ARIZONA

ABSTRACT

Variation in the sources of water used by tree species has important ramifications for forest water balances. The fraction of tree transpiration water derived from the unsaturated soil zone and groundwater in a riparian forest was quantified for *Populus fremontii*, *Salix gooddingii*, and *Prosopis velutina* across a gradient of groundwater depth and streamflow regime on the San Pedro River in southeastern Arizona, U.S.A. The proportion of tree transpiration derived from different potential sources was determined using oxygen ($\delta^{18}\text{O}$) and hydrogen (δD) stable isotope analysis in conjunction with 2- and 3-compartment linear mixing models. Comparisons of $\delta^{18}\text{O}$ and δD of tree xylem water with that of potential water sources indicated that *Salix gooddingii* did not take up water in the upper soil layers during the summer rainy period, but instead used only groundwater, even at an ephemeral stream site where depth to groundwater exceeded 4 m. *Populus fremontii*, a dominant 'phreatophyte' in these semi-arid riparian ecosystems, also used mainly groundwater, but at the ephemeral stream site during the summer rainy season this species derived between 26 and 33 % of its transpiration water from upper soil layers. Similarly, at the ephemeral stream site during the summer rainy period,

Prosopis velutina derived a greater fraction of its transpiration water from upper soil layers, than at a perennial stream site where groundwater depth was less than 2 m. Measurements of transpiration flux combined with stable isotope data revealed that *Populus fremontii* transpired a greater *quantity* of water from upper soil layers at the ephemeral stream site than at the perennial stream site. These results imply that transpiration from groundwater and unsaturated soil layers by riparian vegetation may depend on the interaction between site conditions and species assemblage.

Key words: $\delta^{18}\text{O}$, δD , plant water sources, cottonwood, willow, mesquite, *Salix gooddingii*, *Populus fremontii*, *Prosopis velutina*, leaf water potential, phreatophytes

INTRODUCTION

Riparian forests in the southwestern United States are characterized by a mixed assemblage of obligate phreatophytes (plants that send their roots into or below the capillary fringe to use groundwater) and facultative phreatophytes (plants that can also survive in upland environments where groundwater is not available). However, extreme spatial and temporal dynamics of water within riparian ecosystems in arid and semi-arid regions can place severe constraints on the ability of trees to meet transpiration requirements during key periods of the growing season. For example, groundwater pumping and surface water diversions have produced dramatic changes in stand structure and species composition of riparian areas in this region (Stromberg and Patten 1990). Successful conservation of these forests will require knowledge on the dependence of riparian species on groundwater and conversely, on the feedback between riparian vegetation and stream and groundwater dynamics. Not all woody species in these forests use only groundwater for transpiration as the term 'phreatophyte' implies. Use of growing season precipitation can vary considerably among different woody species in the riparian environment (Smith *et al.* 1991; Busch *et al.* 1992; Thorburn and Walker 1994; Kolb *et al.* 1997). However, many widely used hydrologic models assume that riparian trees derive water principally from the saturated zone (McDonald and Harbaugh 1988), which is clearly an oversimplification. Characterization of the conditions that promote use of alternative water sources and identification of those species most likely to use

these water sources in riparian forests is necessary to accurately assess water budgets at the catchment level in semi-arid and arid basins.

The long-term reliability of groundwater may encourage riparian trees to develop roots predominantly in the capillary fringe and saturated zone rather than throughout the soil profile, especially if precipitation during the growing season is unreliable (Ehleringer and Dawson 1992). Conversely, plants that maintain roots in many soil layers, or that can rapidly deploy roots into moisture-rich patches in the soil, may respond opportunistically to precipitation. Research on riparian trees using stable isotope methodology has provided evidence for both modes of root system function. In Australia, *Eucalyptus* spp. used various combinations of groundwater, rainfall-derived shallow soil water, and stream water (Mensforth *et al.*, 1994; Thorburn and Walker 1994; Dawson and Pate 1996; Jolly and Walker 1996). Trees along a perennial, montane stream in California took up water from upper soil layers early in the growing season, then used primarily groundwater when soil dried (Smith *et al.* 1991). In western Arizona, *Populus fremontii* and *Salix gooddingii* used groundwater throughout the entire growing season at perennial and ephemeral streams, regardless of depth to groundwater (Busch *et al.* 1992). However, responses of these trees to precipitation events were not assessed in this Mojave Desert environment. Similarly, mature *Acer negundo* trees in northern Utah used only groundwater, and did not appear to use perennial stream water or shallow soil water (Dawson and Ehleringer 1991). In contrast, this species did use soil water from precipitation at ephemeral and perennial stream reaches in Arizona (Kolb *et al.* 1997).

Although previous studies addressed various components of riparian ecosystems and tree water sources, questions still remain about the integrated effects of groundwater depth and flow regime on the ability of riparian trees to take up precipitation during the growing season. Knowledge of species-specific responses to growing season precipitation and constraints on deep and shallow root function for water uptake will facilitate predictions of how habitat variation and declining groundwater affect patterns of transpiration in riparian ecosystems.

The upland species *Quercus gambelii*, *Juniperus osteosperma*, and *Pinus edulis* in the southwestern United States derived greater proportions of their transpiration water from summer precipitation as the amount of these rains increased across a broad climatic gradient (Williams and Ehleringer, 2000). Intraspecific variation of this type implies that the distribution of functional roots in woody species varies significantly across resource supply gradients. However, the tradeoffs associated with this variation have not been addressed. For instance, it is not known if development of roots in one zone of the rhizosphere comes at the expense of root activity elsewhere. Additionally, plants with roots deployed in upper soil layers should experience a greater amplitude of soil water availability compared to plants that access a deep, stable water source. Plant predawn water potential (Ψ_{pd}), therefore, should be correlated with plant water sources (Dawson and Ehleringer 1998). A better understanding of the tradeoffs between plant water balance and active rooting depth will help resolve linkages between groundwater, soil water, and transpiration in riparian forests.

The riparian forest sites addressed in the current study receive a high proportion (approximately 60%) of annual precipitation from summer 'monsoon' storms. Thus it was predicted that at least some of the dominant riparian trees would use summer precipitation. As in most riparian environment in semi-arid regions, the floodplains varied in depth to groundwater that may further differentiate shallow soil water use among the dominant riparian species. This study addressed seasonal patterns of water-source use by dominant riparian tree species at sites with contrasting groundwater depths and streamflow conditions. This study was part of the Semi-Arid Land Surface Atmosphere (SALSA) Program (USDA-ARS, Tucson, AZ) whose primary aim was to validate models for basin-wide water balances (Goodrich *et al. this issue*). The specific objectives of this study were to: (1) determine if riparian tree species along the San Pedro River in southeastern Arizona use growing season precipitation; (2) assess whether depth to groundwater influences the capacity for these trees to use precipitation; and (3) characterize physiological consequences associated with specific rooting patterns.

METHODS

Study Sites

Three sites were selected to represent a gradient in streamflow and groundwater availability. All sites were located within the San Pedro Riparian National Conservation Area at elevations between 1150 and 1250 m (ca. 31° 33' N; 110° 07' W). The San Pedro

River is a low-gradient alluvial drainage that flows from Sonora, Mexico north to the Gila River in southern Arizona. Mean precipitation at the three sites is 330 mm per year, with 60% of the rain falling in the summer and most of the remainder falling in winter months (NOAA 1996). The hydrologic regime at the three sites was perennial (Lewis Springs), intermittent (Boquillas Ranch), and ephemeral (Escapule Wash). The riparian floodplain vegetation consisted of *Populus fremontii* (Frémont cottonwood) and *Salix gooddingii* (Goodding willow) as the dominant and sub-dominant overstory species. In this region these species, both classified as obligate phreatophytes, generally form a narrow gallery forest in active floodplains (Stromberg 1993). *Prosopis velutina* (Velvet mesquite) was present as a sub-dominant tree and as an understory shrub within the *Populus/Salix* gallery forests. *Prosopis velutina* is a facultative phreatophyte that also occurs in xeric upland sites where groundwater is unavailable. Hereafter, these taxa will be referred to as *Populus*, *Salix*, and *Prosopis*.

Sampling Methods

Between five and eleven tree clusters with each of the three species present were randomly selected at each site. Clusters were not considered blocks in a statistical sense because species were analyzed separately. Trees were sampled in spring (April 19-24), summer (June 7-11, July 9-11, August 8-11) and fall (September 20-27) in 1997. April sampling occurred after *Populus* experienced spring leaf-flush. June and July sampling periods coincided with the annual dry season for this region. Sampling in August was

performed during the height of the growing season and after a large monsoon precipitation event which did not produce over-bank flooding. During this single precipitation event, 27 mm of rainfall was measured at the perennial stream site, which fell during a single hour; trees were sampled 7, 8, and 10 days after the rainfall event at the ephemeral, intermittent and perennial stream sites, respectively. September sampling followed a series of storms that produced over-bank flooding along the perennial reach at the end of the growing season.

Stable isotopes of oxygen in xylem water extracted from twigs were used as natural tracers for determining the fraction of water taken from groundwater and unsaturated soil layers (Ehleringer and Dawson 1992; Brunel *et al.* 1995). Plant stems of approximately 0.5 cm in diameter were sampled at midday from sunlit branches and stored in airtight glass vials for subsequent analysis of hydrogen and oxygen isotope ratios (δD and $\delta^{18}O$). Soils were collected at each site from 5-, 10-, 25-, 50-, and 100-cm depths for analysis of δD and $\delta^{18}O$ of water and gravimetric water content (θ_g). Local floodplain groundwater was collected from wells at each sampling period and at all sites. At the perennial reach, regional groundwater was collected from a deep (11 m) well located approximately 300 m from the stream and outside the local floodplain. Precipitation was collected at all sites in standard rain gauges that contained a layer of mineral oil to minimize evaporation. These integrated precipitation samples were collected monthly throughout 1997. A Scholander-type pressure chamber (PMS, Corvallis, OR) was used to measure predawn leaf water potentials (Ψ_{pd}) on every sampling date.

Stable Isotope Analysis

Water was extracted from plant stems and soils by cryogenic vacuum distillation (Ehleringer and Osmond 1989; Smith *et al.* 1991). Water samples from plant, soil, precipitation, stream and groundwater were analyzed for oxygen isotope ratios ($\delta^{18}\text{O}$) on a Finnigan Delta-S isotope ratio mass spectrometer using CO_2 equilibration (University of Arizona Geosciences Stable Isotope Facility). A subset of samples was also analyzed for hydrogen isotope ratios (δD) using a chromium reduction furnace (HD-Device, Finnigan-Mat, Bremen, Germany) to convert liquid water to hydrogen gas.

Data Analysis

Plant $\delta^{18}\text{O}$ values were compared with those of soil water from different depths and groundwater to determine sources of plant transpiration water. One-sided t-tests ($\alpha = 0.05$) were used to determine if mean $\delta^{18}\text{O}$ of a species at a given site was more positive (enriched) than $\delta^{18}\text{O}$ of floodplain groundwater in June, July and August. Normality of $\delta^{18}\text{O}$ data was determined with the Shapiro-Wilk test. δD - $\delta^{18}\text{O}$ relationships of isotopic values from 10-, 25- and 100-cm soil depths, extracted plant water, precipitation and groundwater were used to determine further differences in plant water-source use (Craig 1961; Clark and Fritz 1998). Source waters with similar $\delta^{18}\text{O}$ or δD values may have different δD - $\delta^{18}\text{O}$ relationships due to evaporation conditions.

Although $\delta^{18}\text{O}$ and Ψ_{pd} values are presented for all sampling periods, statistical analyses were restricted to June, July and August. This restriction was due to phenological differences among species (*Prosopis* and *Salix* were not fully leafed out in April), and because, large storms produced over-bank flooding at the perennial site in September. Over-bank flooding percolated through the soil creating similar $\delta^{18}\text{O}$ values to a depth greater than 1 m. Therefore, it was not possible to distinguish use of monsoon precipitation from groundwater and floodwater use. Repeated measures analysis of variance in a multivariate framework (MANOVA; $\alpha = 0.05$) was used to analyze the effect of site and sampling date (June, July and August) on the difference between plant $\delta^{18}\text{O}$ and groundwater $\delta^{18}\text{O}$ for each species (Von Ende 1993). This difference was used to standardize plant response across sites even when there were shifts in local groundwater $\delta^{18}\text{O}$ values. ANOVA models ($\alpha = 0.05$) were used to compare sites within sampling dates if MANOVA revealed a significant interactive effect. Since it was expected that sampling date would produce an effect on $\delta^{18}\text{O}$, differences within a site across dates were not analyzed. Contrasts ($\alpha = 0.05$) were used to determine differences between sites. Plots of residuals indicated that calculated $\delta^{18}\text{O}$ differences were linear and normally distributed with homogenous error variances, thus meeting the assumptions of MANOVA.

In cases where plant $\delta^{18}\text{O}$ differed significantly from that of local groundwater, the fraction (f) of total plant xylem water derived from shallow soil layers (0-50 cm) was calculated using a 2-compartment linear mixing equation of the form:

$$\text{[Eqn. 1]} \quad \delta^{18}\text{O}_t = f(\delta^{18}\text{O}_s) + (1-f)(\delta^{18}\text{O}_{\text{gw}})$$

where $\delta^{18}\text{O}_t$ was the measured $\delta^{18}\text{O}$ of tree xylem sap, $\delta^{18}\text{O}_s$ was a weighted average of the measured $\delta^{18}\text{O}$ values of soils sampled at 5-, 10-, 25-, and 50-cm depths. Soil $\delta^{18}\text{O}$ values were weighted by gravimetric water content (θ_g) at each depth by dividing mean θ_g at each depth by the sum of θ_g at all depths. $\delta^{18}\text{O}_{\text{gw}}$ was the measured $\delta^{18}\text{O}$ value of groundwater. The equation was solved for f , with f being the fraction of total plant water obtained from water in shallow soil layers. Differences between sites were determined for each species using t-tests.

At the intermittent stream site, the use of a 2-compartment model was inappropriate because of the existence of a third source of plant water. Therefore a 3-compartment linear mixing model was used. This was done using plant and source $\delta^{18}\text{O}$ and δD to produce two equations for tree water source. These two equations, in combination with a third equation (for constraining the sum of the source fractions to one) produced a system of three equations and three unknown variables of the form:

$$\text{[Eqn. 2]} \quad \delta^{18}\text{O}_t = a(\delta^{18}\text{O}_{\text{S1}}) + b(\delta^{18}\text{O}_{\text{S2}}) + c(\delta^{18}\text{O}_{\text{S3}})$$

$$\text{[Eqn. 3]} \quad \delta\text{D}_t = a(\delta\text{D}_{\text{S1}}) + b(\delta\text{D}_{\text{S2}}) + c(\delta\text{D}_{\text{S3}})$$

$$\text{[Eqn. 4]} \quad 1 = a + b + c$$

where $\delta^{18}\text{O}_t$ and δD_t are the oxygen and deuterium values of extracted plant water. The subscripts S1, S2, S3 are sources 1, 2, and 3, respectively. a, b, c are the fractions of total

plant xylem water derived from sources 1, 2, and 3. Equations were solved simultaneously using algebraic substitution. Results were checked using MatLab (MathWorks, Inc., Natwick, MA).

To analyze effects of site and date (June, July, August) on Ψ_{pd} , MANOVA, ANOVA and contrasts were used as described above. Data were transformed as necessary to meet the assumptions of MANOVA based on inspection of residual plots. Non-transformed values are presented in the figures and text. For each species within a site, linear regression analysis was used to quantify the relationship between Ψ_{pd} and $\delta^{18}\text{O}$ measured throughout the growing season.

RESULTS

Mean depth to groundwater was 1.80, 2.61 and 4.26 m at the perennial, intermittent, and ephemeral stream sites, respectively (Table 1). Median streamflow along the perennial reach of the San Pedro River measured downstream of our study site at the Charleston gauge for the period January 1997 to October 1997 was $0.2 \text{ m}^3 \text{ s}^{-1}$. (Tayadon *et al.* 1998). Streamflow was observed along the intermittent reach for 11 months of 1997, but there was no flow in July. Flow occurred only briefly during storm events at the ephemeral stream site. Streamflow was observed twice in August at this site, but may have occurred during other storms as well.

Monsoon rains had $\delta^{18}\text{O}$ values ranging from 2.0 ‰ to -3.5 ‰ (Fig. 3-1), while winter precipitation had more negative $\delta^{18}\text{O}$ values ranging from -4.4‰ to -8.7‰ (data

not shown). $\delta^{18}\text{O}$ values of groundwater sampled from the local floodplain did not vary much over the growing season at the perennial and ephemeral reach, averaging -8.3 ± 0.2 ‰ (± 1 SE) and -8.4 ± 0.2 ‰, respectively (Fig. 3-1). In late September, after a particularly large series of storms, groundwater at the ephemeral reach was enriched (more positive) ($\delta^{18}\text{O} = -7.8$ ‰) relative to previous sampling periods. $\delta^{18}\text{O}$ of local floodplain groundwater at the intermittent stream site varied substantially (Fig. 3-1). During April, June, and July, the local floodplain groundwater ($\delta^{18}\text{O} = -7.3 \pm 0.15$ ‰) isotopically resembled $\delta^{18}\text{O}$ of winter precipitation (-6.1 ± 0.35 ‰) at the intermittent stream site. However, during the summer rainy season in response to storm and flow events, $\delta^{18}\text{O}$ of local floodplain groundwater at this site was substantially enriched (-4.9 ± 0.07 ‰) and reflected $\delta^{18}\text{O}$ of monsoon precipitation and recent streamflow. Isotopic composition of regional groundwater was stable throughout the growing season, with mean $\delta^{18}\text{O}$ of -9.7 ± 0.07 (‰). Stream water is tightly linked with groundwater in this region, and $\delta^{18}\text{O}$ values of stream water (data not shown) were generally related to those of floodplain groundwater; therefore, stream water was not treated as a separate source.

$\delta^{18}\text{O}$ of Tree Xylem Water

Salix gooddingii

Salix (willow) exhibited little variation in water-source use over the growing season among the three sites and relied predominantly on groundwater (Fig. 3-1). $\delta^{18}\text{O}$

values of this species were not significantly enriched above those of groundwater at any site or any sampling period ($p > 0.13$). $\delta^{18}\text{O}$ values of *Salix* were not different among sites or sampling periods ($p > 0.07$), and this species did not appear to use significant amounts of precipitation at any site, even in August after a significant precipitation event. Although $\delta^{18}\text{O}$ values of *Salix* increased at the intermittent and ephemeral stream sites in August, these shifts mirrored changes in groundwater $\delta^{18}\text{O}$ within the floodplain.

Populus fremontii

$\delta^{18}\text{O}$ values of xylem water in *Populus* (cottonwood) varied seasonally and apparently responded to monsoon precipitation events (Fig. 3-1). *Populus* relied on groundwater during the rainless period in June and July, but showed evidence of water use from shallow soil during the rainy season. The $\delta^{18}\text{O}$ values of *Populus* did not differ from that of floodplain groundwater in June and July at any of the three sites ($p > 0.19$). However, use of water from shallow soil layers during the summer rainy period (August) developed differently among the three riparian habitats studied. These habitat-related differences in $\delta^{18}\text{O}$ of *Populus* were reflected statistically in a significant interaction between sampling date and site ($p = 0.05$). Specifically, the difference between $\delta^{18}\text{O}$ of *Populus* and that of floodplain groundwater varied between all sites in August ($p < 0.0025$). $\delta^{18}\text{O}$ values of *Populus* were 0.4 ‰ more positive than that of floodplain groundwater at the perennial stream site ($p = 0.01$), and showed the greatest difference (+2.1‰) above floodplain groundwater at the ephemeral stream site ($p < 0.0001$).

However, $\delta^{18}\text{O}$ of *Populus* was more negative than that of floodplain groundwater at the intermittent site (2-sided t-tests; $p < 0.03$).

Prosopis velutina

Similar to *Populus*, water sources of *Prosopis* (mesquite) varied seasonally in response to monsoon rain events (Fig. 3-1). $\delta^{18}\text{O}$ values of *Prosopis* were affected by the interaction between site and sampling date ($p = 0.04$). During the dry season (June and July), mean $\delta^{18}\text{O}$ of *Prosopis* was similar to floodplain groundwater ($p > 0.12$) except at the ephemeral stream site in July ($p = 0.04$). *Prosopis* $\delta^{18}\text{O}$ values were enriched relative to floodplain groundwater in August at the intermittent and ephemeral site ($p < 0.02$) indicating use of monsoon-derived shallow soil water. *Prosopis* $\delta^{18}\text{O}$ did not differ from floodplain groundwater at the perennial stream site in August ($p = 0.11$). However, there was considerable variation among $\delta^{18}\text{O}$ values of individual trees at the perennial stream site that indicated some trees used groundwater while others used a combination of shallow soil water and groundwater. This was reflected in the difference between plant $\delta^{18}\text{O}$ and groundwater $\delta^{18}\text{O}$. These data indicated that $\delta^{18}\text{O}$ values of *Prosopis* were similarly enriched relative to floodplain groundwater in August at the intermittent and perennial stream sites ($p > 0.11$). However, trees at the ephemeral site were more enriched relative to groundwater than at the other two sites ($p < 0.01$).

δD - $\delta^{18}O$ Plots

Isotope values of *Populus*, *Salix*, and *Prosopis* water in δD - $\delta^{18}O$ space plotted near groundwater and soil water from 100-cm depth at the perennial stream site (Fig. 3-2). At the intermittent stream site, values for *Populus* and *Salix* were between local floodplain groundwater (sampled in July and August) and water from the 100-cm depth, but *Prosopis* plotted between soil water from the 10-, 25- and 100-cm depths. *Salix* values were similar to those of groundwater at the ephemeral stream site and *Populus* values were between those of groundwater and 100-cm soil demonstrating potential use of shallow soil water. *Prosopis* plotted with soil water from the 25- and 100-cm at the ephemeral stream site.

$\delta^{18}O$ and Gravimetric Content of Soil Water

The large rainfall event in August caused gravimetric water content (θ_g) in the shallow soil layers to increase at all sites (Fig. 3-3). θ_g increased from 5% in June to 18% in August in the upper 10 cm at the perennial reach, whereas θ_g at this depth increased from 2% to 6% and from 2% to 8% at the ephemeral and intermittent stream sites, respectively. Changes in θ_g at the 25-, 50-, and 100-cm depths between June and August were minimal along the perennial and intermittent reaches. θ_g increased slightly over the same period at the 25- and 50-cm depths along the ephemeral reach. Soil water $\delta^{18}O$

values were more positive in upper than in lower layers (Fig. 3-3), and became more negative with depth. $\delta^{18}\text{O}$ values at 100 cm (-8.0‰) were similar to those of groundwater at the perennial site, however $\delta^{18}\text{O}$ values at the same depth were approximately -6.0‰ at the ephemeral and intermittent reaches. $\delta^{18}\text{O}$ values of soil water in the upper 25 cm of soil resembled $\delta^{18}\text{O}$ of summer monsoon rainfall (Fig. 3-1).

Fraction of Transpiration Water Derived from Shallow Soil

$\delta^{18}\text{O}$ of *Salix* did not differ from groundwater at any period and therefore the fraction of water derived from shallow unsaturated soil layers was assumed to be zero. When $\delta^{18}\text{O}$ of *Prosopis* and *Populus* differed from groundwater at the perennial and ephemeral stream sites, the percentage of shallow (0-50 cm) soil water use was calculated from the 2-compartment mixing model (Eqn. 1 and Table 2). Initially, the percentage of transpiration water derived from shallow soil was calculated using $\delta^{18}\text{O}$ of floodplain groundwater. However, trees at all sites appeared to have access to a source of water that was more negative in $\delta^{18}\text{O}$ than local floodplain groundwater, such as regional groundwater. This produced negative values for soil water use for three trees, which were set to zero. Therefore, the average of local floodplain groundwater and regional groundwater $\delta^{18}\text{O}$ was used for comparison, which may overstate the percentage of soil water used by *Populus* and *Prosopis*. The average difference between the two models was 7%.

In August after a summer rainfall event, *Populus* derived 8 to 16% of its transpiration from shallow soil layers at the perennial stream site and between 26 to 33% from soil water at the ephemeral stream site (Table 2). The proportion of *Populus* transpiration water from shallow soil layers at the perennial site was significantly less than that at the ephemeral stream site ($p < 0.03$). After the August rain event, shallow soil water comprised 53 to 57% of transpiration water of *Prosopis* at the ephemeral stream site. (Table 2). *Prosopis* was not different from groundwater ($p = 0.11$) at the perennial site therefore mixing was assumed to be zero. In July at the ephemeral stream site, $\delta^{18}\text{O}$ of *Prosopis* was significantly enriched relative to floodplain groundwater, but since shallow soil water content was low ($< 2\%$) at this time, this source was unlikely to have contributed to plant transpiration.

The fraction of transpiration water derived from shallow soil layers was calculated with the 3-compartment mixing model [Eqn. 2-4] at the intermittent site. Groundwater $\delta^{18}\text{O}$ at this site changed substantially in August from prior periods (Fig.1), yet many trees in August had $\delta^{18}\text{O}$ values comparable to floodplain groundwater sampled in June and July. It appears that the August runoff moving through the stream channel flowed laterally through the gravelly substrate at this site, creating a saturated zone of water that was above the floodplain groundwater and had more positive $\delta^{18}\text{O}$ values than the underlying aquifer. Therefore, $\delta^{18}\text{O}$ values from three sources were used in the model: shallow soil water in August; shallow groundwater sampled in August; and deeper floodplain groundwater sampled in July. If $\delta^{18}\text{O}$ of a species differed significantly from $\delta^{18}\text{O}$ of shallow groundwater sampled in August then the 3-compartment mixing

model was applied. $\delta^{18}\text{O}$ of *Prosopis* was enriched relative to August groundwater ($p=0.02$). However, $\delta^{18}\text{O}$ of *Populus* was more negative than August groundwater ($p = 0.03$). $\delta^{18}\text{O}$ of *Salix* did not differ from August groundwater, and therefore shallow soil water use was assumed to be zero. *Prosopis* used 67% of the shallow August groundwater, 30% shallow soil water, and 2% of the deeper floodplain groundwater sampled in July (Table 3). *Populus fremontii* used -5% of the shallow August groundwater, 9% shallow soil water, and 97% of the deeper floodplain groundwater (Table 3). Negative percentages are an artifact produced by inherent matrix sensitivity. The 3-compartment mixing model had high matrix sensitivity, which produced large standard errors (Table 3). This was likely due to the limited variation in the isotopic composition of water sources. To synthesize 2- and 3-compartment model results and provide a conceptual interpretation of root function in this system, the fraction of transpiration water derived from shallow soil water was plotted in relation to groundwater depth (Fig. 3-4).

Predawn Water Potential

Predawn water potentials (Ψ_{pd}) in *Salix* and *Populus* were high (between -0.75 and -0.25 MPa) and changed little over the growing season (Fig. 3-5). These two species apparently had access to groundwater even at the ephemeral stream site, where groundwater depth averaged 4 m. In June, when streamflow was still present and groundwater tables were less than 3 m deep at the intermittent site (Table 1), the effect of

site ($p < 0.045$) on Ψ_{pd} was consistent across all species. During June, Ψ_{pd} values did not differ between the perennial and intermittent reaches ($p > 0.12$) and trees at these sites exhibited higher water potentials relative to those at the ephemeral stream site ($p < 0.02$). Ψ_{pd} of *Salix* and *Populus* did not vary between sites in July ($p > 0.10$). In August, *Salix* and *Populus* Ψ_{pd} values were similar at the ephemeral and perennial reaches ($p > 0.73$), but both species exhibited slightly higher water potentials along the intermittent reach compared to those measured at the other two sites ($p < 0.05$). Ψ_{pd} of *Prosopis* showed considerable seasonal variation (Fig. 3-5). The lowest Ψ_{pd} values (between -1.0 to -1.75 MPa) were observed in July just before the onset of the summer rainy period. The lowest Ψ_{pd} values in *Prosopis* during the dry season were observed at the intermittent stream site, but did not differ significantly from those at the ephemeral site ($p = 0.27$). In July, *Prosopis* along these reaches had lower water potentials than at the perennial reach ($p = 0.0005$).

The relationship between Ψ_{pd} and $\delta^{18}\text{O}$ of xylem water during the growing season was evaluated for trees at the perennial and ephemeral stream sites. Mean values at each sampling period are shown (Fig.6), however regression analyses were based on all observations. There were no significant relationships between these variables at either of the two sites in *Salix* ($p > 0.09$). Ψ_{pd} and $\delta^{18}\text{O}$ likewise were not correlated in *Populus* and *Prosopis* at the perennial stream site. However, at the ephemeral stream site, seasonal changes in water source availability that produced enriched $\delta^{18}\text{O}$ values of plant

were accompanied by less negative Ψ_{pd} values for both *Populus* ($p = 0.0091$; $\Psi_{pd} = -0.14 + 0.04 \delta^{18}\text{O}$, $r^2 = 0.15$) and *Prosopis* ($p = 0.0007$; $\Psi_{pd} = 0.02 + 0.17 \delta^{18}\text{O}$, $r^2 = 0.43$).

DISCUSSION

Reed (1988) classified these dominant species of low-elevation riparian ecosystems in southern Arizona as obligate wetland (*Salix gooddingii*), facultative wetland (*Populus fremontii*) and facultative upland (*Prosopis velutina*) based on their probability of occurrence within the heterogeneous floodplain environment. This study provides new insight into belowground responses of these species across sites with very different patterns of groundwater availability and within a climatic region that receives a substantial input of precipitation late in the growing season from the regional monsoon. Within this setting, substantial variation among species and populations was found for fractional use of soil water derived from these late summer storms. Sites where groundwater was deep and streamflow duration was intermittent or ephemeral promoted greater fractional use of soil water by *Populus* and *Prosopis*, but not *Salix*. This study, as well as others (Dawson and Ehleringer 1991; Smith *et al.* 1991; Busch *et al.* 1992; Mensforth *et al.*; 1994; Thorburn and Walker 1994; Dawson and Pate 1996; Jolly and Walker 1996; Kolb *et al.* 1997), confirms that 'phreatophytes' encompass a wide spectrum of functional types that respond uniquely to spatial and temporal variation in the distribution of available water in the rhizosphere.

Salix gooddingii appeared to be most critically tied to groundwater among the three species examined at our sites within the San Pedro River drainage system. Predawn leaf water potentials in this species were high and did not change in response to seasonal inputs of precipitation, and isotopic composition of xylem water mirrored that of groundwater in the local floodplain aquifer. *Salix* apparently used water only from the saturated zone or capillary fringe above the water table (Figs. 1 and 2), and therefore did not respond to precipitation, even at the ephemeral stream site where groundwater depth was greater than 4 m. *Salix* apparently develops roots for water uptake only in the capillary fringe or saturated zone in these alluvial soils.

Populus fremontii and *Prosopis velutina* exhibited greater flexibility for use of precipitation than did *Salix* along the San Pedro River. Use of monsoon precipitation may have reduced water stress as evidenced by the relationship between Ψ_{pd} and $\delta^{18}O$ in these species at the ephemeral stream site (Fig. 3-6). Greater depth to groundwater was associated with greater fractional use of soil water during the monsoon season for these species (Fig. 3-4). Thus, groundwater depth may be a good predictor of fractional water-source use in this ecosystem. *Prosopis* exhibited a linear response in the proportion of soil water used for transpiration along a gradient of greater depth to groundwater. The response of *Populus* indicated that this species used shallow soil water at all sites, but used a greater proportion of soil water at the ephemeral stream reach when depth to groundwater exceeded 4 m. *Populus* potentially exhibits a threshold response in contrast to the linear response observed in *Prosopis*. In contrast, Busch *et al.* (1992) observed no shallow soil water use by *Populus* on the Lower Colorado and Bill Williams Rivers in

western Arizona. Our sites in southeastern Arizona receive summer precipitation inputs substantially greater than in the arid Mojave desert, where Busch *et al.* conducted their study. Leaffler and Evans (1999) found that adult *Populus* along the Rio Grande River floodplain in central New Mexico responded photosynthetically to growing season precipitation in years when streamflow along the river was low, but not when streamflow was high. Temporal variation for precipitation use in *Populus* observed in the Rio Grande study apparently was similar to the spatial variation that was observed along the San Pedro River.

Prosopis velutina exhibited substantial use of soil water during the summer rainy period at the intermittent and ephemeral stream sites (Fig. 3-4). δD - $\delta^{18}O$ plots (Fig. 3-2) from ephemeral and intermittent stream reaches indicated that soil water from the 10-, 25- and 100-cm depths were important sources of transpiration water for this species. Along the perennial reach the amount of shallow soil water use was assumed to be zero because $\delta^{18}O$ was not different than that of floodplain groundwater (Fig. 3-4). This was likely due to the high variability in $\delta^{18}O$ values of *Prosopis* at this site, which indicated that some individual trees were using shallow soil water (up to 54%), while several trees relied solely on groundwater. *Prosopis* apparently is highly flexible in its use of various water sources. *Prosopis* predawn water potentials (Fig. 3-5) declined abruptly in July when water table levels dropped and streamflow ceased at the intermittent site (see also Stromberg *et al.* 1993). However, monsoon rains quickly ameliorated water stress in this species at all sites. These data suggest that *Prosopis* may not develop extensive roots into saturated zones within the soil profile and likely uses water from the capillary fringe.

The amount of summer rainfall in southeastern Arizona is enough to promote soil water use in *Populus fremontii*. While the proportion of soil water use was higher at sites with greater depth to groundwater, isotopic data do not indicate whether the *amount* of soil water extracted by these trees differed between sites. This is an important distinction because the amount of water gained from a soil compartment by root systems illustrates the potential constraints and allocation tradeoffs that may explain root system dynamics in these heterogeneous environments. The amount of water moving through *Populus* trees from shallow soil layers was calculated by multiplying the fraction of xylem water from this source and maximum sap flux in August at the perennial and ephemeral stream sites. Sap flux data were obtained from Schaeffer and Williams (*this issue*) using the same trees that were sampled for water sources. Three trees of similar size (0.5–0.8 m stem diameter) were selected at each site to minimize tree size effects. The total amount of transpiration flux derived from soil water based on this calculation was 2.8 and 10.5 g cm⁻² sapwood area hr⁻¹ at the perennial and ephemeral stream sites, respectively. Based on this simple calculation, nearly four times as much water moving through the sapwood of *Populus* trees was coming from shallow soil at the ephemeral stream site compared to that at the perennial stream site. However, maximum sap flux was approximately the same at the perennial and ephemeral stream reaches (35.0 and 40.7 g cm⁻² sapwood area day⁻¹, respectively). Total daily transpiration was compared by selecting days with similar solar radiation and vapor pressure deficit. Total transpiration flux was 415.9 and 458.9 g cm⁻² sapwood area day⁻¹ at the perennial and ephemeral stream sites, respectively. Hence, 382.7 and 338.9 g cm⁻² day⁻¹ were derived from groundwater, and 33.2 and 119.1

g cm^{-2} sapwood area day^{-1} were derived from shallow soil water at the perennial and ephemeral reach respectively. Total water use was similar, but the amount of water from shallow soil layers was greater, while the amount from groundwater was less at the ephemeral reach relative to the perennial reach. Root excavation studies of *Populus* trees along the Mojave River in southern California (G. C. Lines 1999) revealed a greater percentage (between 10-50%) of roots in the upper meter of soil at sites where groundwater levels were more than 3 m in depth, compared to sites where groundwater was only 1.5 m deep. Taken together, these data suggest that there may be allocation tradeoffs associated with deploying roots in a particular soil layer. Exploitation of groundwater may come at the expense of water use from other sources, or, alternatively, when groundwater becomes deep or unreliable, trees may develop more shallow roots to exploit other water sources.

Declining groundwater in the San Pedro River system will have a strong negative impact on the survival of *Salix*, because water sources appear to be limited to groundwater. If mature *Populus* is able to continue deep root growth during groundwater recession by augmenting transpiration needs with precipitation taken up by shallow lateral roots, this species may be able to tolerate some recession of groundwater depth. However, it appears that *Populus* must keep roots in the saturated zone to maintain high predawn water potentials. Therefore, persistent long-term declines in groundwater levels will likely limit this species as well (*see also* Stromberg *et al.* 1996). *Prosopis* is highly flexible in its use of water sources and is likely to become more dominant in the riparian landscape if declining groundwater levels persist. However, *Prosopis* tree growth may be

limited in stature relative to historic riparian mesquite woodlands if groundwater is limited (Stromberg *et al.* 1993; Stromberg 1993b).

In conclusion, some riparian trees, even those commonly associated with shallow groundwater, can use substantial amounts of precipitation to meet transpiration requirements. One of the main goals of the SALSA program is to estimate and model transfer of groundwater *via* riparian evapotranspiration on a basin-wide scale (*see* Goodrich *et al.*, *this issue*). These data suggest that species composition in riparian forests (abundances of *Salix*, *Populus*, and *Prosopis*) together with habitat conditions (depth to groundwater) will interactively determine the fraction of transpiration derived from groundwater at the stand level. Integrated over a large area, the contribution of soil water to transpiration in these forests can be substantial, yet easily misrepresented in scaling algorithms. Results of this study indicate that water balance calculations in riparian forests from semi-arid regions should take into consideration species-environment responses within the heterogeneous floodplain ecosystem.

Table 3-1 Depth to groundwater (m) from the ground surface at the three study sites along the San Pedro River, Arizona in 1997. Data were obtained from a single well that penetrated the local floodplain groundwater at the perennial and intermittent site. Data at the ephemeral study site are an average of 2 wells. All wells were located within 10 meters of the stream channel.

| Month | Perennial | Intermittent | Ephemeral |
|---------|-----------|--------------|-----------|
| Feb. | -- | 2.28 | 3.86 |
| Mar. | 0.95 | 2.27 | 3.90 |
| Apr. | 1.98 | -- | 4.21 |
| June | 1.42 | 2.61 | 4.43 |
| July | 2.43 | 3.32 | 4.69 |
| Aug. | 2.08 | 2.67 | 4.48 |
| Sept. | 1.91 | 2.53 | 4.31 |
| Average | 1.8 | 2.61 | 4.26 |

Table 3-2 Percentage of xylem water (± 1 SE) derived from shallow soil layers in August after a monsoon rain event.^a

| Species | Perennial Stream Site | | | Ephemeral Stream Site | | |
|--------------------------|-----------------------|------|-------------------------------|-----------------------|------|--------------------------------|
| | June | July | August | June | July | August |
| <i>Salix gooddingii</i> | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Populus fremontii</i> | 0 | 0 | 8 \pm 8 a (16 \pm 2) a | 0 | 0 | 26 \pm 7 b (33 \pm 7) b |
| <i>Prosopis velutina</i> | 0 | 0 | 0 | 0 | 0 | 52 \pm 5 (57 \pm 7) |

^a Percent shallow soil water was calculated with a 2-compartment mixing model (Eqn. 1). Normal type indicates the percent of water derived from shallow soil layers with local floodplain groundwater as one end member of the model. Different letters indicate significant differences between the ephemeral tributary and the perennial reach of the San Pedro River, Arizona. Values in parentheses are estimates using an average of regional groundwater and local floodplain water as one end member of the model.

Table 3-3 Percentage of xylem water (± 1 SE) derived from shallow soil layers in August after a monsoon rain event for trees along an intermittent reach of the San Pedro River, Arizona. Percent soil water was calculated with a 3-compartment linear mixing model [Eqn. 3] for species whose mean $\delta^{18}\text{O}$ values were significantly different than that of groundwater ($p < 0.05$).

| Intermittent Stream Reach | | | |
|---------------------------|----------------------|--------------------|-----------------------|
| Species | % shallow soil water | % deep groundwater | % shallow groundwater |
| <i>Populus fremontii</i> | 9 ± 4 | 97 ± 16 | -5 ± 13 |
| <i>Prosopis velutina</i> | 30 ± 14 | 3 ± 27 | 67 ± 40 |

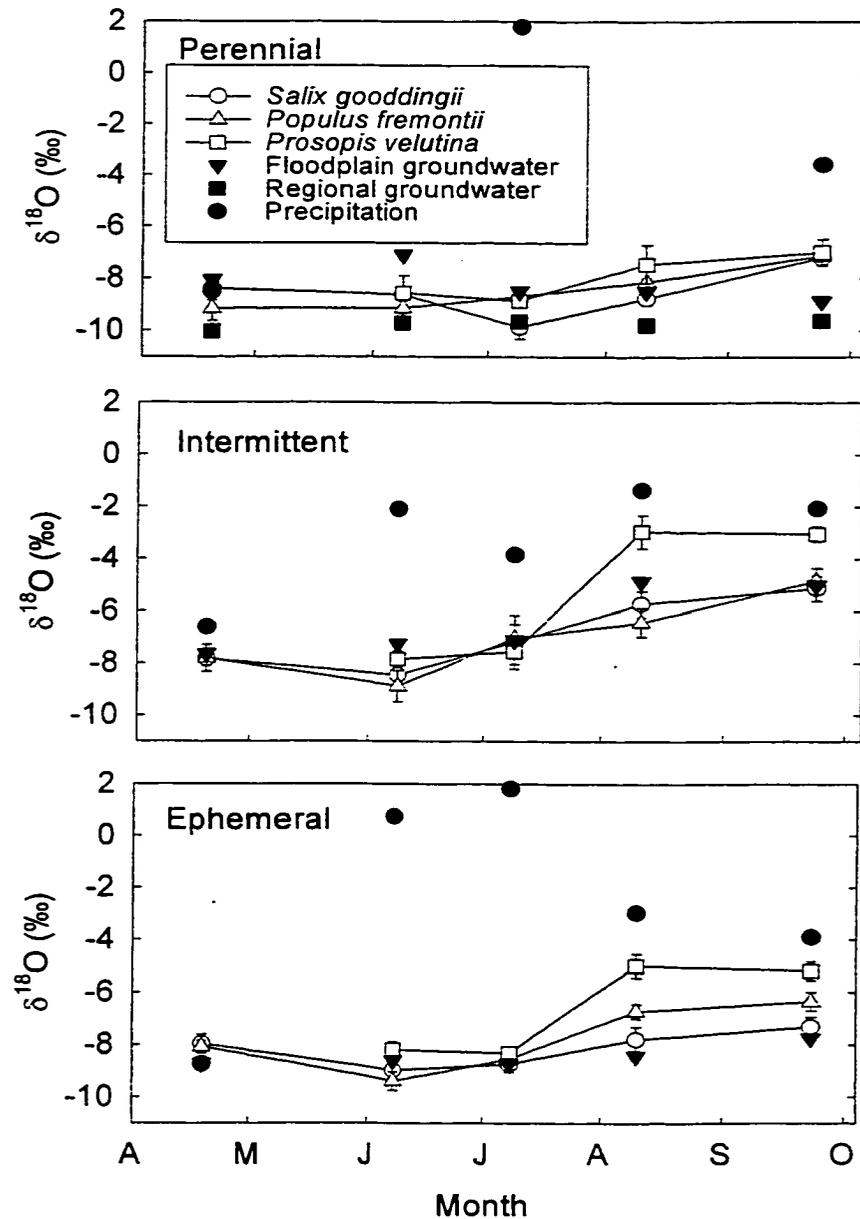


Figure 3-1 Mean $\delta^{18}\text{O}$ values of *Salix goodingii*, *Populus fremontii*, and *Prosopis velutina*, regional groundwater, floodplain groundwater, and precipitation during the 1997 growing season along perennial, intermittent and ephemeral reaches of the San Pedro River in southeastern Arizona. Vertical bars represent ± 1 SE of the mean.

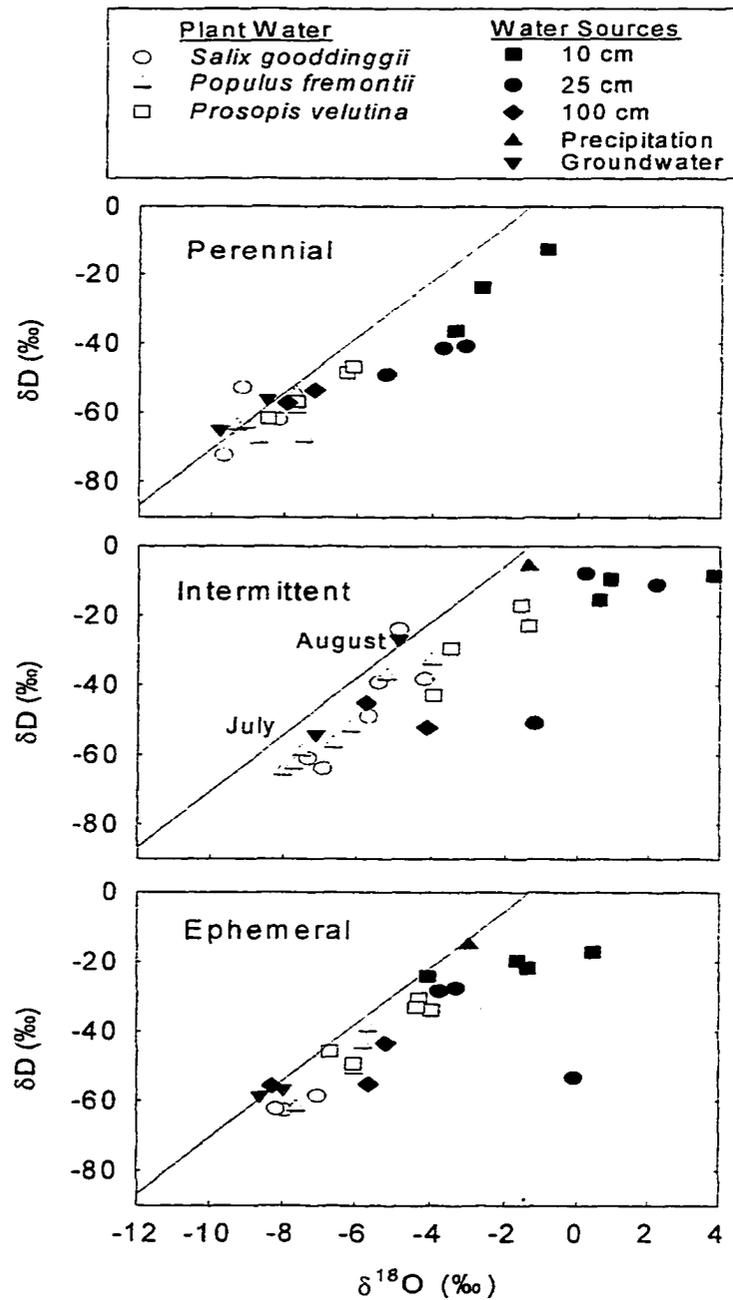


Figure 3-2 $\delta\text{D} - \delta^{18}\text{O}$ values from trees, soil, groundwater, and rainfall in August 1997 along perennial and intermittent reaches and an ephemeral tributary of the San Pedro River in southeastern Arizona. Fitted line is the global meteoric water line (GMWL) ($\delta\text{D} = 8.31 \cdot \delta^{18}\text{O} + 10.8$) (Clark and Fritz 1998). Soil water was extracted from 10-, 25-, and 100- cm depths.

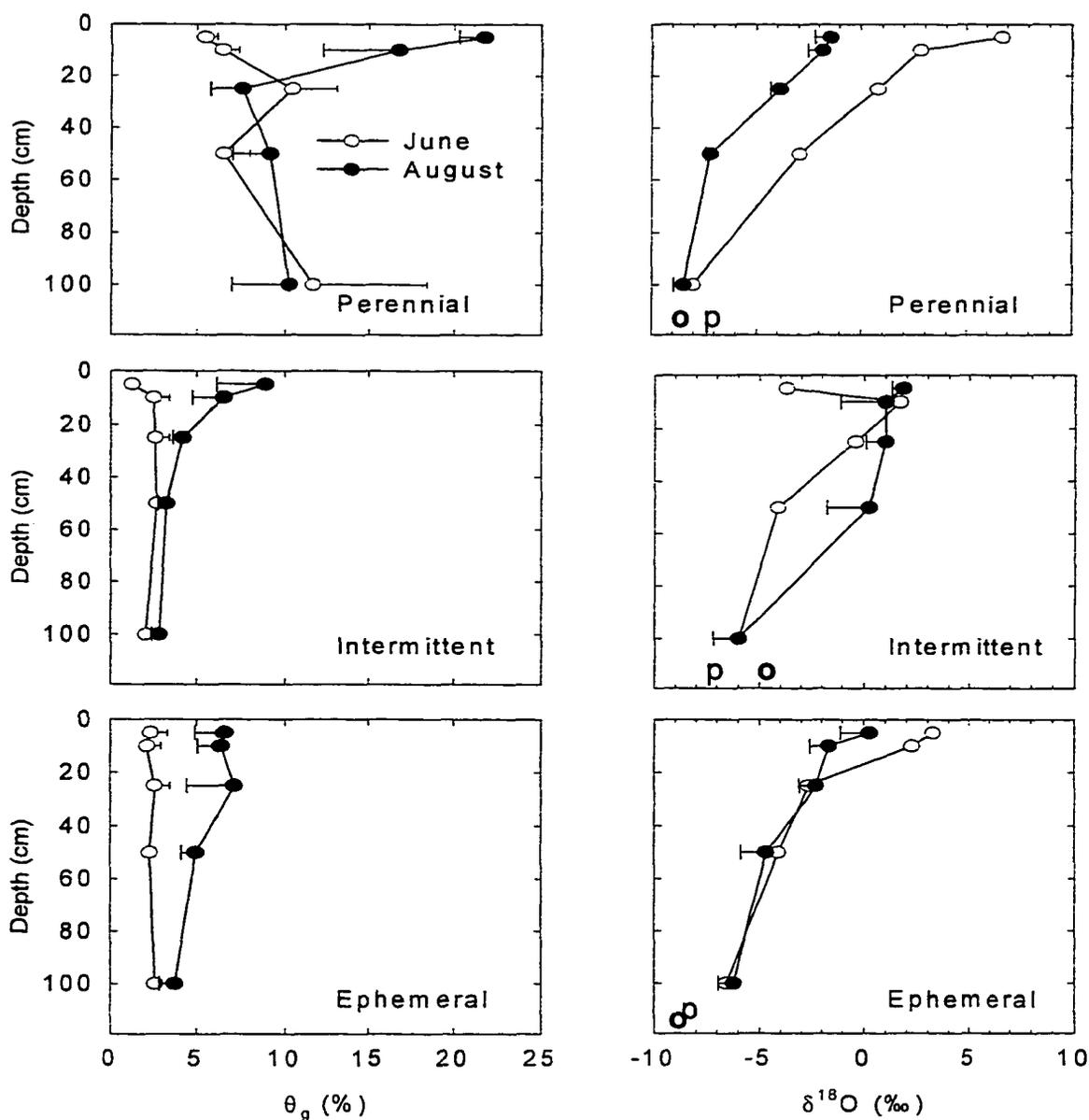


Figure 3-3 Gravimetric soil water content (θ_g) and soil water $\delta^{18}\text{O}$ at 5-, 10-, 25-, 50-, and 100-cm depths in June prior to the onset of the summer 'monsoon' rainy season, and August after a large precipitation event. The arrows on $\delta^{18}\text{O}$ plots indicate the $\delta^{18}\text{O}$ values of local floodplain groundwater in June (*open arrow*) and August (*closed arrow*). Soils were sampled in 1997 along a perennial and intermittent reach and ephemeral tributary of the San Pedro River in southeastern, Arizona. Horizontal bars represent 1 SE of the mean and are presented when possible with the exception of soil $\delta^{18}\text{O}$ values sampled in June.

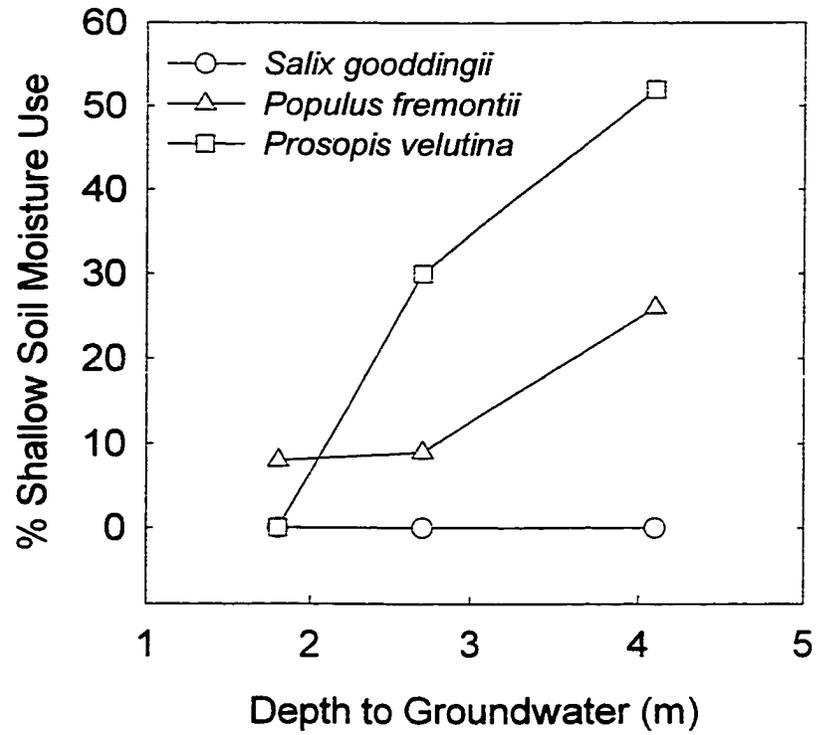


Figure 3-4 The relationship between depth to groundwater and the percentage of plant transpiration water derived from shallow soil water in August after a monsoon rain event for *Salix gooddingii*, *Populus fremontii*, and *Prosopis velutina* sampled along the San Pedro River in Arizona.

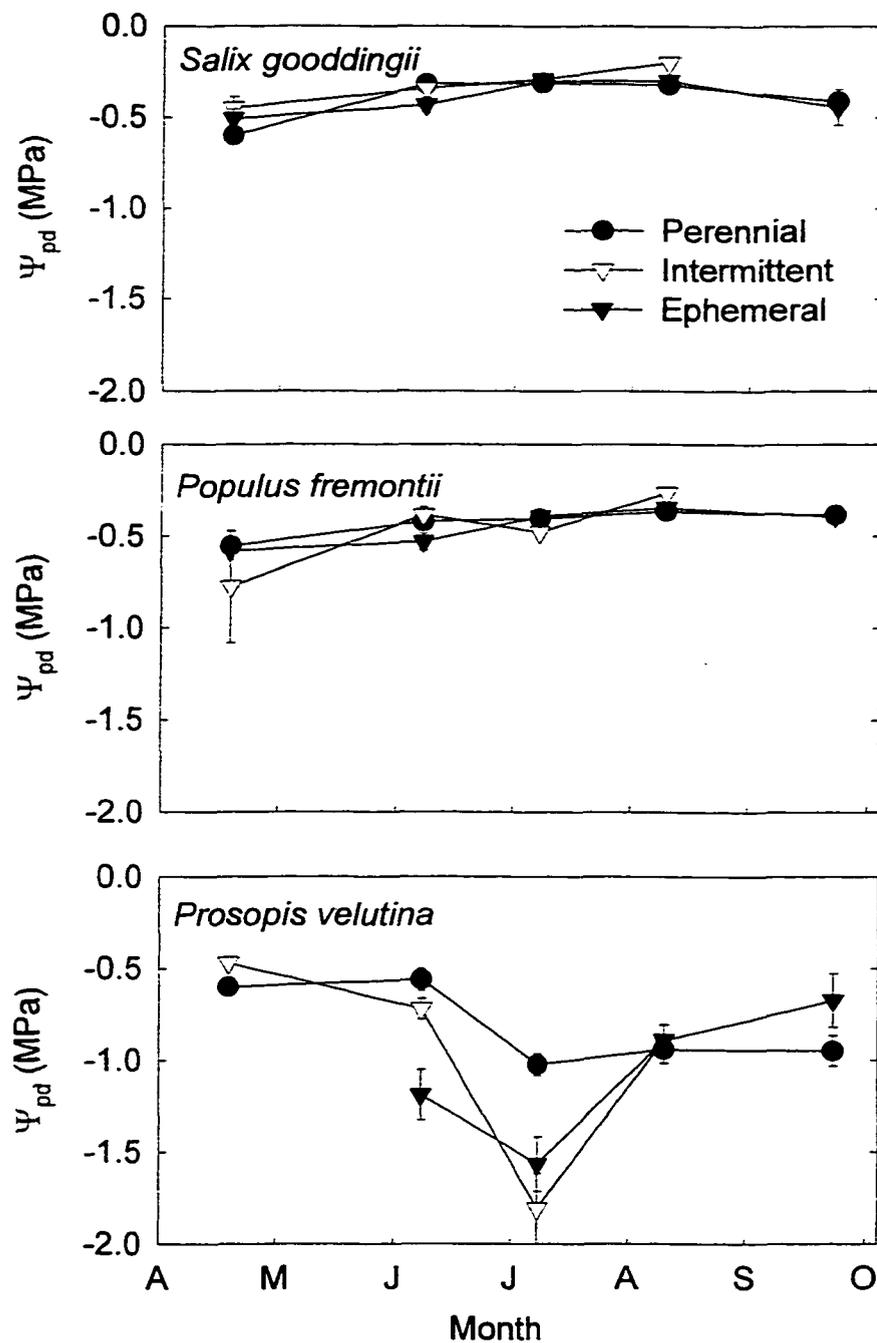


Figure 3-5 Mean predawn leaf water potential (Ψ_{pd}) of *Salix gooddingii*, *Populus fremontii*, and *Prosopis velutina* along perennial and intermittent reaches, and an ephemeral tributary of the San Pedro River in southeastern Arizona in 1997. Horizontal bars represent 1 SE of the mean.

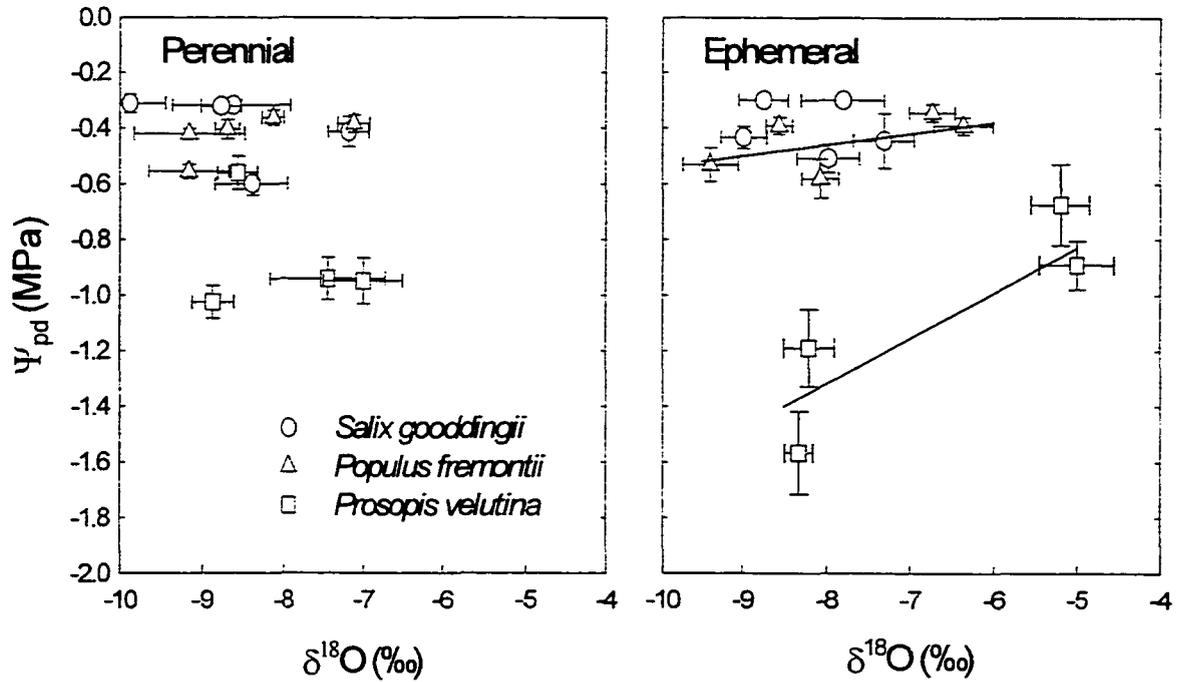


Figure 3-6 Relationship between mean $\delta^{18}O$ and mean predawn leaf water potential (Ψ_{pd}) at each sampling period over the 1997 growing season for *Salix gooddingii*, *Populus fremontii*, and *Prosopis velutina* at a perennial reach and an ephemeral tributary of the San Pedro River in southeastern Arizona. Horizontal and vertical bars represent ± 1 SE of the mean. Fitted lines are the statistically significant regression relationships for a given species ($\alpha = 0.05$)

CHAPTER 4

EFFECTS OF EXPERIMENTAL DEFOLIATION ON WATER UPTAKE BY DEEP AND SHALLOW ROOTS OF MESQUITE (*PROSOPIS VELUTINA*)

ABSTRACT

Prosopis velutina Woot. (velvet mesquite) develops taproots, that extract water from deep soil layers and shallow surface roots, that capture rainfall in shallow soil layers. Growth and maintenance of roots in these different soil layers comes at a physiological cost to the plant, but tradeoffs associated with the differential activity of these roots are not well understood. We predicted that water extracted by deep and shallow roots of mesquite at sites with different access to groundwater would be sensitive to defoliation treatments that reduced the carbon available for root growth and function. δD and $\delta^{18}O$ values in the xylem sap of *Prosopis* after natural rain events and after irrigation with deuterium-labeled water indicated that defoliated *Prosopis* trees took up water in shallow soil layers to a greater extent than undefoliated control trees. This suggests that reductions in photosynthate pools differentially alter activity of deep and shallow roots. Defoliation reduced deep taproot activity and defoliated trees may have become disconnected from deep groundwater. These data indicate that short-term

adjustments in carbon allocation belowground alter patterns of water-source use from different soil layers.

INTRODUCTION

In arid and semi-arid ecosystems, water is the most limiting resource to plant growth (Noy-Meir 1979), consequently the ability to respond rapidly to infrequent pulses of water resulting from growing season precipitation may confer an important advantage to species possessing this flexibility. Global climate change, as well as other anthropogenic changes, may alter water and nutrient availability throughout the soil profile. Therefore, root system architecture, root hydraulic conductivity, the vertical and horizontal distribution of roots, and the degree of plasticity in these attributes may affect the pattern and efficiency of soil resource extraction (Curtis *et al.* 1994; Pregitzer *et al.* 2000; Williams and Snyder *in press*).

Phreatophytes in arid and semi-arid ecosystems have access to groundwater, as well as to shallow soil water derived from growing-season precipitation. It has been proposed that a stable source of groundwater will encourage long-lived perennials to produce deep taproots instead of shallow lateral roots (Ehleringer and Dawson 1992; Williams and Ehleringer 2000). The sporadic nature of growing-season precipitation may make the carbon costs incurred for the growth and maintenance of shallow roots greater than the benefits of water uptake from these roots.

Allocation can be viewed in terms of optimality theory (Bloom *et al.* 1985; Bazzaz 1997). Plants have a certain “carbon budget” and they must allocate this carbon to various competing sinks both above- and belowground. Many woody plants in arid ecosystems have a dimorphic root system with shallow lateral roots and deeper taproots. Tradeoffs associated with producing an extensive or deep root system or maintaining small lateral roots in the upper soil layers are not well-quantified (Fitter 1994; Dawson and Pate 1996). However, the dynamic nature of root growth and proliferation in resource-rich zones of the soil is linked to carbon assimilation by leaves, though the response to defoliation varies among species (*see review by* Eissentat and Yanai 1997).

Pulses of soil water are heterogeneously distributed in space and time. Because roots compete internally for limited plant carbon and respond to source-sink relationships, allocation patterns within the belowground compartment by woody plants in water-limited ecosystems should reflect optimal foraging for water and roots should preferentially grow into water-rich patches (Fitter 1994). When two water sources are available, plants should allocate resources to maximize water revenues by growing into the more stable source of water (Williams and Snyder *in press*). However, this pattern may be mediated by the efficiency of resource capture of different roots (i.e., shallow lateral versus deep roots) such that plants face allocation tradeoffs between root growth in various soil regions. As well, there may be different carbon costs (Poorter and Villar 1997) associated with different types of roots, which may change the costs and benefits of root growth within two competing patches of soil moisture. Lastly, although many studies have documented variations in water-source use, questions still remain about the

degree to which plants can exhibit plastic allocation responses to changing environmental conditions. Plants may be genetically and ontogenetically constrained in their ability to shift tissue production to roots for water uptake (Donovan and Ehleringer 1994; Weltzin and McPherson 1997). Gedroc *et al.* (1996) demonstrated that root/shoot partitioning was partially consistent with optimal allocation, but also highly ontogenetically constrained.

We observed that water-stressed mesquite trees at sites with greater depth to groundwater used more shallow soil water than unstressed trees (Snyder and Williams 2000). Therefore it seemed likely that reduced deep-water availability resulted in greater root proliferation and water uptake from shallow soil layers. We wanted to explore the roles of defoliation and variation in groundwater on the water uptake behavior of *Prosopis velutina* (Woot.) in order to test predictions about optimal rooting behavior. We predicted that one of several scenarios might occur: plants with limited photosynthate to allocate to various plant organs would limit root growth in less optimal (drier) soil layers if root costs in deep and shallow soil layer were approximately equal (i.e. “optimal” behavior). Conversely, if the carbon costs of lateral roots and deep roots differ substantially then reductions in photosynthate pools may favor the least-cost form of root growth. Deep roots grow rapidly, but require large amounts of carbon inputs for construction and maintenance (Fitter 1994). Therefore, defoliation would favor shallow lateral roots and promote use of shallow soil moisture. Alternatively, shallow lateral roots, which are usually short-lived, and generally have high respiration rates (Nobel *et al.* 1992) may require a greater outlay of photosynthate through time compared to coarser

deep roots. However, the initial high investment and coarser structure of deep roots may make them relatively insensitive to short-term perturbation to carbon assimilation, and therefore defoliation would favor use of deep water.

To examine these predictions, we explored the roles of defoliation and variations in groundwater availability on the water-use patterns of *Prosopis velutina*. *Prosopis* was chosen as the target species because it develops deep taproots, and shallow lateral surface roots and it is known to grow in a variety of habitats, yet its morphological and physiological characteristics vary across habitats, which suggests the potential for plastic response to local conditions (Midwood *et al.* 1993; Stromberg *et al.* 1993; Atchley *et al.* 1999). We chose sites that differed in groundwater availability to assess the plasticity of response. We predicted that defoliation-induced limitation on carbon assimilation would promote root uptake of groundwater at a site with a more stable source of groundwater (perennial reach) because water uptake from shallow roots would be less beneficial than maintenance of deep roots. We predicted defoliation-induced reductions in carbon assimilation at a site where groundwater was apparently less accessible to the plant and more variable (ephemeral reach), would increase root function in upper soil layers.

METHODS

Water-source use of *Prosopis* was determined throughout the 1997-growing season at two sites with differing groundwater availability. Selected trees at these sites were repeatedly defoliated throughout the 1998-growing season and compared to

undefoliated controls for their ability to use shallow soil water from natural precipitation events and from deuterium-labeled irrigation water.

Site Description

A perennial reach (1250 m) and an ephemeral tributary (1207 m) of the San Pedro River within the San Pedro Riparian National Conservation Area (ca. 31° 33' N; 110° 07'W; 31° 36' N; 110°09' W; respectively) were selected as study sites, and are hereafter referred to as the perennial and ephemeral reaches, respectively. The San Pedro River is a low-gradient alluvial drainage that flows from Sonora, Mexico, north to the Gila River in southern Arizona. Mean precipitation at the two sites is 330 mm per year, with 60% falling between July and September and most of the remainder falling in winter. At each site, *Prosopis* trees were selected on secondary alluvial terraces which no longer receive flood flow. The terraces were formed by alluvial processes before the entrenchment of the San Pedro River in the late 1800s. *Prosopis* was the dominant tree species on both sites, and the understory was dominated by sacaton grass (*Sporobolus wrightii* Munro ex Scribn.) and *Acacia greggii* Gray.

Field Sampling for Water Sources

A general survey of water-source use was done in 1997, four to six trees were randomly selected at each site. Trees along the ephemeral reach were approximately 100

m from the ephemeral channel, while trees at the perennial reach were approximately 300 m from the main channel. Plant stems and soil samples were collected and stored for later isotopic analysis on four dates in 1997 (June 7-11, July 9-11, August 8-11, September 20-27). Plant twigs of approximately 0.5 cm in diameter, including bark and phloem, were sampled at midday from a sunlit branch and stored in airtight glass vials for subsequent analysis of hydrogen and oxygen isotope ratios (δD and $\delta^{18}O$). Soils were collected at each site using a soil auger from 5-, 10-, 25-, 50-, and 100-cm depths for analysis of δD and $\delta^{18}O$ of water and gravimetric water content (θ_g). Local floodplain groundwater was collected from a well at the ephemeral reach, and regional groundwater was collected from a deep well located approximately 10 m from sampled trees at the perennial reach at each sampling period. Depth to groundwater at this well during this study was approximately 11 m. Precipitation was collected at all sites in standard rain gauges that contained a layer of mineral oil to minimize evaporation. These integrated precipitation samples were collected monthly throughout the growing season. A Scholander-type pressure chamber (PMS, Corvallis, OR) was used to measure predawn leaf water potentials (Ψ_{pd}) on every sampling date.

Water was extracted from plant stems and soils by cryogenic vacuum distillation (Ehleringer & Osmond 1989; Smith *et al.* 1991). Plant, soil, stream, and groundwater samples in 1997 were analyzed for oxygen isotope ratios ($\delta^{18}O$) on a Finnigan Delta-S isotope ratio mass spectrometer using CO_2 equilibration (Compston and Epstein 1958) at the University of Arizona Geosciences Stable Isotope Facility.

The August sample period corresponded to the peak of the growing season and occurred soon after a large precipitation event; 27 mm of rainfall fell during a single hour on August 1st at the perennial reach. Trees were sampled 7 and 10 days after the storm at the ephemeral and perennial reaches, respectively. September sampling followed a series of summer thunderstorms. Stable isotopes of oxygen in xylem water extracted from twigs were used as natural tracers to determine the fraction of water derived from groundwater and unsaturated soil layers (Ehleringer and Dawson 1992; Brunel *et al.* 1995).

Defoliation Experiment

In 1998 a defoliation experiment was conducted at the two sites where trees had exhibited different water-source use in 1997. Defoliated trees and control trees were selected at random from within large macro plots (90 x 25 m at the ephemeral reach and 135 x 25 m at the perennial reach). We restricted our sampling to trees that were between 1 and 2 m in height, in open grass-dominated patches in the savanna mosaic (i.e., they received sunlight from the top and at least 1 side). At each site, 10 trees were repeatedly mechanically defoliated starting in June just after the first leaf flush and continuing through the growing season until August, and 10 trees of similar size remained as controls. Defoliation was performed weekly or every two weeks as determined by re-growth of individual trees, and defoliation treatments consisted of hand-removal of all leaves. Removed leaves were collected and deposited off site. Defoliated trees were

allowed to re-grow leaves for 2 weeks prior to isotope sampling to permit transpirational movement of xylem sap.

Trees were sampled after naturally occurring rainfall events in July, August and September to compare the ability of undefoliated and defoliated trees to use a surface pulse of moisture. In addition, trees were sampled after a simulated rainfall during the third week of August; five trees in each treatment group were irrigated with 305 liters each of deuterium-enriched irrigation water ($\delta D \cong + 66\text{‰}$). Irrigation water was evenly applied in 5-m diameter circles around target plants. The simulated rainfall corresponded to a 14-mm rainfall event. Stems, precipitation, and regional groundwater were collected for isotopic analysis (see methods above) from irrigated and non-irrigated trees immediately prior to irrigation and 2, 4, and 6 days after irrigation. Soils for isotopic analysis and gravimetric soil moisture were collected using a hand-auger from 5-, 10-, 25-, 50- and 100-cm depths in July, 10-, 25-, 50-, and 75-cm in August, and 10-, 20-, and 30-cm depths in September. Plant, soil, stream, and groundwater samples in 1998 were analyzed for hydrogen isotope ratios (δD) using a dual inlet isotope ratio mass spectrometer (Delta-S, Finnigan –MA, Bremen, Germany). A chromium reduction furnace attached on-line to the mass spectrometer was used to convert liquid water to hydrogen gas (HD-Device, Finnigan-MAT, Bremen, Germany). A subset of the 1998 samples was analyzed for oxygen isotope ratios (see methods above). Transpiration was measured continuously with heat balance collars on watered individuals (Dynamax Inc., Houston, TX) and predawn leaf water potential was assessed as above.

Data Analyses

For both the 1997 survey and the 1998 defoliation experiment the percentage of xylem water derived from shallow soil layers was calculated using either δD or $\delta^{18}O$ values with a 2-ended linear mixing model of the form:

$$[\text{Eqn. 1}] \quad \delta X_t = f(\delta X_s) + (1-f)(\delta X_{gw})$$

where δX_t was the measured $\delta^{18}O$ or δD value of tree xylem sap, and δX_s was a weighted average of the measured isotopic values of soils sampled at 5-, 10-, 25-, and 50-cm depths. Soil $\delta^{18}O$ or δD values were weighted by gravimetric water content (θ_g) at each depth by dividing mean θ_g at each depth by the sum of θ_g at all depths. δX_{gw} was the measured $\delta^{18}O$ or δD value of groundwater. The equation was solved for f , the fraction of total plant water obtained from water in shallow soil layers. For the 1997 survey the value of local groundwater was used at each site. However, in 1998 trees at the ephemeral reach appeared to have access to regional water as evidenced by the stable isotopic composition of tree xylem sap during dry periods, therefore regional groundwater was considered as the main source of groundwater at both sites. Differences between sites were determined with t-tests. Use of mixing models allows comparisons between sites because differences in the absolute isotopic values of groundwater and shallow soil water are removed when calculating the percentage use from each source.

Dual isotope plots of $\delta^{18}\text{O}$ - δD (Clark and Fritz 1998) were used to evaluate the possibility that observed changes in δD values resulted from evaporative enrichment in stems. Figure 4-1 illustrates the range of potential plant responses to the irrigation. The labeled water had $\delta^{18}\text{O}$ values close to that of the regional groundwater. Therefore, uptake of irrigation water should cause $\delta^{18}\text{O}$ of trees to shift towards the $\delta^{18}\text{O}$ values of groundwater and artificially enriched δD values. Use of natural rainfall in shallow soil layers and/or groundwater would place tree xylem values near the line between these values. Substantial stem evaporation would place tree isotopic values below the fitted soil evaporation line. The fitted soil evaporation line was drawn between groundwater and soil isotope values taken from beneath non-watered trees, whereas plotted soil isotope values represent actual soil water in a given watering treatment.

Transpiration per unit sapwood area was measured with heat balance collars 2 days following irrigation in August. Transpiration was partitioned between deep and shallow sources using results of the isotope analyses. Transpiration was multiplied by the proportion of water derived from shallow and deep soil to obtain the absolute volume of water from these soil layers. This was done for an average flow rate at midday between 1000 and 1500 h. The total daily flux of water from different soil layers was determined with the same analysis, but using total transpirational water loss over the entire day.

For the natural rainfall events in 1997, $\delta^{18}\text{O}$ values of tree xylem sap were compared with those of local groundwater at each site, using 1-sided t-tests, to determine if trees used a significant proportion of rainfall-derived shallow soil moisture. Repeated measures analysis of variance in a multivariate framework (MANOVAR; $\alpha = 0.05$) was

used to analyze the effects of site and sampling date (June, July and August, September) on Ψ_{pd} in 1997 (Von Ende 1993). Student t-tests ($\alpha = 0.05$) were used to compare sites within sampling dates in the presence of a significant interaction between site and sampling date. This same analysis was used to determine the effects of site and sampling date on the percentage of shallow soil water use determined in 1997.

Two-way analysis of variance (ANOVA) was used to assess the effects of site and defoliation at each sampling date in 1998 on the percentage of shallow moisture use calculated with the 2-compartment mixing model. LSD *a posteriori* mean separation tests were used to assess significant treatment effects in the presence of a significant interaction between site and defoliation. Repeated measures analysis of variance (MANOVAR) was used to determine the effects of defoliation and irrigation on δD of *Prosopis* xylem sap at each site during the irrigation experiment. ANOVA was used to determine the effect of defoliation and irrigation within a site on initial Ψ_{pd} and 2 days after irrigation (post Ψ_{pd}). The difference between initial and post Ψ_{pd} values was used as the response variable to avoid violating the assumptions of independence.

Data were transformed as necessary to meet the assumptions of MANOVAR and ANOVA based on inspection of residual plots. Non-transformed values are presented in the figures and text.

RESULTS

1997 Field Sampling for Water Sources

$\delta^{18}\text{O}$ of regional groundwater measured at the perennial reach was -9.7‰ throughout the 1997 growing season. Along the ephemeral reach groundwater was relatively stable throughout the growing season (average $\delta^{18}\text{O} = -8.4\text{‰}$), but in September there was a slight shift in groundwater to a more positive value of -7.8‰ (Fig. 3-3). $\delta^{18}\text{O}$ values of *Prosopis* xylem sap increased during the rainy season, showing a shift toward that of summer rainfall, which ranged from -4‰ to $+1.8\text{‰}$ (Fig. 4-2). *Prosopis* during June and July, when there was little or no rainfall, took up less than 20% of its water from shallow soil water (Fig. 4-3), and mean $\delta^{18}\text{O}$ values did not differ from groundwater at either site in June ($p > 0.09$). Mean $\delta^{18}\text{O}$ values of xylem water differed from groundwater in July ($p < 0.06$), August and September ($p < 0.005$). The percentage of *Prosopis* xylem water taken from shallow soil layers did not differ between sites in June, July, and September ($p > 0.38$). However, in August along the ephemeral reach *Prosopis* derived 80% of its transpiration water from shallow soil water while only 20% of transpiration water was derived from shallow soil water at the perennial reach ($p = 0.0004$). This difference in water-source use between sites was reflected in a significant interaction between sampling date and site ($p = 0.003$). The shift in $\delta^{18}\text{O}$ values of *Prosopis* and the increased percentage of xylem water derived from shallow soil water during the summer rainy period indicate that *Prosopis* used rainfall-derived surface soil

water when it became available. *Prosopis* along the ephemeral reach had more negative Ψ_{pd} values than those along the perennial reach ($p = 0.07$); along the ephemeral reach the difference was reduced after the onset of the summer rains (Fig. 4-4).

1998 Defoliation Experiment

Rainfall amounts in July, August, and September 1998 for the 7 days preceding sampling dates were 30.5, 91, and 12 mm, respectively. Gravimetric soil water content were similar between the ephemeral and perennial reaches in July, and δD of soil became more positive with depth down to 25 cm (Fig. 4-5). Below 25 cm, isotopic values became more negative with depth, which reflects an increased contribution of winter precipitation. In August and September, gravimetric soil water content along the perennial reach was higher than along the ephemeral reach and shallow soil moisture δD values reflected precipitation derived from summer thunderstorms.

Trees were sampled 4 to 7 days following precipitation events. δD values of defoliated trees at both perennial and ephemeral reaches were similar to those of shallow soil water and summer rain. However δD of undefoliated controls closely resembled that of groundwater (Fig. 4-6). In August, a storm with very negative δD values that produced very negative δD values in the uppermost soil layers caused the percentage of shallow soil water use calculated with the model to exceed 100%, these values were set to 100%. There was no interaction between site and defoliation on the percentage of shallow soil water use on any sample date ($p > 0.17$) (Fig. 4-7). In July and September,

there was an effect of defoliation across sites with the percent shallow soil water use being greater for defoliated trees than undefoliated controls ($p < 0.0003$). There was also a main effect of site in July with trees at the ephemeral reach using a greater percentage of shallow soil water than those at the perennial reach ($p = 0.05$). Control trees used very little (less than 14%) shallow soil water in July and September and mainly relied on groundwater or unsaturated soil moisture deeper within the soil profile. In contrast, during the August sampling period all trees used the same proportion of shallow soil water regardless of defoliation treatment or site.

Plant responses to irrigation with deuterium-labeled water appeared to be minimal because trees in watered treatments and non-watered treatments did not respond in a consistent manner (Fig. 4-8). Sites were analyzed separately because of the 3-way interaction of defoliation, site, and time ($p = 0.047$). Along the ephemeral reach, undefoliated, irrigated trees were the only trees that clearly responded to irrigation water, with increased δD values 2 and 4 days after irrigation. However, defoliated trees did not appear to respond to the irrigation water but had consistently more positive δD values throughout the 6-day period ($p = 0.02$), indicating that they were using shallow soil water, but not irrigation water. There was a slight effect of irrigation on the δD values of *Prosopis* ($p = 0.08$); irrigated trees used more shallow soil water than non-irrigated trees, however this response was driven by the strong irrigation response of undefoliated trees. Non-irrigated and undefoliated trees maintained constant δD values throughout the 6-day period, with δD values similar to those of local groundwater.

Along the perennial reach, trees in all treatments experienced increased δD values, and non-irrigated trees exhibited a greater increase in δD than irrigated trees ($p = 0.02$). Irrigation water was apparently masked by the large natural rainfall events (91 mm) during the week preceding the irrigation experiment. However, defoliated trees had more positive δD values at both sites throughout the 6 day period ($p < 0.008$). These more positive δD values indicate that defoliated trees relied to a greater extent on shallow soil water than undefoliated trees (Fig. 4-8). Defoliation and irrigation did not affect the percentage of water use from shallow soil layers 2 days after irrigation for all watered treatments ($p > 0.18$) (Table 4-1).

Soil moisture content immediately prior to irrigation (Day 0) was higher along the perennial than along the ephemeral reach (Fig. 4-9). Irrigation effectively wetted the upper 10 cm of soil at the perennial reach and appeared to penetrate to 20 cm by day 4 at the ephemeral reach. A 12 ‰ increase in isotopic composition of soil was observed in the 10-cm soil layer at both sites. However, this shift is slight compared to the δD of irrigation water (+66‰).

Ψ_{pd} values exhibited a slight response to irrigation (Fig. 4-10) at the ephemeral reach ($p < 0.09$) (Fig. 4-10). Wet treatments had less negative Ψ_{pd} values 2 days after irrigation at the ephemeral reach. At the perennial reach both the irrigated treatments and the undefoliated, non-irrigated trees exhibited less negative Ψ_{pd} values 2 days after irrigation, indicating that trees responded to natural rainfall not irrigation. Ψ_{pd} values of trees at the ephemeral reach had more negative water potentials just prior to irrigation (-

1.03 MPa) than trees at the perennial reach (-0.8 MPa) ($p < 0.004$), but this statistically significant difference is very small.

Prosopis $\delta^{18}\text{O}$ - δD plots did not indicate substantial stem evaporation because no treatment fell substantially off the fitted soil evaporation line (values given in parentheses) at either the ephemeral reach ($\delta\text{D} = 3.2 \delta^{18}\text{O} - 27$) or at the perennial reach ($\delta\text{D} = 3.6 \delta^{18}\text{O} - 29$) and into the region reflecting stem evaporation (Figs. 4-1 and 4-11). Along the ephemeral reach, defoliated and undefoliated plants in non-irrigated treatments used shallow soil water 2 days after irrigation. Defoliated plants plotted near 10- and 20-cm soil water indicating that upper soil layers were especially important for water uptake. Undefoliated, non-irrigated trees plotted near 50 cm soil water indicating that these trees also used shallow soil moisture. In irrigated treatments along the ephemeral reach, defoliated and undefoliated trees 2 days after irrigation showed increased use of 10- and 20-cm soil water derived from natural rainfall, respectively.

At the perennial reach, non-irrigated plants at the perennial reach plotted near shallow soil water indicating use of natural rainfall. $\delta^{18}\text{O}$ - δD values of undefoliated plants were near 50 cm soil water, while defoliated plant values shifted towards $\delta^{18}\text{O}$ - δD values of soil water at 10 and 20 cm. Undefoliated trees in irrigated treatments shifted directly towards values of rainfall on Day 2. However, defoliated and irrigated plants were the only treatment group that showed a definite use of the labeled water on Day 2 evidenced by the change in $\delta^{18}\text{O}$ to more negative values.

Defoliation substantially reduced transpiration, with greatest transpiration loss occurring in undefoliated trees along the ephemeral reach (Fig. 4-12). Defoliated trees

along the ephemeral reach had reduced rates of transpiration relative to trees in other treatments and sites, and used almost no groundwater (Fig 4-13, Table 4-1). Defoliation along the perennial reach caused the amount of water derived from groundwater to decline, such that undefoliated trees along this reach used the greatest amount of groundwater and substantial amounts of shallow soil moisture relative to trees in other treatments and sites.

DISCUSSION

Water sources of *Prosopis* were highly variable between years and in response to defoliation. The response to defoliation suggests that internal pool sizes of carbon may be as important as external precipitation inputs in determining whether this species uses specific water sources. Manipulation of the plant's carbon assimilation at the leaf produced significant changes in the sources of water used by *Prosopis*, indicating a rapidly expressed high degree of plasticity in root water uptake in response to carbon limitations. Defoliation resulted in reduced overall water use, but a greater proportion of shallow soil water use relative to undefoliated trees. This suggests shallow root function will be enhanced, or less impaired relative to deep root function by plant stresses that reduce available photosynthate.

In the current study, absolute water uptake by shallow roots of defoliated trees did not increase, but it was not reduced as much as water uptake by deep roots. Transpiration rates and isotope data indicate that all water uptake by deep and shallow roots was

reduced, but that taproot activity was reduced more than shallow root activity. This is in contrast to the prediction and previous empirical evidence that woody plants should allocate carbon resources to more stable (in this case deep) sources of water to buffer themselves from the impacts of seasonal drought (Ehleringer and Dawson 1992). Data from the current study suggest that perhaps the energy required to construct and maintain deep taproots in this species may exceed that of lateral roots. Another explanation is that all root growth may have been curtailed, but the reduction in taproot growth may have been sufficient to disconnect these plants from groundwater. Other studies found that this species while capable of growing roots to great depths (Phillips 1963), generally is rooted in the capillary zone above the water table (Stromberg 1993b). In either case, *Prosopis* did not follow predictions of optimal water uptake based on availability of water sources.

We hypothesized that defoliation would change water-use patterns of *Prosopis* differentially, depending on site conditions. Defoliation did produce changes in water-source use, but they were consistent across sites: defoliated trees appeared to rely to a greater extent than undefoliated trees on shallow soil water derived from summer rain during the entire growing season. However, there were differences in transpiration fluxes from various soil compartments between sites: defoliated trees along the perennial reach used similar amounts of groundwater as undefoliated trees along the ephemeral reach (Fig. 4-13). Even undefoliated trees along the ephemeral reach likely had reduced carbon assimilation because of limited access to groundwater, and perhaps they already favored shallow root activity before treatments were applied. Perhaps defoliation at the perennial

reach created similar carbon limitation to those experienced by undefoliated trees along the ephemeral reach and this was reflected in similar groundwater use. Trees at the ephemeral reach required less frequent defoliation, because leaves did not re-grow as quickly in comparison to trees at the perennial reach, which indicates that trees at the ephemeral site may have already had limited photosynthate and storage pools (pers. obs.). Transpiration in conjunction with the percentage of shallow soil water use indicated that undefoliated trees along the ephemeral reach used greater quantities of shallow soil moisture when available than trees along the perennial reach, but defoliation of trees along the ephemeral reach substantially reduced total water use.

Other irrigation experiments, which did not employ defoliation, found that *Prosopis* in the Chihuahuan desert (Montana *et al.* 1995) and other woody species in the Patagonian steppe (Gollusico *et al.* 1998) did not use irrigation water. This is in direct contrast to undefoliated *Prosopis* trees (control trees) in the current study that used shallow soil water. However, leaf water potential was used as the response variable in experiments by Montana *et al.* (1995) and Gollusico *et al.* (1998), and our data indicate that maintenance of roots in multiple soil layers may buffer changes in leaf water potentials. The ability to use water from deeper sources changed in the current study. Two plausible explanations are 1) reduced photosynthetic production in defoliated trees was first manifested at the taproot, or 2) the water potential gradient from soil to leaf was insufficient to pull water from depths of 10 meters to the soil surface. Groundwater depth was 10 m and it is likely less distance to water in the capillary fringe. The water potential gradient needed to overcome gravity is 0.01 MPa per meter (Nobel 1991), so a water

potential gradient of 0.1 MPa would need to be established. Midday water potentials, measured on a few selected basal shoots covered with foil to minimize transpiration losses, were sufficient to overcome the resistance due to gravity. These estimates on foiled, non-transpiring leaves necessarily understate the true gradient between actively transpiring leaves and average gradients between midday Ψ and Ψ_{pd} of leaves were 0.23 MPa (ephemeral reach) and 0.13 MPa (perennial reach) for defoliated trees. Therefore it is unlikely that physical restrictions alone accounted for the difference in use of deep water.

Defoliation appeared to affect the taproot by reducing its water uptake, and this may have enhanced water uptake and possibly allocation to shallow roots. These results corroborate those of Callaway (1990) who showed that 3 oak species exhibited differential responses to drought at the taproot level in a greenhouse setting. The 2 xeric species experienced a substantial increase in lateral root development when the taproot tip died from exposure to dry soil. However, primary taproot death in the more mesic oak species did not promote increased lateral root growth, which caused a reduction in total carbon allocated to roots. Tischler *et al.* (1996) also found that taproot length of *Prosopis glandulosa* increased by 23% in an elevated CO₂ environment relative to an ambient CO₂ environment, which suggests that increased carbon assimilation facilitated taproot growth.

These data suggest that the availability of, and access to, alternative sources of water, in conjunction with plant carbon balance, help determine whether a plant exploits summer precipitation. Thus, the relative availability of different water sources and the

availability of assimilated plant carbon determine root allocation and function to a greater degree than the absolute availability of precipitation. For example, *Populus fremontii* (cottonwood) and *Prosopis* used an increasing proportion of shallow soil moisture along a gradient of greater depth to groundwater in riparian areas of the Chihuahuan desert region where rainfall was relatively similar between sampled trees (Snyder and Williams 2000). Lack of correspondence between plant water-source use and total rainfall amount was observed across an aridity gradient in Argentina; plants used shallow soil water derived from growing-season precipitation regardless of the 6-fold variation in annual precipitation, however species composition changed across this aridity gradient (Schulze *et al.* 1996).

Defoliation produced a subset of trees along the perennial reach (Fig. 4-13) that behaved more like trees along the ephemeral reach, suggesting that carbon balance and individual plant plasticity play an important role in determining patterns of water-source use. These findings have important implications for scaling plant responses to community and ecosystem levels because they address underlying mechanisms of plant response to environmental changes. The interactive effect of changes to the plant's carbon assimilation capacity and changes in external availability of water resources will need to be considered in mechanistic models of belowground resource acquisition. Substantial intraspecific variation in water-source use indicates that for some species, likely those that grow in a variety of habitats, simple predictions about response to changes in regional climate will have limited utility. The ability to use water in the

rhizosphere may vary as much with internal physiological controls on carbon allocation and specific site conditions as with larger regional patterns of climate.

Table 4-1 Mean (\pm S.E.) percent shallow soil water use calculated with the 2-compartment linear mixing model for irrigated trees. There was no significant effect of site or defoliation or their interaction (ANOVA, $p > 0.18$).

| Sampling Time | <u>Ephemeral Reach</u> | | <u>Perennial Reach</u> | |
|----------------------------|------------------------|------------|------------------------|------------|
| | Undeveloped | Defoliated | Undeveloped | Defoliated |
| 2 days after irrigation | 96(11) | 91 (20) | 70(10) | 79 (4) |

Potential Response to an Artificial Rain Event

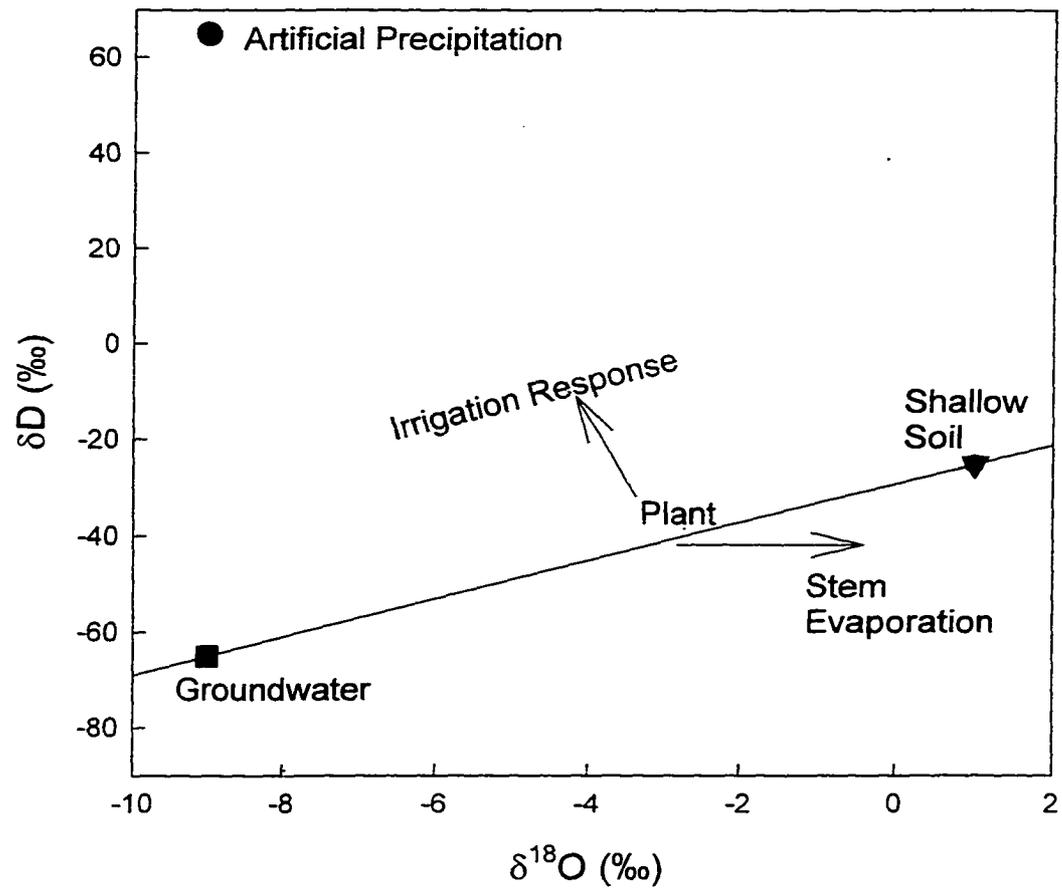


Figure 4-1 Potential responses to irrigation using deuterium-labeled water. Solid line is the fitted soil evaporation line.

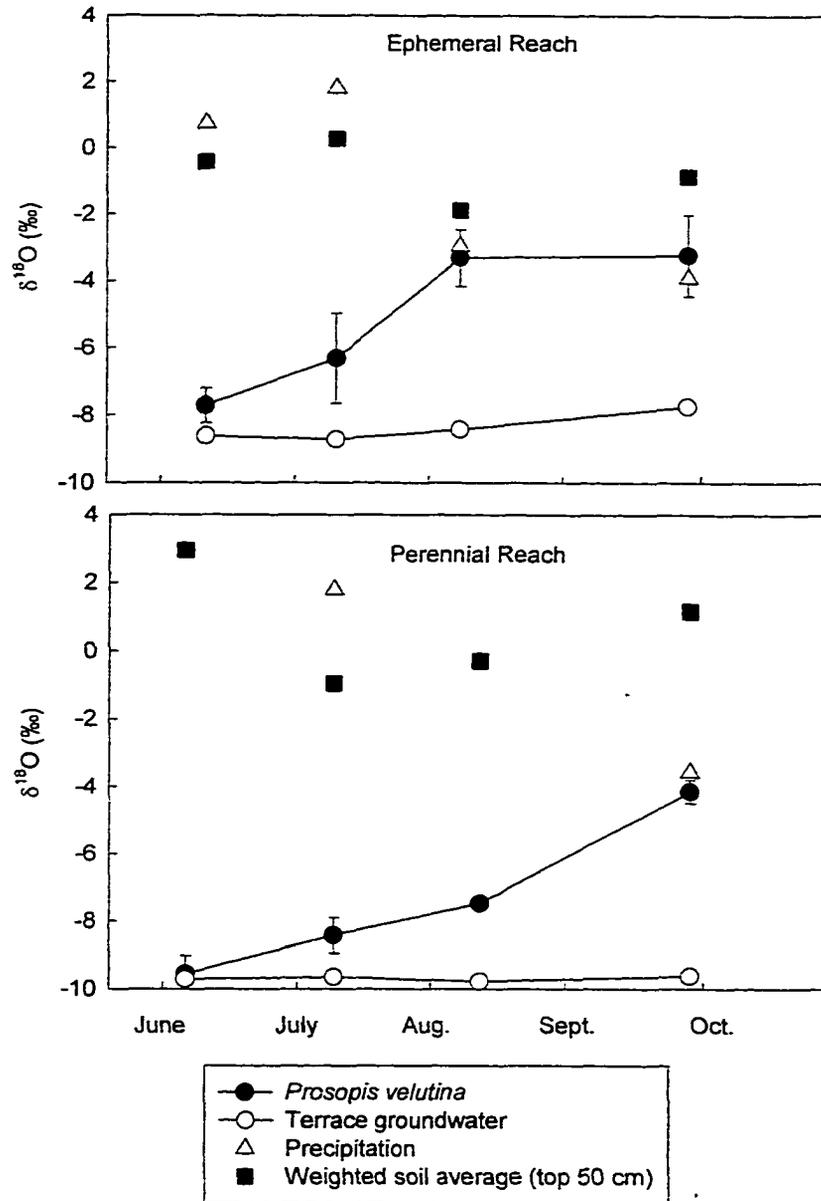


Figure 4-2 Mean $\delta^{18}\text{O}$ (\pm SE) values of *Prosopis velutina* sampled on secondary terraces near a perennial reach and ephemeral tributary of the San Pedro River, Arizona. $\delta^{18}\text{O}$ values of floodplain groundwater, soil (average of top 50 cm values weighted by gravimetric soil moisture) and rainfall (when available) sampled throughout the growing season are shown.

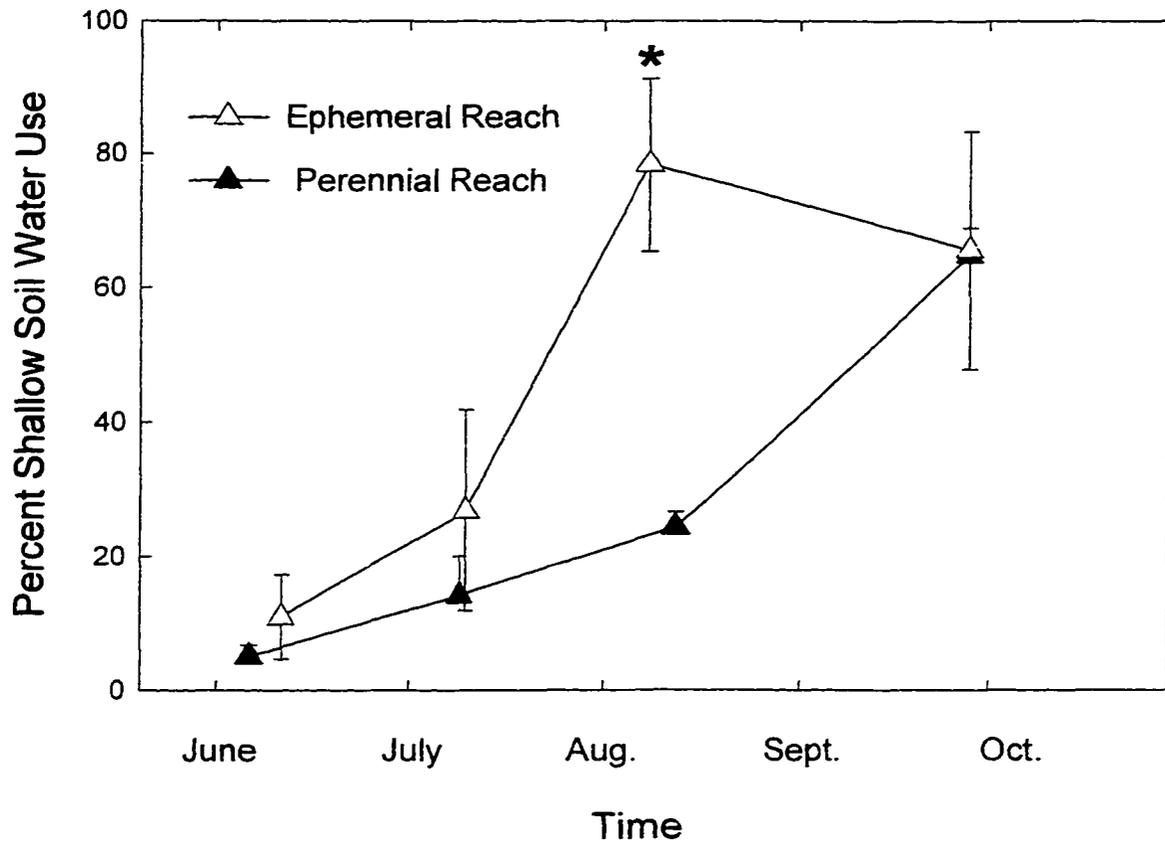


Figure 4-3 The percentage of shallow soil water use for *Prosopis velutina* throughout the growing season in 1997 from trees sampled on secondary terraces near a perennial reach and ephemeral tributary, Arizona. Percent shallow soil water use was calculated with a 2-compartment linear mixing model. Asterisks (*) indicate differences between sites using t-tests.

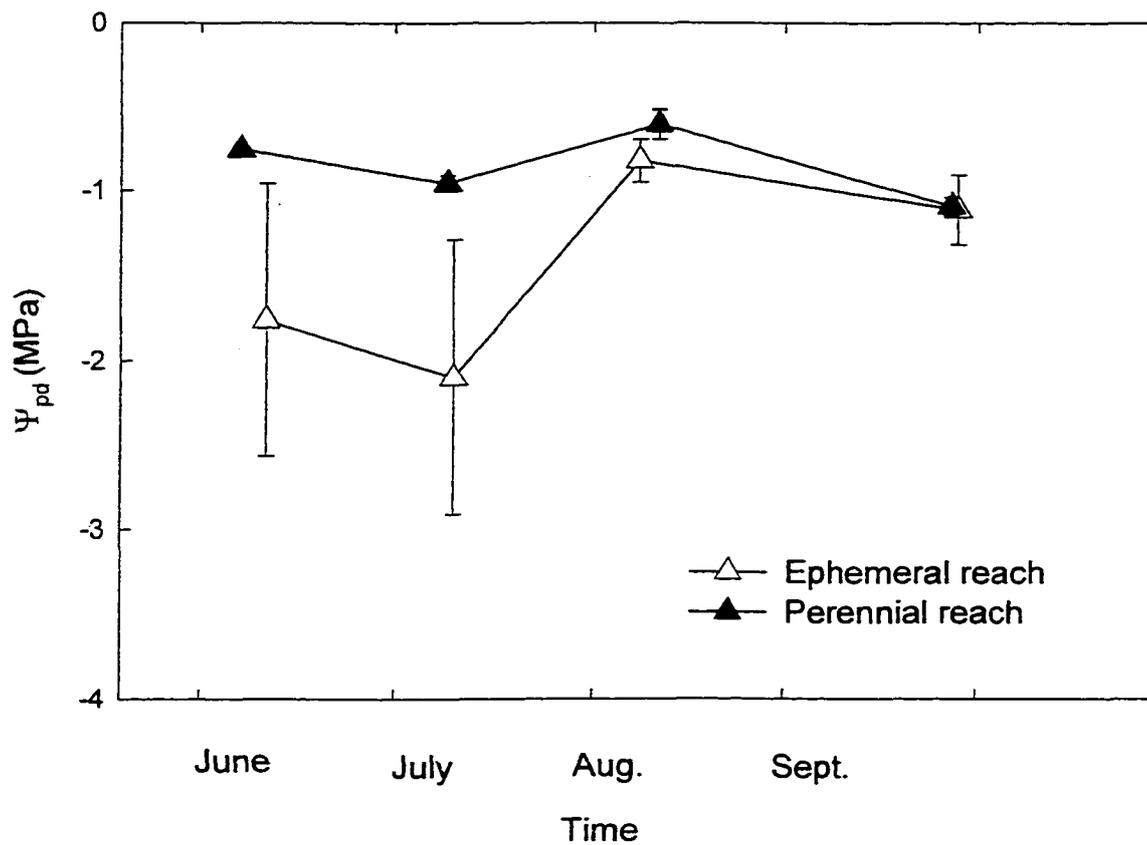


Figure 4-4 Mean Ψ_{pd} (\pm SE) values of *Prosopis velutina* in during the 1997 growing season from trees sampled at secondary terraces along a perennial reach and along an ephemeral tributary of the San Pedro River, Arizona.

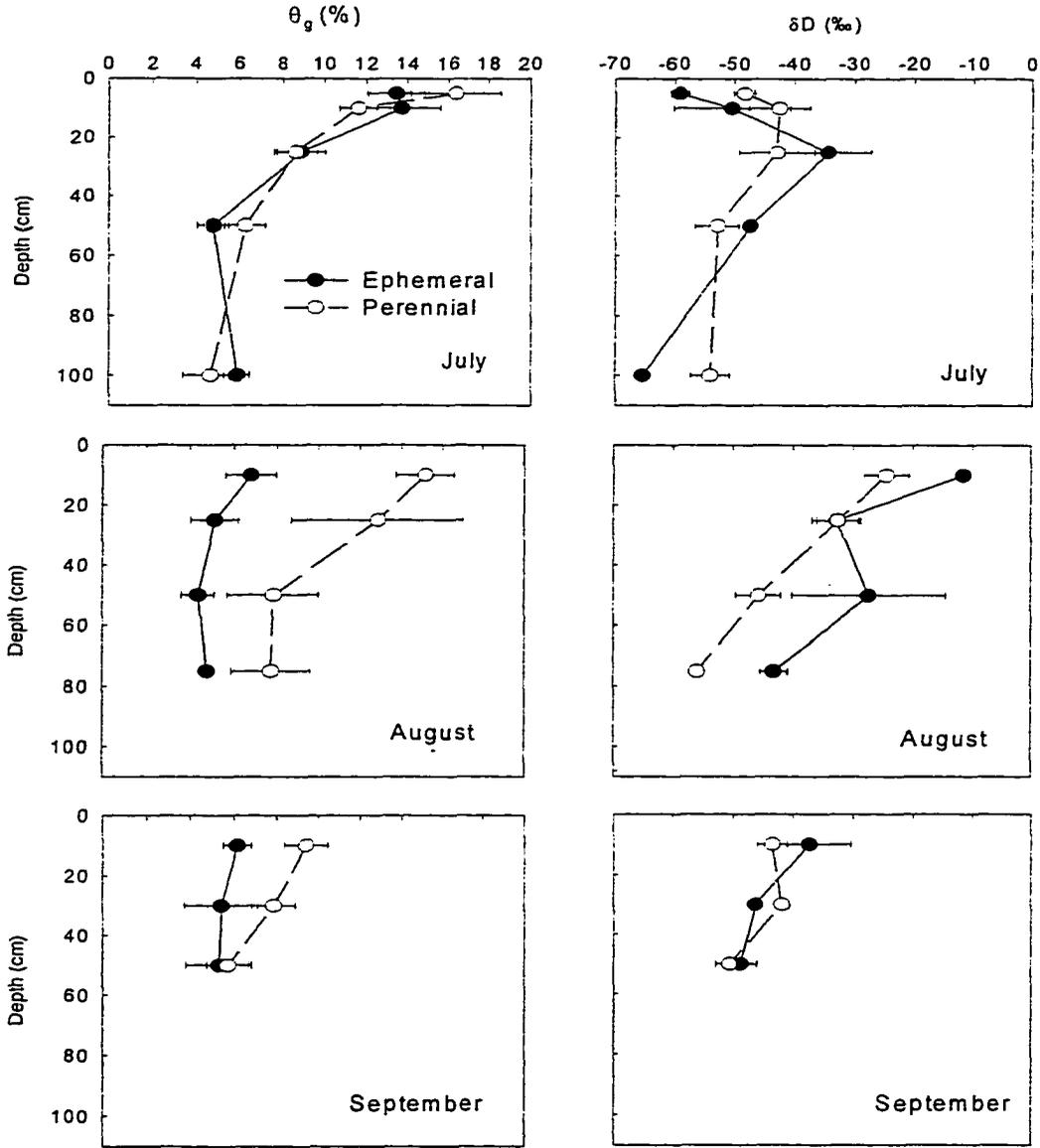


Figure 4-5 Mean gravimetric soil moisture values (\pm SE) and mean δD values (\pm SE) of soils sampled at 5-,10-, 25-, 50-, and 100-cm depths in July, 10-, 25-,50-, and 75-cm 10-, 30-, and 50-cm depths in September. Soils were sampled after natural rainfall of 31-,91- and 12-mm in secondary terraces near a perennial reach and ephemeral tributary of the San Pedro River. Rainfall amounts were measured at the perennial reach.

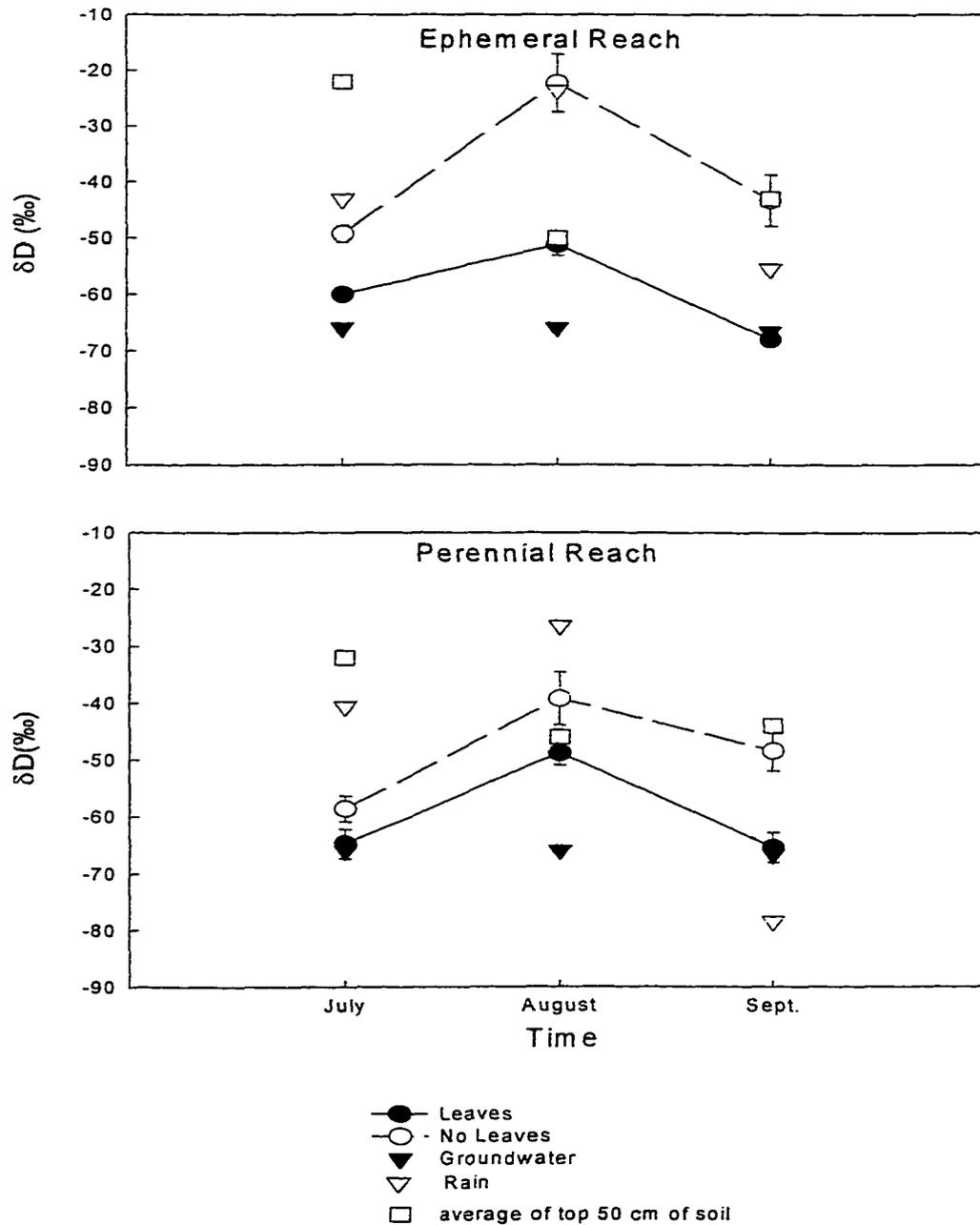


Figure 4-6 The mean δD (\pm SE) values of *Prosopis* xylem sap of undefoliated trees and defoliated trees sampled after natural rain events in 1998 on secondary terraces along a perennial reach and ephemeral tributary of the San Pedro River. δD values of groundwater, precipitation and the upper 50 cm of soil weighted by gravimetric soil moisture are also shown.

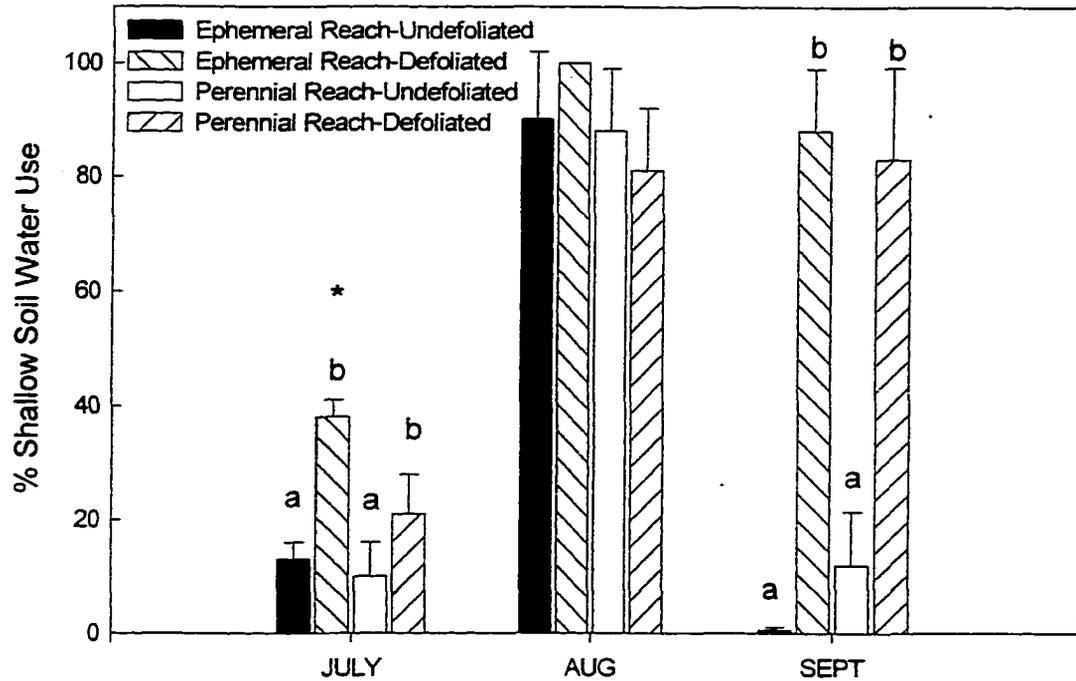


Figure 4-7 The percent shallow soil water use from the upper 50 cm of soil calculated with a 2-compartment linear mixing model for defoliated and undeveloped *Prosopis* trees in 1998 on secondary terraces along a perennial reach and ephemeral tributary of the San Pedro River. Lowercase letters indicate a significant effect of defoliation on the percentage of shallow soil water taken up by *Prosopis*. Asterisk (*) indicates a significant effect of site on the percentage of shallow soil water taken up by *Prosopis*.

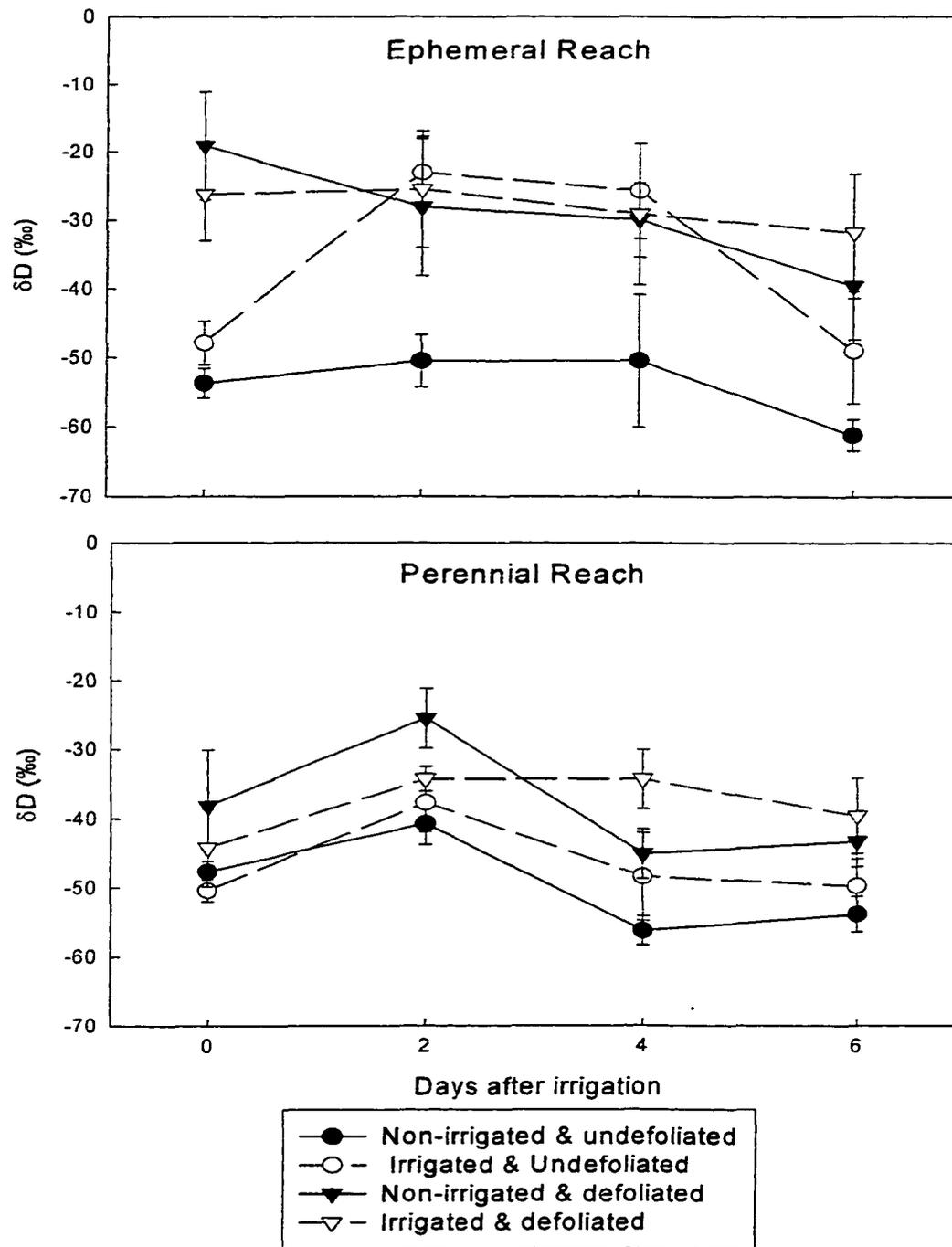


Figure 4-8 Mean δD (\pm SE) values of *Prosopis* xylem sap sampled just prior to irrigation (Day 0) and 2, 4, and 6 days after irrigation with deuterium-labeled water labeled.

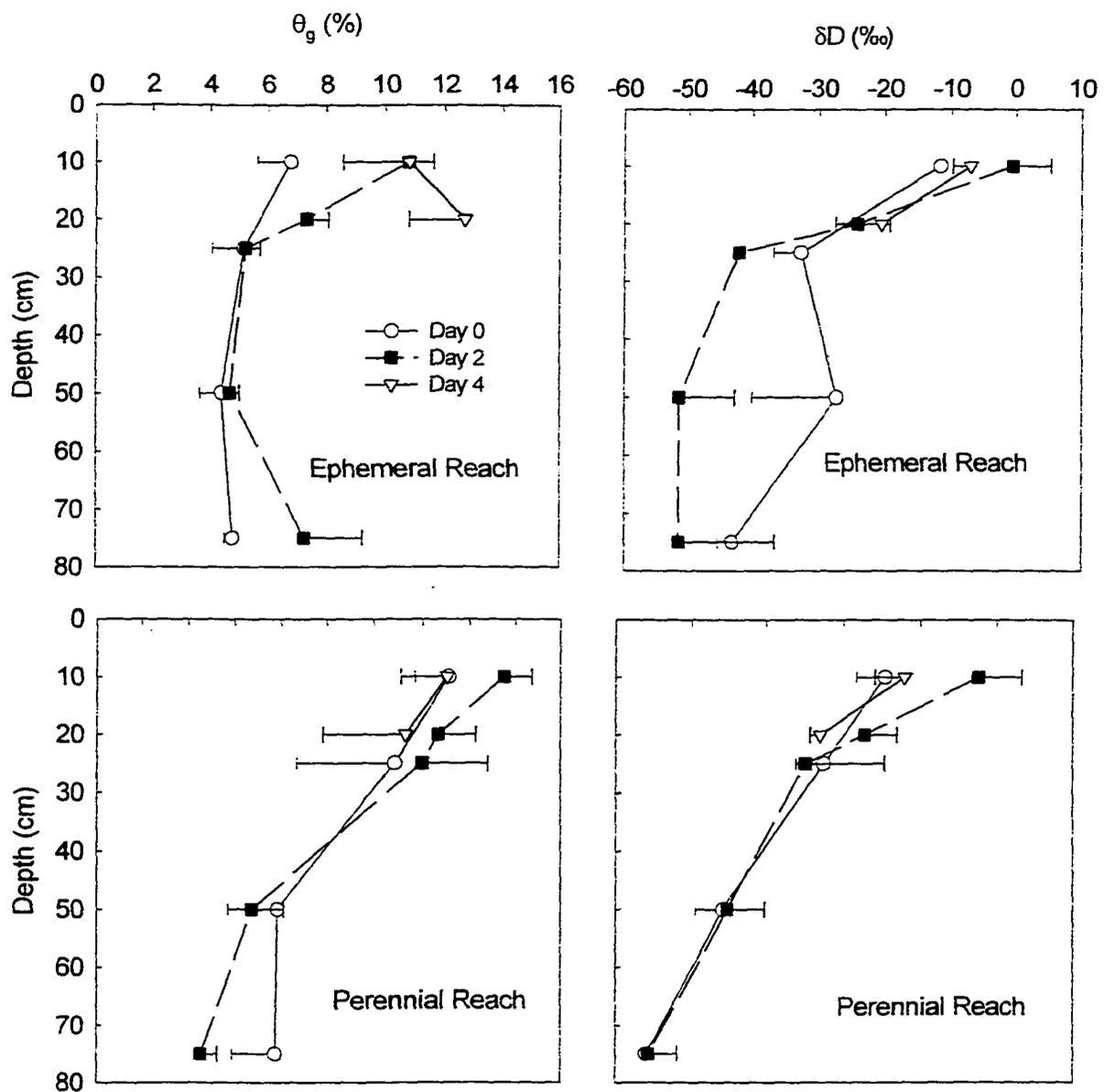


Figure 4-9 Mean gravimetric soil moisture values (\pm SE) and mean δD values (\pm SE) of soils sampled at 10-, 20-, 25-, 50-, and 75-cm depths. Soils were sampled just prior to irrigation, 2 days and 4 days after irrigation with water labeled with deuterium under watered trees on secondary terraces near a perennial reach and ephemeral tributary of the San Pedro River.

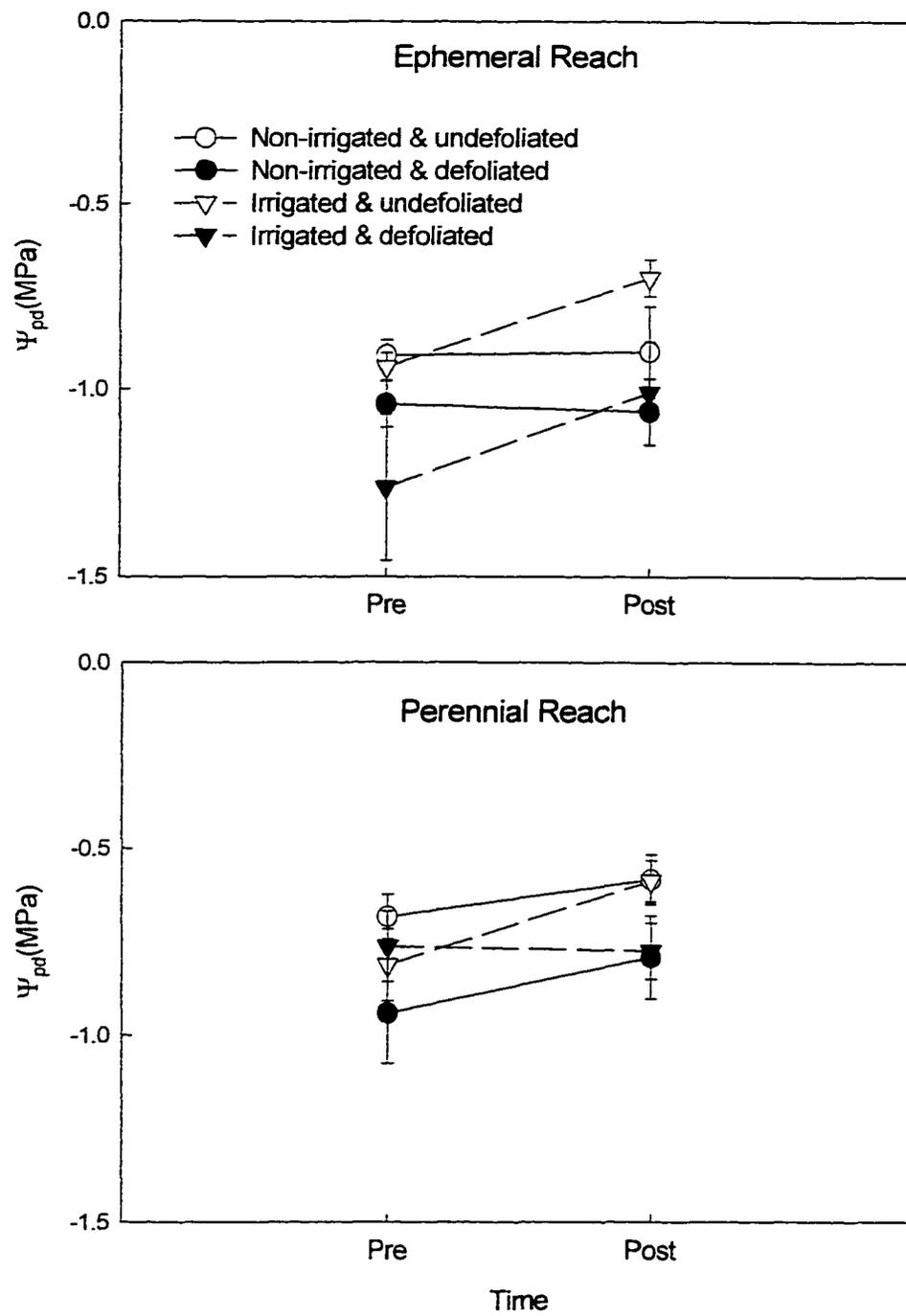


Figure 4-10 Mean Ψ_{pd} (\pm SE) values of *Prosopis* sampled just prior to irrigation (Pre) and 2 days after irrigation (Post).

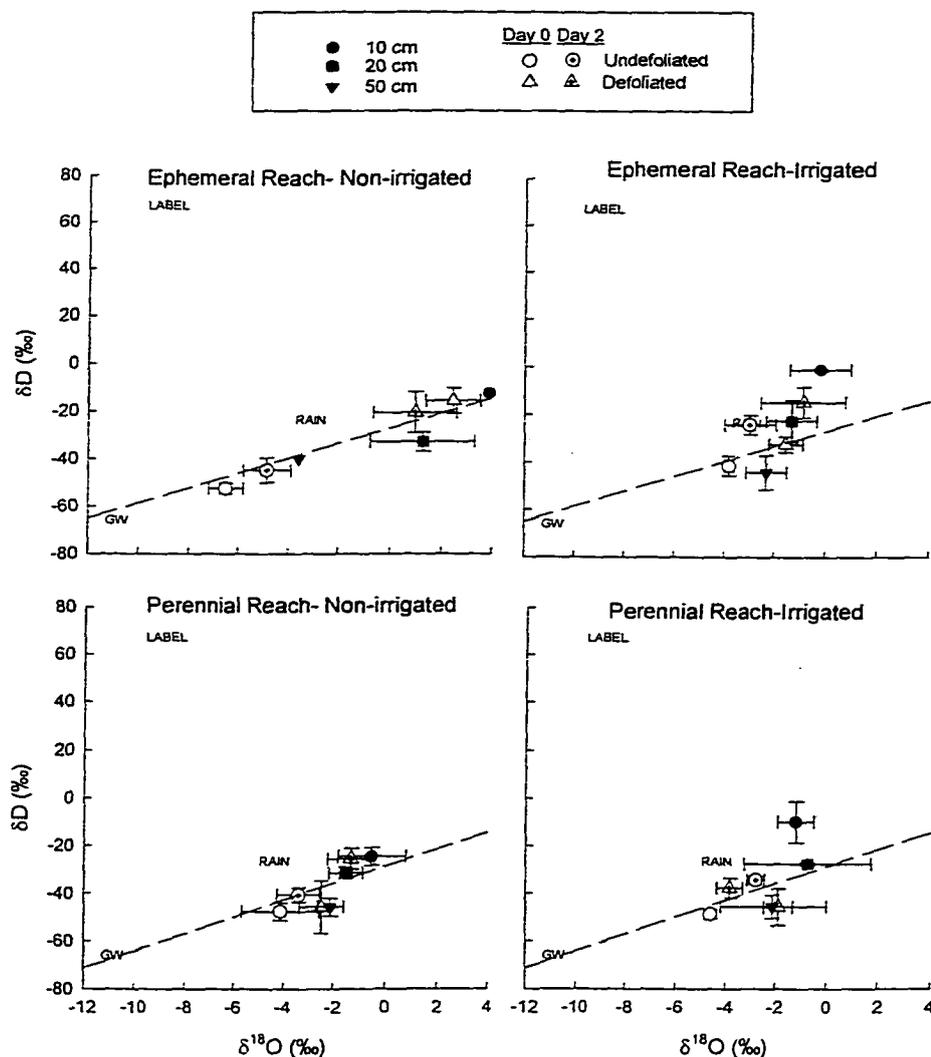


Figure 4-11 $\delta D - \delta^{18}O$ values from *Prosopis*, soil, groundwater, and rainfall in August 1998 at terraces along perennial and intermittent reaches and an ephemeral tributary of the San Pedro River in southeastern Arizona. *Prosopis* values of defoliated trees and undeveloped trees just prior to irrigation (Day 0) and 2 days after irrigation with deuterium-labeled water. Soil values are from the 10 cm, 20 cm, and 50 cm depths sampled in the non-irrigated treatments, but after the natural rainfall. Dashed line is the fitted soil evaporation line between groundwater values and soil water values at the ephemeral reach ($\delta D = 3.2 \delta^{18}O - 27.1$) and at the perennial reach ($\delta D = 3.6 \delta^{18}O - 28.6$).

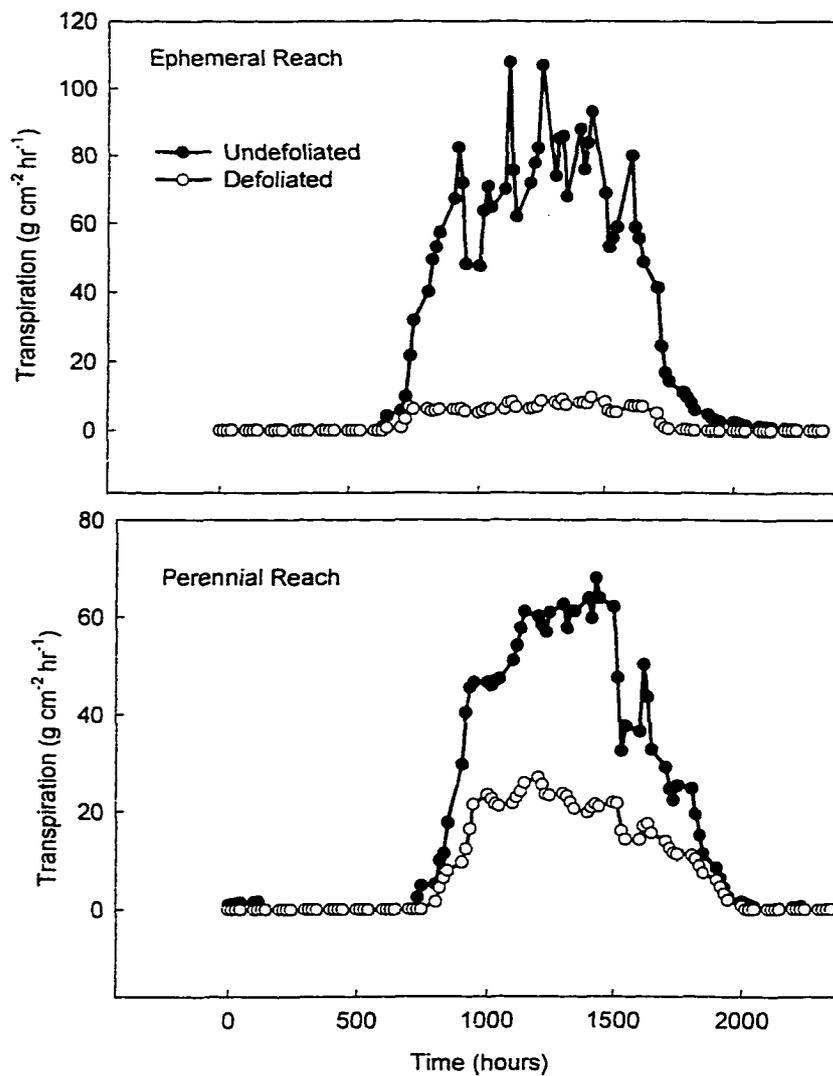


Figure 4-12 Daily course of transpiration per sapwood area of *Prosopis* measured along the ephemeral reach 2 days after irrigation (DOY= 233) and along the perennial reach 1 day after irrigation (DOY= 231) in 1998.

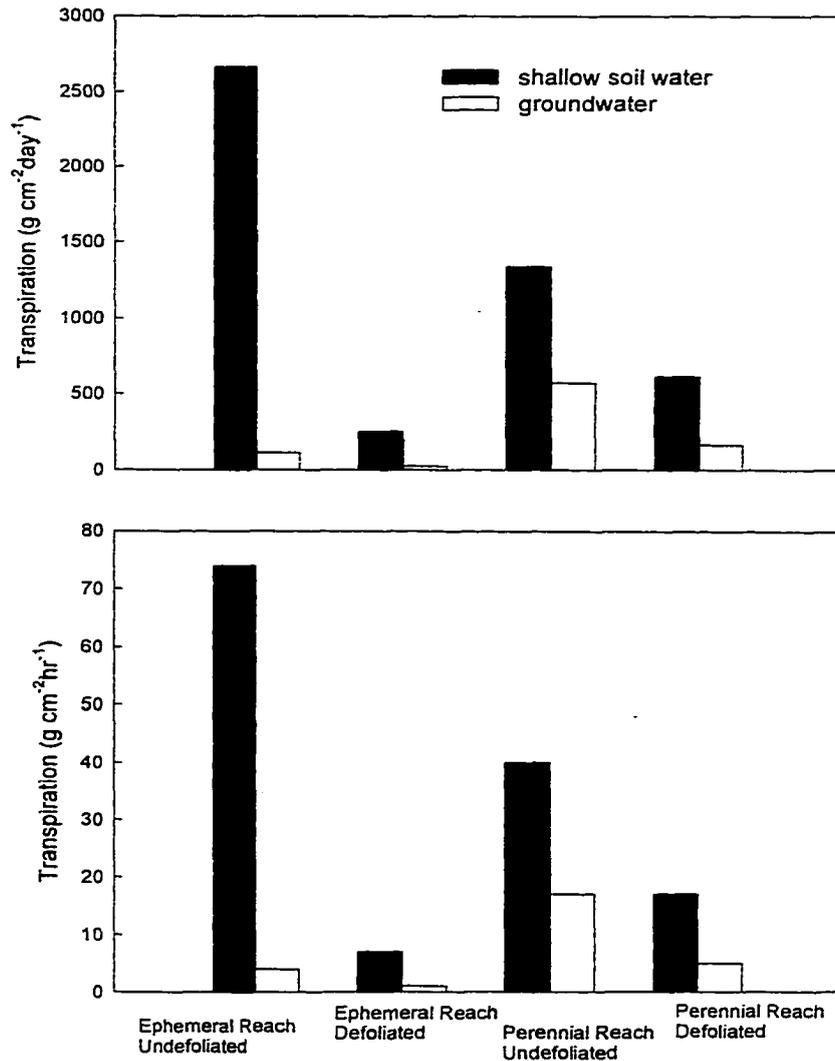


Figure 4-13 Amount of transpiration derived from groundwater and soil water in August, 1998 for two populations of *Prosopis* on secondary terraces near a perennial reach and ephemeral tributary of the San Pedro river after irrigation with deuterium-labeled water. Data are expressed as transpiration flux (grams H_2O per unit xylem area per unit time) at the scale of single trees rather than for whole canopies or per unit ground area. Values were derived by combining averaged rates of water flux at midday (panel a) (1000-1500 h) and by summing transpiration losses over an entire day (panel b) with the percentage of water used from deep and shallow layers derived from isotopic data and the 2-compartment-linear mixing model.

CHAPTER 5

ROOT ALLOCATION AND WATER UPTAKE PATTERNS IN RIPARIAN TREE SAPLINGS: RESPONSE TO IRRIGATION AND DEFOLIATION IN A GLASSHOUSE ENVIRONMENT

ABSTRACT

We conducted a greenhouse experiment with *Populus fremontii* (Frémont cottonwood) and *Prosopis velutina* (velvet mesquite) to determine if allocation to fine and coarse roots, and water uptake from hydraulically separated upper (0-57 cm) and lower soil compartments (57-110 cm), would be altered by available soil moisture and defoliation. The lower soil compartments were irrigated to maintain high ("wet") or low ("dry") moisture availability. Half of the plants in each watering treatment were defoliated at regular intervals. Isotopically labeled water was supplied in pulses to upper soil compartments to determine the fraction of total transpiration water derived from each compartment. Above- and belowground resource limitations differentially altered pulse utilization and root proliferation of these two species. Changes in biomass allocation in drought-stressed *Populus*, specifically decreased fine-root biomass in the upper soil compartment reduced use of surface irrigation pulses, while increased use of surface pulses was associated with greater allocation to fine roots in shallow soil layers.

Defoliation of *Populus* plants reduced the proportion of shallow soil water use in both wet and dry treatments, suggesting that uptake of water by shallow roots is not favored when plants have limited carbon to allocate to roots. In contrast, watering and defoliation did not produce a consistent effect on the biomass partitioning or water-source use of *Prosopis*. *Populus* exhibited belowground allocation tradeoffs in response to spatial heterogeneity of soil water and consequently water-source use in one part of the rhizosphere came at the expense of water-source use elsewhere in the rhizosphere in this obligate riparian species.

INTRODUCTION

Water is often the most limiting resource for plant growth in semi-arid and arid regions (Noy-Meir 1979). Rainfall is bimodal in the semi-arid southwestern United States, with precipitation distributed primarily in winter and summer seasons. Coexistence of species in this region has been attributed to the pattern of bi-modal precipitation (Archer 1990; Ehleringer *et al.* 1991), with C₃ woody species using winter rains that percolate deep into the soil profile, while shallow-rooted herbaceous plants and grasses use shallow soil water derived from summer rainfall. However, many woody species use summer rainfall to augment transpiration demands. Yet, there is a high degree of intra- and interspecific variation among woody plants for use of shallow soil water derived from summer rainfall (Kolb *et al.* 1997; Dawson and Ehleringer 1998; Snyder and Williams 2000; Williams and Ehleringer 2000). Understanding what

conditions promote use of shallow and deep water sources and determining if we can expect uniform responses by species and populations is critical to establishing predictive relationships under future climate change scenarios or changing groundwater levels. If the functional development of root systems exhibits limited ability to respond to changes in soil moisture patterns, then changes in the amount of precipitation received in either season may substantially affect the distribution of species. Addressing these issues requires a detailed understanding of how woody plants use ephemeral pulses of water within the soil environment.

Roots are major sinks for carbon assimilated by plants (Eissenstat and Yanai 1997). It is generally thought that plants proliferate roots in resource rich patches (*see review* by Fitter 1994). However, even in regions where water is often the most limiting resource to plant growth, not all sources of water are used equally. Proliferation of roots into moist patches carries a cost to the plant: and if the moist patch is short lived, the cost of growing and maintaining the new root may not be repaid (Nobel 1992; Fitter 1994).

Plants have a limited carbon pool and they must allocate this carbon to various competing sinks both above- and belowground (Bazzaz 1997). Optimization models of plant carbon allocation predict that a plant should continue to produce roots until the marginal cost of root production equals the marginal benefit of root production (Bloom *et al.* 1985). Therefore, plants with access to different sources of water should preferentially allocate carbon to the more stable source of water because that is where the greatest benefit is derived. Does this foraging for water in one patch come at the expense of root growth in another part of the rhizosphere? It seems plausible that as a plant

becomes increasingly carbon-limited, there will be tradeoffs between root growth and function in various soil layers, because plants must now optimize available resource use to an even greater extent.

Many woody plants in semi-arid and arid systems have a dimorphic root system with shallow lateral roots and deep sinker or taproots. Empirical evidence from field studies indicates that these woody plants may be redirecting limited carbon resources in response to the relative availability of water resources (Dawson and Ehleringer 1998; Kolb *et al.* 1997; Snyder and Williams 2000). This evidence was derived from stable isotopic analyses, which uses hydrogen and oxygen isotope ratios as natural tracers for determining the proportional uptake of water from various soil layers (Brunel *et al.* 1995). More traditional studies of roots used belowground harvesting, and proliferation of roots into either water or nutrient rich patches was equated with resource use from a particular patch (Fitter 1994). However, few studies to date have determined if changes in water-source use patterns as determined with stable isotopic analyses are associated with changes in root biomass and structure (but see Dawson and Pate 1996).

Therefore, we were interested in understanding how root development and water uptake of woody phreatophytes with dimorphic root systems, would be affected by the presence or absence of a stable water source and perturbations to the plant's carbon budget imposed through defoliation. We asked the following questions: 1) does the presence of a stable deep water source affect a plant's ability to use pulses of shallow soil water; 2) do plants exhibit plastic responses to changes in water availability belowground and are these changes manifested in water-source use and allocational patterns; and 3)

does defoliation affect patterns of biomass allocation and pulse use? We predicted that plants with access to a stable deep water source would optimally allocate to roots in wetter soil layers, and that this allocation would come as a tradeoff to allocation to drier soil layers. Plants without a stable deep-water source should exhibit enhanced use of surface soil water. As plants became increasingly carbon-limited they should exhibit more efficient foraging for water because limited carbon assimilation will force more allocation tradeoffs between root growth in different soil layers.

To address these predictions we conducted a greenhouse experiment with *Populus fremontii* and *Prosopis velutina* to determine if allocation to fine and coarse roots, and water uptake from hydraulically separated upper and lower soil compartments, would be altered by available soil moisture and defoliation. These species occur in riparian ecosystems of the southwestern United States. *Prosopis* is a facultative phreatophyte, and *Populus* is an obligate phreatophyte.

METHODS

A glasshouse experiment was conducted to determine the role of water availability and defoliation on the response of *Prosopis* and *Populus*. We used specially constructed pots to create different water availability in upper and lower compartments. The objective was to create similar moisture conditions in the upper compartments, and different conditions in the lower compartment, and to determine how deep water availability and defoliation affected the growth responses and subsequent use of shallow

pulses of soil water administered from the surface. We were interested in assessing the effects of water availability and defoliation on belowground biomass allocation, not the effect of drought on transpiration, therefore when assessing response to surface pulses all plants were provided ample water in the bottom compartment.

Experimental Design and Pot Construction

Pots were constructed polyvinyl chloride tubes 0.46 m in diameter and 1.1 m deep (Fig. 5-1). Pots were split in half vertically and bolted back together to facilitate removal of root biomass at the end of the experiment; seams were sealed with silicone to prevent leakage. Pots were filled with 5 cm of coarse rock at the bottom and then lined with a mesh screen to permit drainage and aeration at the bottom while minimizing soil loss through the drain holes. Pots were filled to a height of 0.53 m with clayey-sand field soil from an old river channel in south Tucson (Triple A Fertilizer, Tucson, AZ) and then flooded from the bottom and allowed to drain and settle to remove macropores. After drainage, more soil was added to ensure pots were filled to a specified height of 0.53 m. A melted paraffin wax and petroleum jelly layer (1:6.5 by mass, wax:jelly) was poured into each pot and allowed to cool (Bryla *et al.* 1997). This layer prohibited water exchange between the top and bottom of the pot, but allowed unimpeded root growth. The remainder of the pot (0.49 m) was filled with the same field soil to within 0.08 m from the top. Soil was obtained from a local source and mixed with time-release nutrient pellets (Sierra 17-6-10 with micronutrients, Scotts Chemical Co., Marysville, OH) to

maintain adequate levels of nutrients. A drain-hole through the polyvinyl chloride tube in the upper compartment allowed drainage from above the wax layer. The bottom compartment contained three large drain-holes at the pot bottom, that were sealed during bottom irrigation with rubber stoppers and one other inlet/outlet hole that connected via tubing to the central reservoir. Thus, pots could be watered from the bottom, through a large central reservoir (Mahoney and Rood 1991). Four small holes drilled through the tube below the wax layer facilitated gas exchange in the lower compartment. Pots were planted with cuttings of *Populus*, obtained from a nearby field sites (see Snyder and Williams 2000), and with established 1-gallon *Prosopis* seedlings from a local nursery.

Plants initially grew under similar conditions and received ample water from the top and bottom of the pots. Greenhouse temperature were between minimum of 20° and maximum of 30° C. When plants no longer wilted with the removal of surface water, roots were assumed to have penetrated to the lower compartment and treatments were initiated. *Populus* treatments were initiated 150 days after planting and *Prosopis* treatments were initiated 300 days after planting. Due to the slower growth of *Prosopis*, the different species were treated as two separate experiments.

Two treatments were employed in each experiment: watering and defoliation. All plants were given 1.25 liters of water every other day from the top of the pots throughout the experiment, administered via a drip irrigation system. This water was supplied to plants to maintain live roots in the tops of pots. The amount of water supplied to the lower compartment differed. That is, watering treatments consisted of a dry lower compartment or a wet lower compartment. The wet treatment maintained a high water

content in the lower compartment with weekly flooding from the central reservoir. The dry treatment was wet thoroughly once and then allowed to dry-down until each sampling date.

Leaf removal treatments consisted of undefoliated and defoliated plants.

Defoliated plants had half of the leaves hand-removed every 2 weeks. The experimental design was a replicated fully factorial experiment, with 12 individuals of each species randomly assigned to all combinations of bottom water conditions and leaf removal: 1) dry-undefoliated, 2) dry-defoliated, 3) wet-undefoliated and 4) wet-defoliated ($n = 3$ per treatment combination). There was one extra *Prosopis* tree ($n=4$) in both the wet-undefoliated and dry-undefoliated treatments.

Measurements

After plants grew under treatment conditions, they were pulsed (irrigated) with isotopically labeled water at the soil surface to determine which plants used the pulse of shallow soil water. *Populus* plants were sampled for isotopic composition of xylem sap according the following protocol 14 and 19 weeks after initiation of treatments. *Prosopis* plants were sampled 15 and 28 weeks after initiation of treatments. Three days before sampling, normal maintenance watering was ceased. Predawn leaf water potentials (Ψ_{pd}) values were measured with a Scholander-type pressure chamber. Then the lower compartments of all plants were fully saturated and allowed to drain to minimize water limitations in the lower compartment 24 hours before the application of the surface pulse.

Plant stems of approximately 0.5 cm diameter were sampled prior to surface irrigation from sunlit branches and stored in airtight glass vials for subsequent analysis of hydrogen isotope ratios (δD). Deuterium-enriched water was mixed in a large container and then hand-applied to the soil surface. To minimize ponding at the soil surface and preferential flow, the 3-liter surface pulse delivered to each plant was applied in the form of 1 liter every 2 hours beginning at 9:00 am. The next day, between 9:00 and 10:00 am, plant stems were sampled for later isotopic analyses. In the laboratory, water was extracted from plant stems by cryogenic vacuum distillation (Ehleringer and Osmond 1989; Smith *et al.* 1991). Water extracted from plant stems and irrigation waters were analyzed for hydrogen isotope ratios (δD) using a dual inlet isotope ratio mass spectrometer (Delta-S, Finnigan –MA, Bremen, Germany). A chromium reduction furnace attached on-line to the mass spectrometer was used to convert liquid water to hydrogen gas (HD-Device, Finnigan-MAT, Bremen, Germany).

Transpiration was measured continuously during isotopic labeling with heat balance collars (Dynamax Inc., Houston, TX). Additional measurements of Ψ_{pd} values were made for *Prosopis* 1 day after surface irrigation. Gas exchange was measured with a portable infrared gas analyzer (PP Systems, CIRAS-1, Herts, UK) immediately prior to the surface pulsing and bottom watering, and after all plants were well-watered. After the final pulsing experiment, all above- and belowground biomass was harvested, oven-dried and weighed. Pots were opened along the length of the pot and roots were washed and separated into fine (< 5mm in diameter) and coarse (> 5 mm in diameter) fraction within upper and lower compartments.

Data Analyses

Two-way analysis of variance (ANOVA) was used to assess the effects of watering and defoliation on three response variables. If a significant interaction was present, contrasts were used to determine which treatment combinations differed. Ψ_{pd} values immediately before irrigation were used as response variables. Photosynthetic rate (A) was also used as a response variable. The difference between δD immediately before surface watering and after irrigation with labeled water was used to determine which plants used surface pulses. The change in δD values was used because it reflects the result of the surface pulse, which minimizes differences in the initial δD values that were a function of differences within pots prior to the surface pulse. Data were transformed as necessary to meet the assumptions of ANOVA based on inspection of residual plots. Non-transformed values are presented in the figures and text.

Mass ratios between various plant structures (shoots, fine roots, coarse roots) and ratios between roots in various soil layers as a proportion of total root biomass were examined to determine changes in allocation. Ratios were used to counteract differences in absolute plant size. Ratios were constructed within each compartment as well as by summing upper and lower compartments. ANOVA models, as described above, were used to assess the effects of watering and defoliation on these ratios.

A 2-compartment linear mixing model was used to calculate the proportion of xylem water derived from the surface pulse and deeper soil layers. The model was of the form:

$$[\text{Eqn. 1}] \quad f = (\delta D_{p0} - \delta D_{p1}) / (\delta D_{\text{pulse}} - \delta D_{\text{tap}}),$$

where f is the fraction of water derived from the labeled pulse water, δD_{p0} is the δD value of stem xylem sap prior to application of labeled water, δD_{p1} is the δD values of stem xylem water 1 day after application of labeled water, δD_{pulse} is the value of labeled water, and δD_{tap} is the δD values of the greenhouse tap water used to water plants, with the exception of labeled surface pulses. The proportion of water derived from deeper soil layers (Y) was calculated as:

$$[\text{Eqn. 2}] \quad Y = (1-f).$$

Transpiration on a per-unit sapwood area basis was partitioned by multiplying the proportion of water derived from shallow and deep soil with the average flow rate at midday (1000-1500 h). This quantified the absolute volume of water derived by plants from shallow and deeper soil layers.

RESULTS

Populus Experiment

There was no interactive effect of defoliation and watering on Ψ_{pd} ($p = 0.75$). Ψ_{pd} values of *Populus* were more negative in dry treatments than wet treatments on both sampling dates, regardless of defoliation treatment ($p < 0.001$) (Fig. 5-2), which indicates that there were differences in available soil water between the two watering treatments. Assimilation rates (A) at all sampling dates were predominately affected by watering regime ($p < 0.004$), though immediately after irrigation in treatment week 13 there was an effect of defoliation, with defoliated plants having lower A than undefoliated plants (Table 1). A increased after irrigation in all treatments, except wet-undefoliated plants (week 13), likely due to the surface pulse and that the lower compartment was also irrigated. Wet-undefoliated plants experienced little increase in A likely because A was high previous to watering. By treatment week 19, dry-defoliated plants experienced little increase in A after watering, apparently because these plants were unable to recover from the multiple stresses of defoliation and drought. Plants in the other three treatments experienced increased A in response to surface and bottom watering.

The change in δD values after surface irrigation in treatment week 13 indicated that all plants used a similar proportion of irrigation water regardless of defoliation or watering and there were no interactive effects ($p > 0.20$) (Fig. 5-3). By treatment week

19, δD of undefoliated plants increased more than defoliated plants in response to surface irrigation ($p = 0.10$) (Fig 5-3). There were no interactive effects of defoliation and bottom water treatments ($p = 0.62$).

Percent water-source use (Table 5-2), in conjunction with transpiration data, indicated that wet-defoliated, dry-defoliated, and dry-undefoliated plants used approximately the same amount of deep water 13 weeks after treatment initiation while, wet defoliated plants used less deep water (Fig. 5-4). The amount of shallow pulse use was approximately the same in wet-defoliated, wet-undefoliated and dry-undefoliated plants, while dry-defoliated plants used less pulse water. Plants in both wet treatments had a much greater use of deep water and shallow pulse water by treatment week 19 than plants in dry treatments. Within the dry treatment, defoliated plants used less shallow and deep water than undefoliated dry plants.

Total biomass was reduced in dry treatments relative to wet treatments (Fig. 5-5). Total shoot to total root ratios increased in defoliated plants relative to undefoliated plants, indicating that defoliation produced a greater allocation to aboveground stems and leaves (Table 5-3). There was no effect of watering or defoliation on the allocation to fine or coarse roots as a proportion of total root biomass. The ratio of fine roots to coarse roots in the top compartment and aggregated across compartments was unaffected by watering and defoliation. Therefore, total allocation to fine and coarse roots did not change. In contrast, allocation to fine roots was affected interactively by watering and defoliation: dry-undefoliated plants allocated more biomass to shallow fine roots than to deep fine roots compared to plants in other treatments.

Coarse root biomass in the bottom compartment was affected only by watering. Plants in wet treatments had greater ratio of coarse root to total root biomass than those in dry treatments. Four of the six plants in dry treatments had no coarse roots in the lower compartment, while all plants in wet treatments had coarse roots in the lower compartment. In contrast, coarse root growth in the upper compartment was unaffected by watering and defoliation.

Prosopis Experiment

Ψ_{pd} values of *Prosopis* were not affected by the interactive effects of defoliation and watering 15 weeks after the initiation of treatments ($p = 0.19$) (Fig. 5-6). There were significant main effects of both water and defoliation ($p < 0.07$). Defoliated plants had less negative Ψ_{pd} values than defoliated plants ($p = 0.06$), and wet treatments had more negative Ψ_{pd} values than dry treatments ($p = 0.02$). After surface irrigation and bottom irrigation, Ψ_{pd} of defoliated *Prosopis* plants were still less negative than those of undefoliated plants ($p = 0.04$). After 28 weeks of treatments, Ψ_{pd} values of defoliated *Prosopis* were less negative than those of defoliated *Prosopis* prior to irrigation ($p = 0.045$). After irrigation, all plants had similar Ψ_{pd} values, regardless of defoliation or watering treatment ($p > 0.10$).

Assimilation rates at each sampling period were unaffected by watering ($p > 0.24$) (Table 5-4). Defoliated plants had higher A compared to undefoliated plants immediately prior to irrigation in week 15 ($p < 0.03$).

The change in δD values of *Prosopis* and the percentage of shallow soil water use after 15 weeks indicated that all plants used the same proportion of the surface pulse regardless of defoliation, watering or the interaction between defoliation and watering ($p > 0.33$) (Fig. 5-6, Table 5-5). By treatment week 28, there was a significant interactive effect of defoliation and watering on the ability of plants to use pulses of shallow soil water ($p < 0.10$). Specifically, wet-undefoliated plants used a greater proportion of the surface irrigation pulse than plants in other treatments ($p = 0.0011$).

Transpiration from shallow and deep soil layers was reduced in dry-undefoliated plants relative to the plants in other treatments by treatment week 28 (Fig. 5-8). Defoliated plants in both the wet and dry treatments had similar fluxes of water from shallow and deep layers. Wet-undefoliated plants had reduced transpiration rates, but used a greater amount of the surface pulse in comparison with defoliated plants. Wet-undefoliated plants used approximately equal amounts of surface pulse water and deeper soil water.

There were no significant main or interactive effects of defoliation or watering on any of the 10 calculated biomass ratios therefore data are not presented ($p > 0.36$) (see Table 5-2 for list of ratios). The allocation to aboveground versus belowground biomass did not change across plants in any treatments (Fig. 5-9). As well, allocation to fine and coarse roots as a proportion of total plant biomass or in relation to each other were unaffected by defoliation and watering in both top and bottom compartment and when integrated across both compartments.

DISCUSSION

There were three important findings from the pot experiment with *Populus*. First, water-source use of these plants is flexible and changes in response to water availability and defoliation. Second, changes in fine root biomass allocation were associated with changes in water-source use for water-stressed plants. Third, defoliation reduced the amount of shallow soil water use by plants

Our data suggest phenotypic plasticity in the allocation of carbon to root growth that expresses itself on short time scales (months). Other studies of the genus *Populus* found root production was responsive to environmental conditions such as atmospheric CO₂, soil N (Pregitzer *et al.* 2000; Zak *et al.* 2000), and water availability (Mahoney and Rood 1991; Shafroth *et al.* 2000). As well, excision of leaves was found to cease root growth, and other studies confirm a relationship between leaf and root growth (*see review* by Pregitzer and Friend 1996).

In the current study, lack of deep water reduced coarse root growth, and promoted redistribution of fine roots. Interestingly, total allocation to the root system did not change in response to watering or defoliation; the ratio of fine root to coarse root biomass, as well as the total proportion of carbon allocated to fine and coarse roots aggregated across upper and lower compartments, was unchanged. In contrast, there was a shifting of where plants grew fine roots when plants faced stresses of defoliation and water limitations. Patterns of fine root growth appeared to explain differences in water-source use. This suggests that measures of root structure, such as root volume and root

depth, may not adequately characterize plant water-source use, and that the ratio of fine roots in different soil layers may need to be considered.

Reduced availability of deep water produced differential responses depending on whether a plant was carbon-limited as a result of defoliation. Dry-undefoliated plants, that likely had more carbon to allocate belowground than dry-defoliated plants, increased allocation to shallow roots when deep water was limited, but decreased allocation to deep fine roots. These changes in biomass were associated with changes in water-source use. These plants used a similar amount and proportion of the surface pulse as plants with access to a reliable source of deep water, but at the expense of root development in deeper soil layers. This is consistent with other experimental work in which taproot desiccation promoted lateral root growth both in *Quercus* (Callaway 1990), and *Salix* (Horton and Clark 2000).

Dry-defoliated plants allocated more biomass to deep fine roots and had similar allocation to shallow fine roots as the plants in wet treatments, but less than dry-undefoliated plants. Contrary to our original predictions, these plants did not use the surface pulse, which indicates that roots present in the shallow soil layers contributed little to plant water uptake. The increased allocation to deep roots by dry-defoliated plants may be a result of the way treatments were applied. The “dry” lower compartment, while drier than the wet compartment, was still likely wetter than top compartment during the experiment. It seems plausible that dry-defoliated plants, which had the lowest A and likely the least photosynthate to expend, maintained fine roots in the lower compartment because it was the optimal soil layer. The shallow roots may have

been produced earlier in the experiment and although apparently not functional for water uptake were still present in the soil at time of harvest. It is possible that some of the roots were dead. Another explanation for the lack of water uptake by the shallow roots is that although roots were present in the upper soil layers, and appeared alive, they may not have been functional for water transport due to root embolism (Sperry *et al.* 1998). Low water availability has been found to cause stem xylem cavitation in *Populus fremontii* (Tyree *et al.* 1994; Pockman *et al.* 1995).

Effects of defoliation on shallow root function were also reflected in a main effect of defoliation across watering treatments, with defoliated plants using a lower proportion of pulse water. Within the wet treatments, defoliated plant used a lower proportion of pulse water than undefoliated plants, although the proportion of water transpired from both the pulse water and deeper water was similar between defoliated and undefoliated plants, and allocation to roots did not change. This suggests that the function of shallow lateral roots may have been reduced or that deep root water uptake was enhanced. Rates of carbon assimilation (A) were affected to a greater extent by watering than by defoliation. In week 13, when dry plants were provided ample water, A was higher in defoliated plants than undefoliated plants. This indicates that there may have been some photosynthetic compensation (Field and Mooney 1986) for defoliation by remaining leaves, which likely explains why wet-defoliated plants had almost identical patterns of biomass allocation as wet-undefoliated plants.

All *Populus* plants except those in the dry-defoliated treatment used the shallow pulse of water. Our predictions, based on optimality theory, that dry-defoliated plants

would use the highest proportion of the surface pulse, while wet treatments would use the lowest amount of the pulse were not supported. It appears the presence of a stable deep water source allowed plants to maintain improved carbon assimilation between pulses of shallow water, and this carbon allowed plants to forage in multiple soil layers without facing allocation tradeoffs. In contrast, the response of plants in dry treatments suggests that there are tradeoffs in root biomass allocation that are associated with using water in one part of the rhizosphere versus another part of the rhizosphere.

We predicted plants would exhibit optimal root growth by foraging for water in wetter soil layers at the expense of root growth in drier soil layers. However, an alternative explanation is that plants continuously grow roots in multiple soil layers. In general, root behavior of *Populus* did not appear to follow our simple optimal model of root behavior. When provided with unlimited deep water, plants appeared to maintain root growth in multiple soil layers. Another reason that plants may have proliferated roots in many soil layers is that they are relatively insensitive to soil water potential. It has been proposed that plants adapted to growing in sandy soil, such as phreatophytes in alluvial soils, may be insensitive to soil water potential; in a sandy substrate, drying conditions would cause a physical gap at the root air-interface, making plant response to declines in soil water potential moot (Passioura 1988; Nobel and Cui 1992; Nobel 1994; Schwinning and Ehleringer *in review*). Contrary to this, plants in dry treatments had changes in biomass allocations that were consistent with changes in water-source use and with optimal foraging for water resources.

In contrast to *Populus*, there was no consistent response of *Prosopis* to defoliation and watering. It seems likely that greenhouse conditions were poorly suited for *Prosopis*, as evidenced by the slow growth both above- and belowground for *Prosopis*. Different watering regimes had little effect on *Prosopis* because few plants had roots in the bottom compartment. *Prosopis* exhibited minimal responses to defoliation and there was some evidence of photosynthetic compensation in defoliated plants that may have further minimized the effect of defoliation. It is likely that all plants were light limited, due to reduced solar radiation in the greenhouse. *Prosopis* deep root growth was probably inhibited by wet conditions in the lower compartment of the pots. This result is surprising as these plants are classified as facultative phreatophytes with roots that penetrate to 50 m, presumably to forage for deep water (Jennings 1974) and they exhibit phenological plasticity in morphology and physiology in response to environmental variation (Midwood *et al.* 1993; Stromberg *et al.* 1993; Atchley 1999). However, root growth of *Prosopis velutina* appeared to be inhibited by extremely moist soil, which suggests that when these trees use groundwater in natural ecosystems, they likely are rooted above the saturated zone and are actually using capillary fringe water, and that their connection with groundwater is limited. This agrees with other work on *Prosopis* where roots have been found in the capillary fringe zone, but not into the saturated zone (*see review* Stromberg 1993b). Therefore sudden declines in groundwater, or lack of photosynthetic assimilation that reduces root growth, may disconnect this species from groundwater, at least temporarily. Evidence for this has been demonstrated by field

experiments on this species where defoliation reduced the ability of this species to use groundwater (Snyder and Williams *in prep*).

Studies in ecosystems receiving substantial summer rainfall have found that as the growing season progresses there is increased use of shallow soil water by a number of species (Lin *et al.* 1996; Snyder and Williams 2000; Williams and Ehleringer 2000). A variety of factors such as lower soil temperatures that reduce root respiration rates and the greater amount of rainfall later in the growing season have been proposed to account for this trend. However, this trend may be a result of root growth longevity, which has been tied to the length of time the soil is wet (Eissenstant and Yanai 1997). Coarse roots persist longer in the soil, while fine roots generally turn over faster (Pregitzer and Friend 1996). Therefore, during periods of low soil water availability longer-lived coarse roots appear to supply water to plants. As the frequency of monsoon storms increases later in the growing season, fine root growth in shallow soil layers may be promoted because of the increased availability of shallow soil water, and this proliferation of fine roots increases water uptake from shallow soil layers. This is supported by our data on *Populus* where production and continued growth of fine roots was important for water uptake. This was also observed in cacti where ephemeral roots were produced quickly in response to infrequent soil wetting (Nobel *et al.* 1992).

Fine roots are important for plant water uptake because they are generally unsubsized and therefore highly permeable (Kramer and Boyer 1995). It appears that growth of these fine roots may be very sensitive to changes in plant carbon assimilation. Perturbations to plant carbon assimilation, in conjunction with reduced water availability

were manifested in shifting of fine root production to different soil layers in the current study. This shifting of fine root production resulted in differences in water-source use, which may reflect optimal foraging for water resources.

Table 5-1 Mean (\pm S.E.) photosynthetic assimilation rates (A ; $\mu\text{mol m}^{-2} \text{s}^{-1}$) of *Populus fremontii* plants grown under different watering and defoliation treatments. Treatment effects were determined with a 2-way ANOVA model.

| Weeks of treatment | Treatments | | | | Treatment Effects (p-values) | | |
|--------------------------------|------------------|----------------|------------------|----------------|------------------------------|-------------|-----------------------|
| | Wet-undefoliated | Wet-defoliated | Dry-undefoliated | Dry-defoliated | Watering | Defoliation | Watering* Defoliation |
| Week 13 Prior to irrigation | 12.6 (0.3) | 14.9 (1.6) | 6.1 (1.6) | 1.1 (0.6) | 0.0001 | 0.15 | 0.07 |
| Week 13 After irrigation | 8.7 (2.4) | 19.8 (0.42) | 17.0 (2.1) | 23.2 (4.5) | 0.24 | 0.08 | 0.80 |
| Week 19 Prior to irrigation | 16.4 (3.5) | 12.5 (0.7) | 1.9 (1.2) | 2.2 (0.3) | 0.0003 | 0.37 | 0.31 |
| Week 19 After irrigation | 18.2 (1.05) | 20.3 (2.3) | 5.8 (4.5) | 2.9 (1.2) | 0.004 | 0.76 | 0.60 |

Table 5-2 Mean percentage of total transpiration derived from the deuterium-labeled surface pulse for *Populus fremontii* plants grown under different watering and defoliation treatments. Values in parentheses are ± 1 S.E.

| Weeks of treatment | Treatments | | | |
|--------------------|------------------|----------------|------------------|----------------|
| | Wet-undefoliated | Wet-defoliated | Dry-undefoliated | Dry-defoliated |
| Week 13 | 16.3 (6.8) | 31.7 (11.0) | 27.6 (10.9) | 24.5 (2.1) |
| Week 19 | 14.9 (4.6) | 8.3 (8.3) | 14.1 (1.8) | 1.5 (1.5) |

Table 5-3 Mean (\pm 1 S.E.) biomass ratios of different plant structures for *Populus fremontii* grown under different watering and defoliation treatments and treatment effects. Treatment effects were determined with a 2-way ANOVA model (* ; $p \leq 0.05$). If a significant interactive effect was determined, contrasts were used to determine which treatments differed. Mean values within a treatment followed by the same letter are not significantly different ($\alpha = 0.05$).

| Variable | Treatments | | | | Treatment Effects | | |
|---|------------------|----------------|------------------|----------------|-------------------|-------------|------------------------|
| | Wet undefoliated | Wet defoliated | Dry undefoliated | Dry defoliated | Watering | Defoliation | Defoliation * Watering |
| Shoot:root | 0.81(0.07) | 1.25 (0.07) | 1.14 (0.21) | 1.30 (0.12) | 0.33 | 0.02* | 0.58 |
| All fine roots:total root biomass | 0.60(0.03) | 0.61(0.05) | 0.70 (0.07) | 0.63 (0.04) | 0.21 | 0.58 | 0.81 |
| All coarse roots:total root biomass | 0.31 (0.03) | 0.39 (0.05) | 0.29 (0.07) | 0.36 (0.04) | 0.21 | 0.58 | 0.43 |
| Shallow fine roots:total root biomass | 0.38 (0.04)a | 0.39 (0.04)a | 0.67 (0.07)b | 0.45(0.02)ab | N/A | N/A | 0.04* |
| Shallow coarse roots:total root biomass | 0.37 (0.03) | 0.37 (0.04) | 0.29 (0.06) | 0.36 (0.04) | 0.30 | 0.51 | 0.48 |
| Deep fine roots:total root biomass | 0.22 (0.05)a | 0.22 (0.01)a | 0.04 (0.02)b | 0.19 (0.04)a | N/A | N/A | 0.06* |
| Deep coarse roots:total root biomass | 0.02 (0.007) | 0.03 (0.007) | 0.004 (0.004) | 0.006 (0.006) | 0.03* | 0.52 | 0.33 |
| Fine roots:coarse roots | 1.53 (0.20) | 1.65 (0.30) | 1.83 (0.30) | 1.83 (0.31) | 0.22 | 0.45 | 0.34 |
| Shallow fine roots:shallow coarse roots | 1.03 (0.17) | 1.10 (0.21) | 1.76 (0.34) | 1.29 (0.14) | 0.11 | 0.22 | 0.18 |
| Shallow fine roots:deep fine roots | 1.96 (0.49)a | 1.82 (0.01)a | 13.07 (4.66)b | 2.63 (0.54)a | N/A | N/A | 0.01* |

Table 5-4. Mean (\pm S.E.) photosynthetic assimilation rates (A ; $\mu\text{mol m}^{-2} \text{s}^{-1}$) of *Prosopis velutina* plants grown under different watering and defoliation treatments. Treatment effects were determined with a 2-way ANOVA model.

| Weeks of treatment | Treatments | | | | Treatment Effects (p-values) | | |
|--------------------------------|------------------|----------------|------------------|----------------|------------------------------|-------------|-----------------------|
| | Wet-undefoliated | Wet-defoliated | Dry-undefoliated | Dry-defoliated | Watering | Defoliation | Watering* Defoliation |
| Week 15 Prior to irrigation | 10.1 (2.6) | 23.1 (2.9) | 15.7 (4.5) | 19.9 (3.4) | 0.74 | 0.03 | 0.25 |
| Week 28 Prior to irrigation | 24.5 (4.4) | 32.9 (3.2) | 23.8 (10.4) | 29.0 (2.6) | 0.74 | 0.34 | 0.82 |
| Week 28 After irrigation | 28.9 (1.8) | 20.3 (11.2) | 28.7 (4.9) | 24.1 (2.1) | 0.24 | 0.64 | 0.77 |

Table 5- 5 Values are the mean percentage of total transpiration water derived from the deuterium-labeled surface pulse for *Prosopis velutina* plants grown under different watering and defoliation treatments. Values in parentheses are ± 1 S.E.

| Weeks of treatment | Treatments | | | |
|--------------------|------------------|----------------|------------------|----------------|
| | Wet-undefoliated | Wet-defoliated | Dry-undefoliated | Dry-defoliated |
| Week 15 | 40.0 (8.3) | 27.7 (14.15) | 38.5 (3.9) | 31.1 (10.7) |
| Week 28 | 44.5 (6.1) | 22.6 (3.5) | 19.9 (4.6) | 16.2 (3.7) |

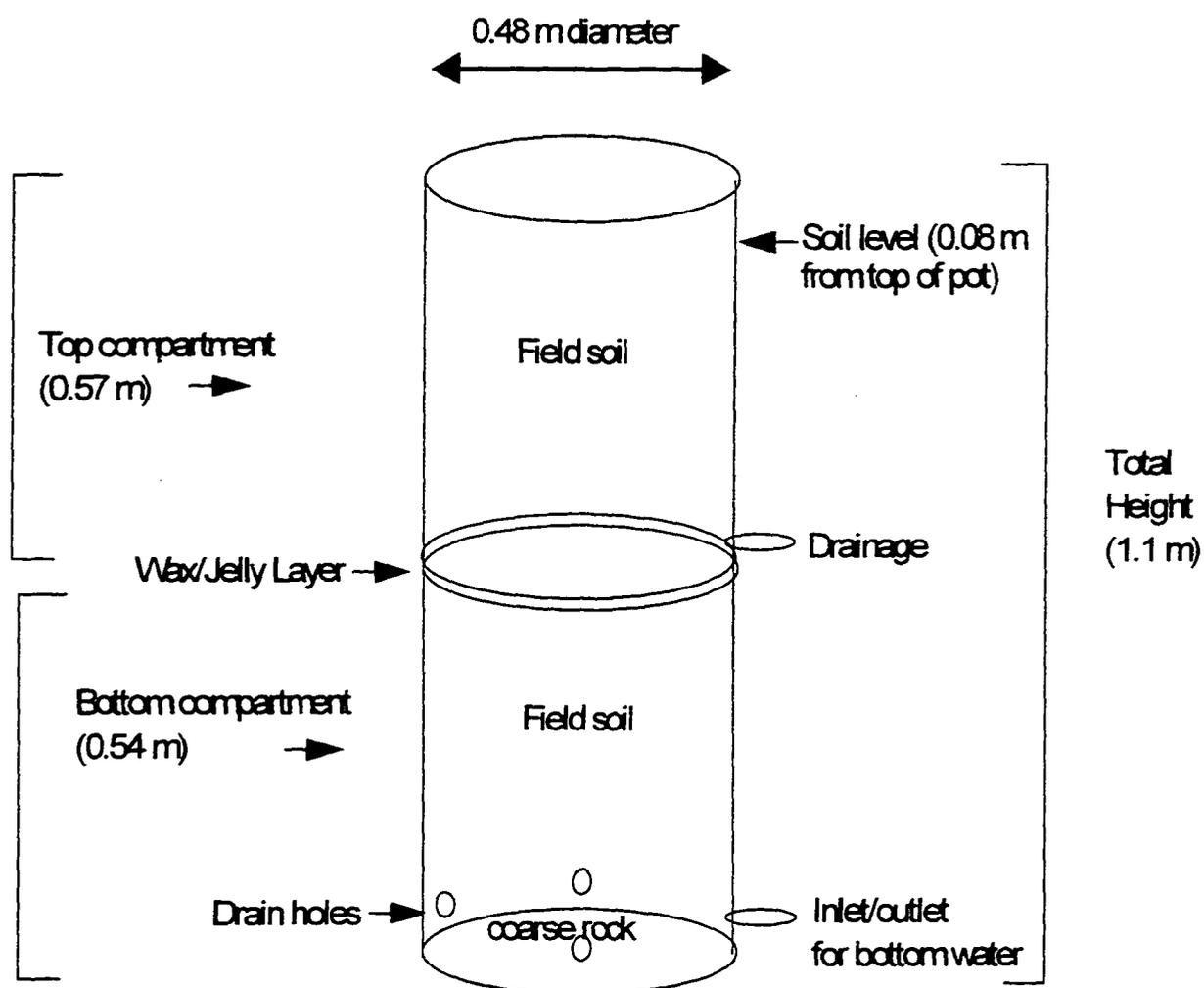


Figure 5-1 A schematic of the pot design used in the glasshouse experiment.

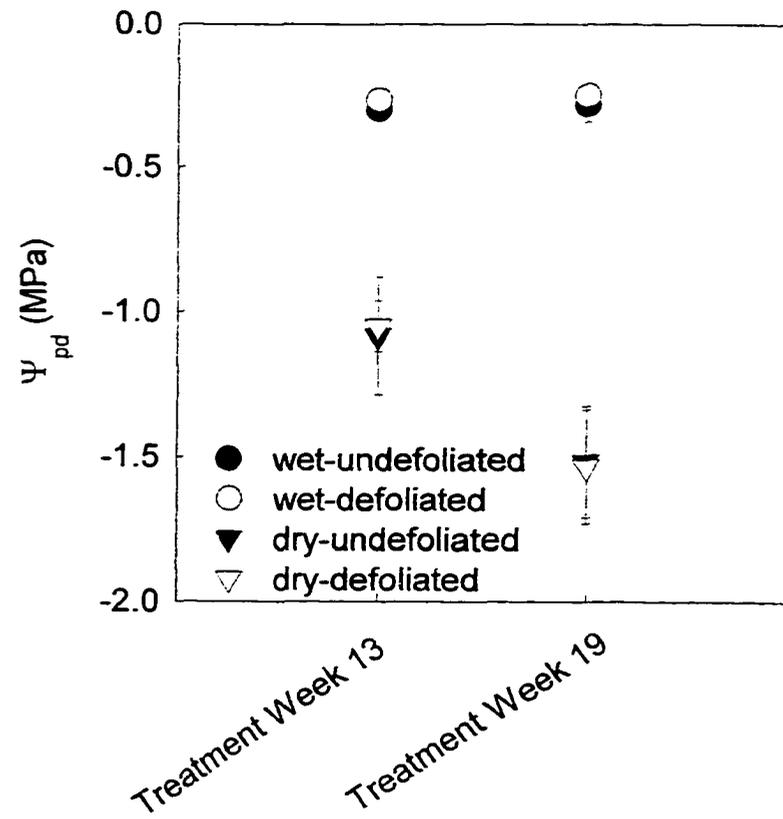


Figure 5-2 Mean predawn leaf water potential (Ψ_{pd}) of *Populus fremontii* for plants grown under different watering and defoliation treatments. Vertical bars represent ± 1 SE of the mean.

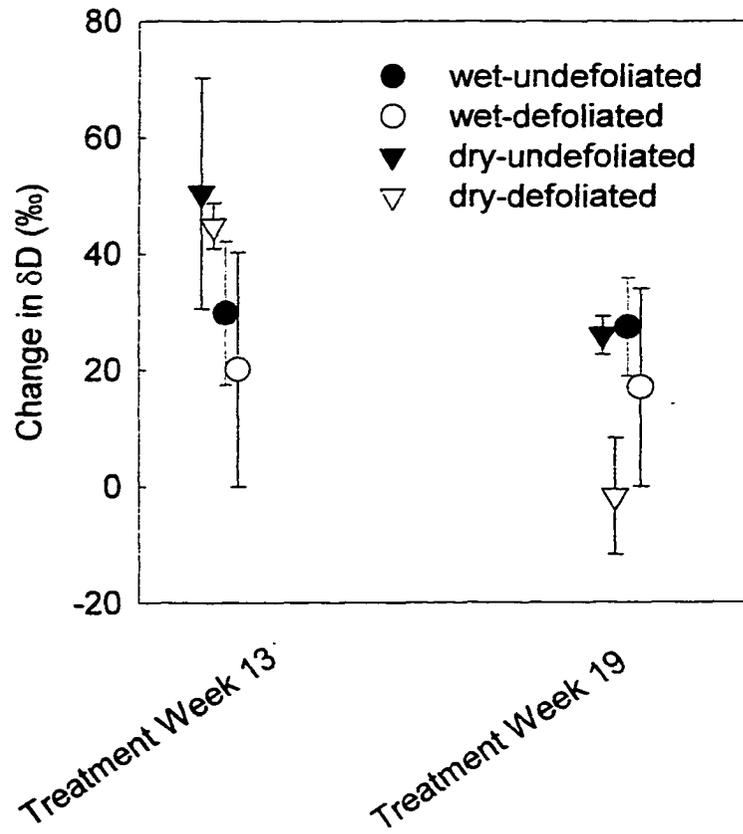


Figure 5-3 The mean change (± 1 S.E.) in δD values of xylem sap of *Populus fremontii* 1 day after surface irrigation with deuterium-labeled water. Plants were grown under different watering and defoliation treatments.

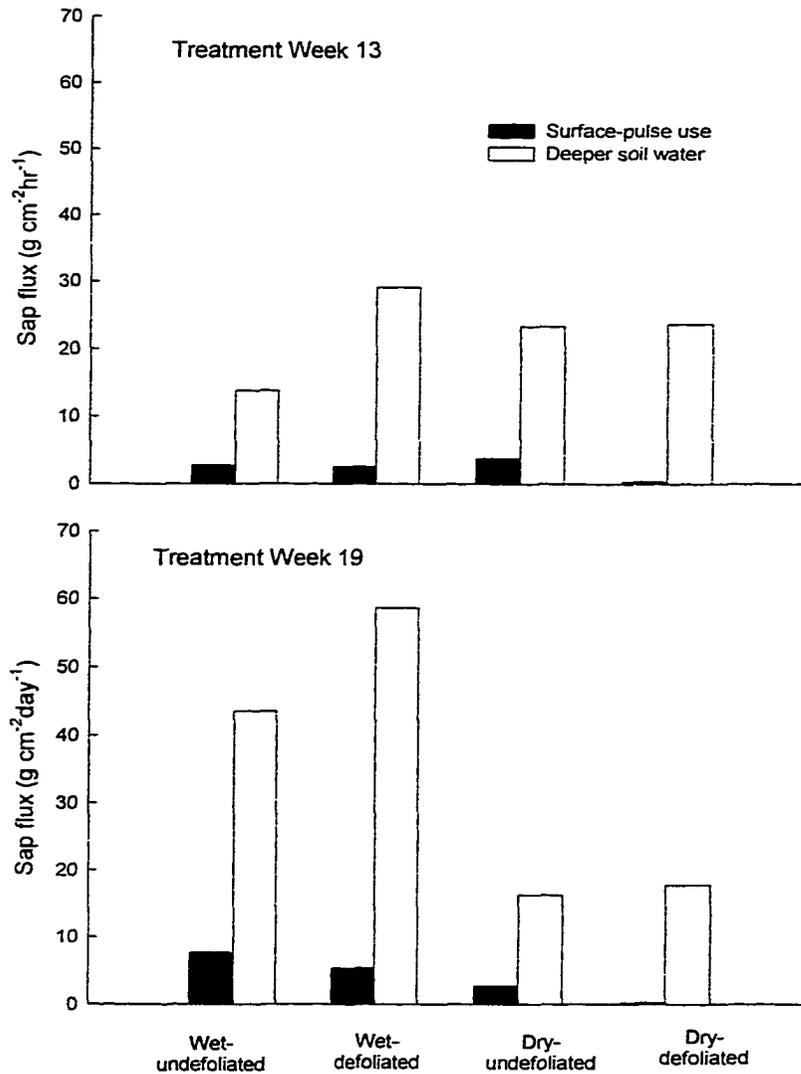


Figure 5-4 Amount of transpiration of *Populus fremontii* derived from the deuterium-labeled surface pulse and from water deeper in the soil. Data are expressed as transpiration flux (grams H₂O per unit sapwood area per unit time) at the scale of single plants. Values were derived by combining averaged rates of water flux at midday (1000-1500-h) with the percentage of water used from deep and shallow layers derived from isotopic data and the 2-compartment-linear mixing model.

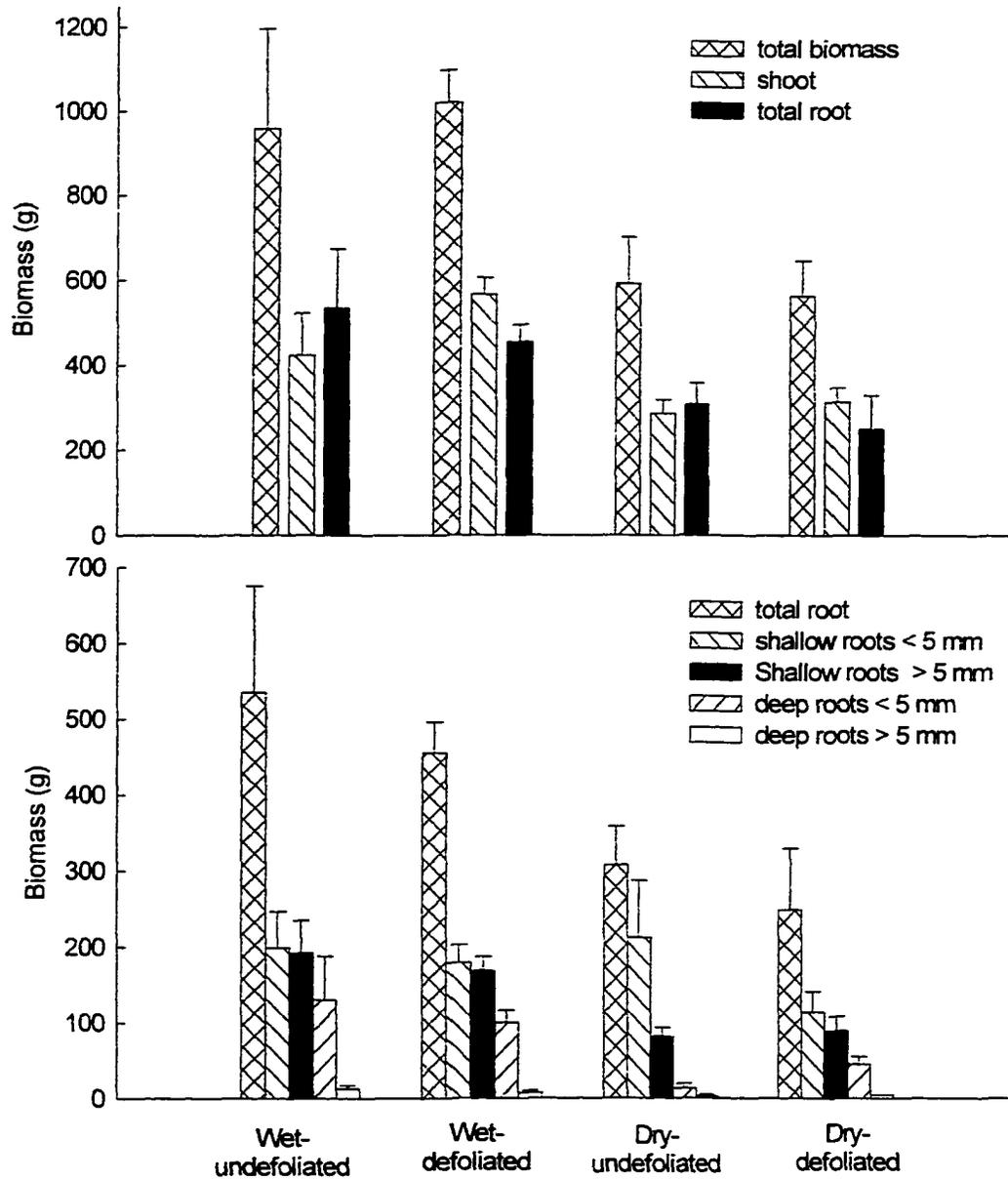


Figure 5-5 Mean biomass (± 1 S.E.) of different plant structures for *Populus fremontii* plants grown under different watering and defoliation treatments. “Shallow” indicates root biomass in the upper half of the pots and “deep” indicates root biomass in the lower half of the pots.

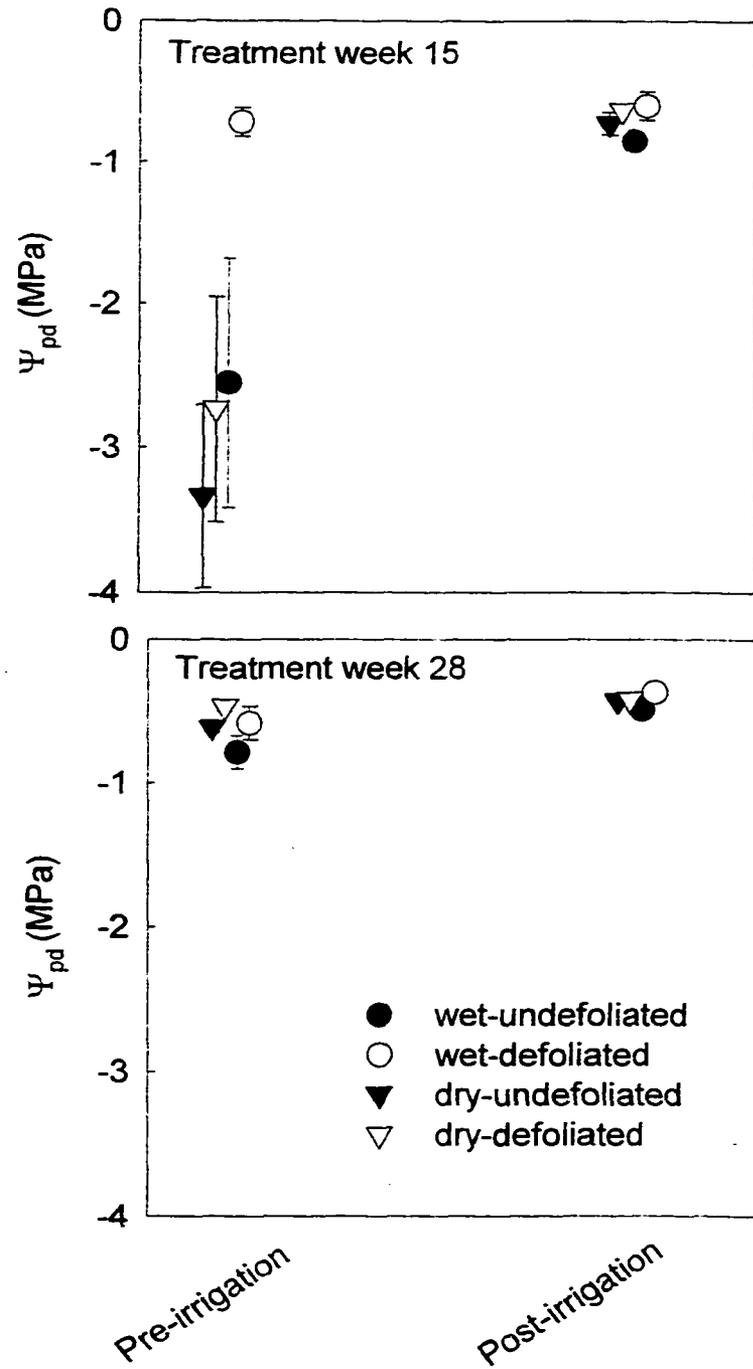


Figure 5-6 Mean predawn leaf water potential (Ψ_{pd}) of *Prosopis velutina* for greenhouse plants grown under different watering and defoliation treatments. Vertical bars represent ± 1 SE of the mean.

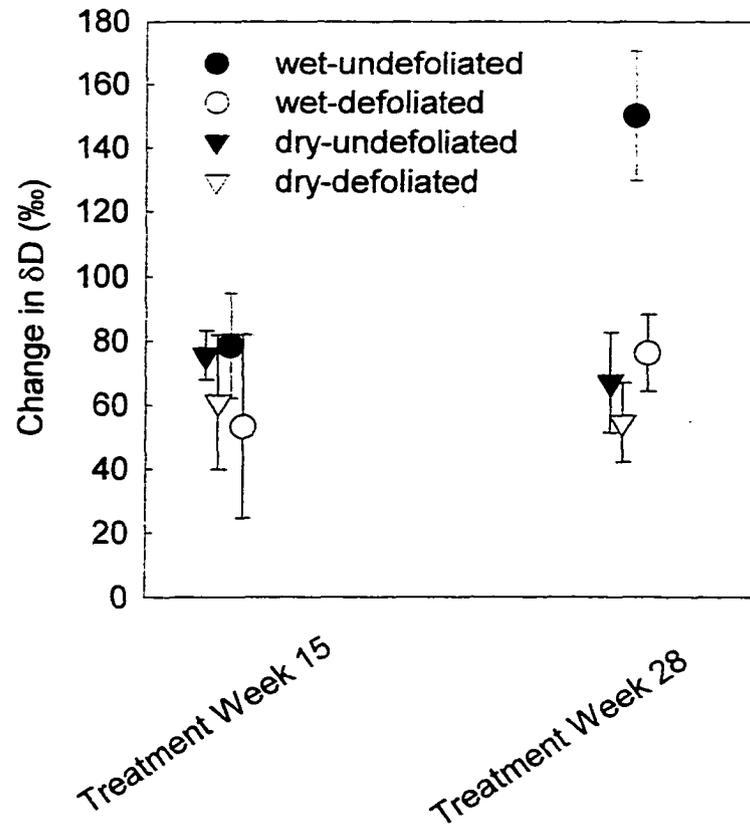


Figure 5-7 The mean change (± 1 S.E.) in δD values of xylem sap of *Prosopis velutina* 1 day after irrigation with deuterium-labeled water at the soil surface. Plants were grown under different watering and defoliation treatments.

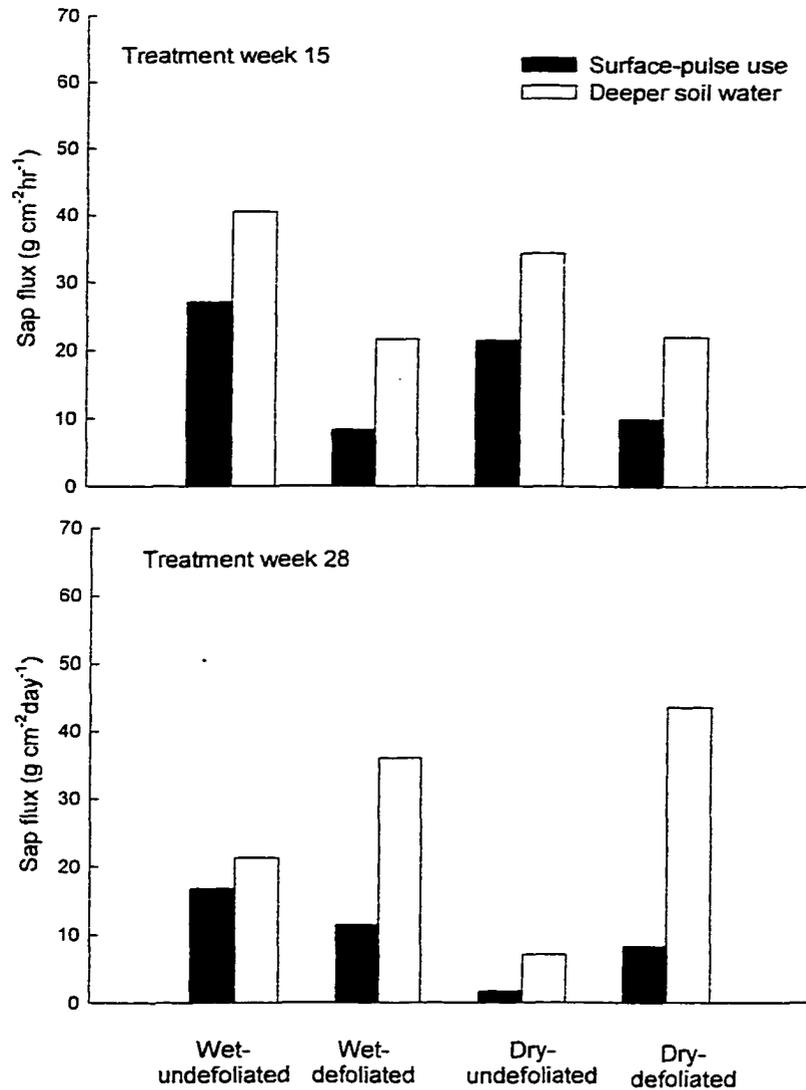


Figure 5-8 Amount of transpiration of *Prosopis velutina* derived from the deuterium-labeled surface pulse and from water deeper in the soil. Data are expressed as transpiration flux (grams H₂O per unit sapwood area per unit time) at the scale of single plants. Values were derived by combining averaged rates of water flux at midday (1000-1500 h) with the percentage of water used from deep and shallow layers derived from isotopic data and the 2-compartment-linear mixing model.

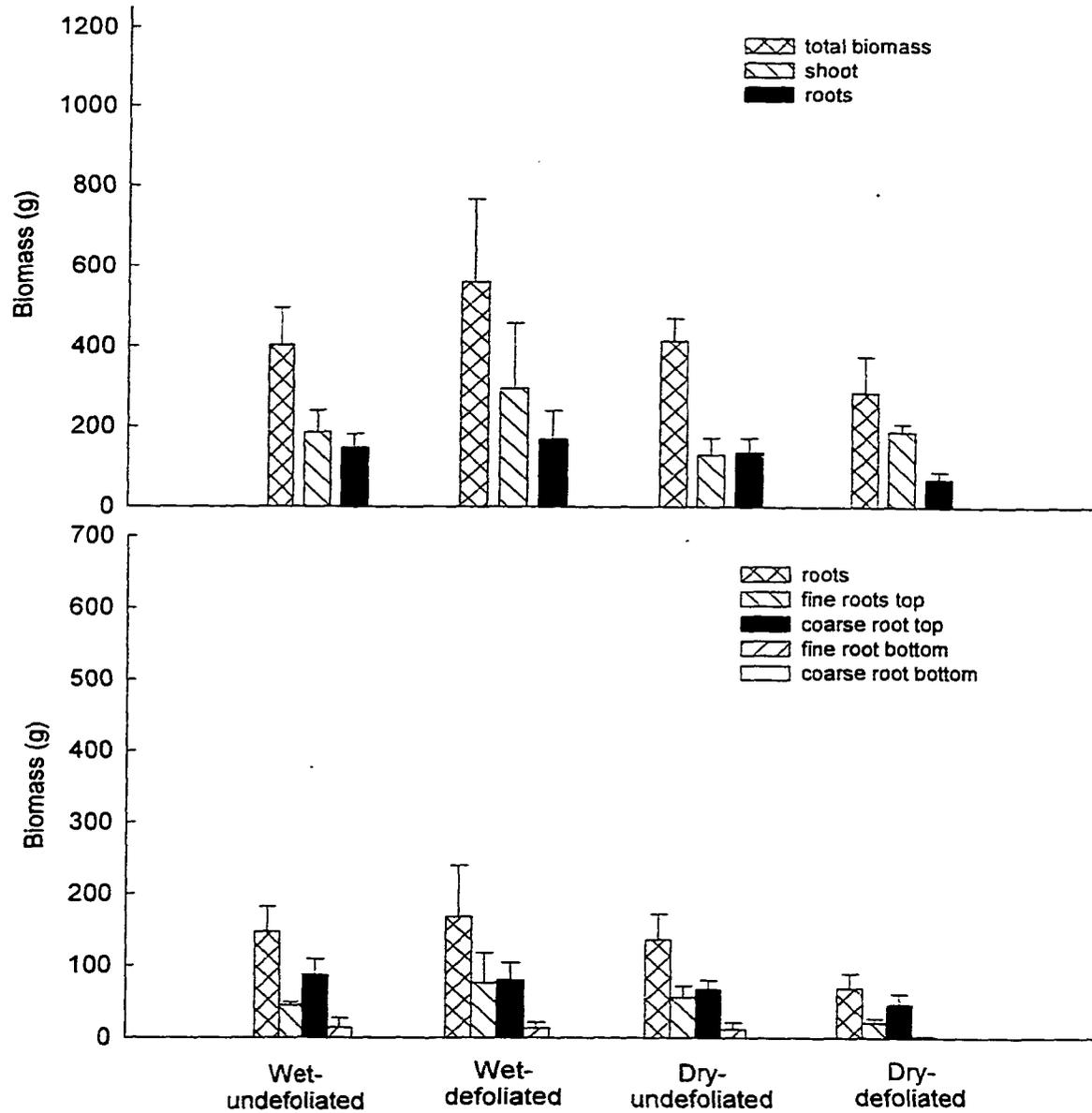


Figure 5-9 Mean biomass (± 1 S.E.) of different plant structures for *Prosopis velutina* plants grown under different watering and defoliation treatments. “Shallow” indicates root biomass in the upper half of the pots and “deep” indicates root biomass in the lower half of the pots.

CHAPTER 6: CONCLUSIONS

SUMMARY

Global climate change, with its suite of potential ramifications to air and soil temperature, regional precipitation patterns, and carbon dioxide levels, is driving the need for better understanding of belowground plant response (Jackson *et al.* 2000). Plants are important regulators of water flux from the soil to the atmosphere, and contemporary treatments of the plant component in hydrologic models are oversimplified (Schulze *et al.* 1996; Dawson and Ehleringer 1998; Jackson *et al.* 2000). This research found substantial intra- and interspecific variation in plant water-source use that has implications for modeling transpiration losses at the ecosystem and larger scales.

The obligate phreatophyte *Populus fremontii* (cottonwood) can use substantial amounts of monsoon rain to augment transpiration demands. Similarly, the facultative phreatophyte *Prosopis velutina* (mesquite) exhibits markedly different amounts of water use from shallow soil layers across different stands, which have similar morphology. This contrasts with other research in which differences in groundwater depth were found to be associated with differences in the morphological stature of mesquite (Stromberg 1993b).

Species of *Populus* and *Salix* are restricted to riparian areas because they are critically tied to shallow groundwater for survival of mature trees (Mahoney and Rood

1990; Stromberg 1993; Scott *et al.* 1999) and streamflow for regeneration of seedlings (Fenner *et al.* 1985; Stromberg and Patten 1991; Freidman *et al.* 1995). However, cottonwood saplings in the current greenhouse study exhibited highly plastic responses to water availability and defoliation. Similarly, mature trees along the San Pedro River used greater amounts of shallow soil water at sites with greater depth to groundwater. These data suggest that in terms of predicting response to future changes in environmental conditions, cottonwood may be more likely to persist than willow under conditions of increased summer rainfall and declining groundwater. However, the rate of groundwater decline may be extremely important for the survival of cottonwood. Shafroth *et al.* (2000) found that the relative change in depth to groundwater overtime, was more important than the absolute depth to groundwater in terms of sapling survival of *Populus fremontii* (see also Scott *et al.* 1999). Responses of this species to rates of groundwater decline are likely to be moderated by soil texture, which influences water holding capacity (Scott *et al.* 1999). In combination with results of the current study, this suggests that this species may be able to adapt to declines in groundwater by redistributing carbon to different soil layers, but that there may be a limit to how quickly trees can redistribute carbon resources. There may also be critical water depths beyond which certain species cannot survive, due to an inability of roots to reach the water table (Graf 1982).

Cover of *Prosopis velutina* has increased an estimated 400% in the San Pedro watershed in the last 30 years (Kepner *et al.* 2000). This species appears to be highly flexible in its use of various water sources, and is capable of existing in xeric habitats

(Stromberg *et al.* 1993). Therefore its continued encroachment into riparian areas typically dominated by cottonwood/willow gallery forests is likely to continue under declining groundwater levels. However, this research suggests that roots of mesquite may be inhibited by saturated or nearly saturated soils. Other research found mesquite rooted primarily in the capillary fringe above the saturated zone (*see review* Stromberg 1993b). Therefore, high groundwater levels that favor cottonwood and willow will likely keep mesquite a sub-dominant species in these systems.

Several conclusions have emerged from this research. Understanding the mechanisms that determine water sources in trees will facilitate restoration and conservation efforts of riparian species, by providing a mechanistic understanding of species response to the spatial and temporal heterogeneity of water availability. This type of understanding will aid in the development of more robust models of water balance. Knowing the amount of plant water use originating from deep or regional groundwater is critical for basin water balances and the application of regional groundwater management models. It is the regional groundwater that is typically subject to possible management through control and regulation of groundwater pumping. On the other hand, it is more difficult to manage the availability of water resources to plants whose primary source of water is derived from precipitation, and these plants are more subject to the variability of climate and weather. In general, water-source use of some species may change in response to variations in depth to groundwater. Additionally, stresses that perturb plant carbon assimilation may also produce changes in water-source use. This suggests that when scaling plant water-source use to community- and ecosystem-levels, variations in

habitat as well as species-specific responses to stress must be considered. Root depth and root distribution are used in global climate change models to determine the terrestrial water balance (Schulze *et al.* 1996). This research indicates that variations in water availability within the rhizosphere, as well as plant regulation of internal carbon pools, may determine whether water within the plant rooting zone is actually used by plants; this has implications for water balance calculations, and for models of plant migration and plant production based on water balance calculations.

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