

SOIL WATER USE AND ROOT SYSTEM
CHARACTERISTICS OF *C. MACULOSA* AND SYMPATRIC PLANTS

by

Megan Cashman Swan

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Jon M. Wraith

Approved for the Department of Land Resources and Environmental Sciences

Jon M. Wraith

Approved for the College of Graduate Studies

Bruce R. McLeod

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Megan C. Swan

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TABLE OF CONTENTS

	Page
1. INTRODUCTION	1
Thesis Objective	3
Literature Review	3
Species	3
Belowground Competition	5
How Plants Use Water	7
Changes in Water Use Efficiency	8
Water Uptake at Critical Periods	10
Morphological Root Plasticity	12
Root Characteristics	13
References	19
2. SOIL WATER UPTAKE BY <i>C. MACULOSA</i> , <i>R. HIRTA</i> AND PERENNIAL GRASSES	
Introduction	24
Methods	28
Study Sites	28
Experimental Design	29
Soil Water Measurements	30
Results	32
Helena Site	32
Site Conditions	32
Soil Water Content	32
Cumulative Changes in Soil Water Storage	33
Leverich Site	36
Site Conditions	36
Soil Water Content	36
Cumulative Changes in Soil Water Storage	37
Discussion	37
Site Conditions	37
Soil Water Content	40
Cumulative Changes in Soil Water Storage	42
Competitive Effects	42
References	46

TABLE OF CONTENTS-CONTINUED

3. ROOT SYSTEM CHARACTERISTICS OF <i>C. MACULOSA</i> AND <i>R. HIRTA</i> IN RESPONSE TO TWO SOIL WATER REGIMES	50
Introduction	50
Methods	52
Species	52
Treatments	52
Harvest	53
Results	55
Total Biomass and Root Mass Ratio	55
Root Mass	55
Root Length	57
Root Length Density	57
Surface Area	59
Root Volume	59
Specific Length	62
Root Diameter	63
Discussion	66
Biomass	66
Root Mass Ratio	68
Root System Size	68
Root System Efficiency	70
Root Diameter Classes	70
References	73
4. SUMMARY	78
APPENDICES	81
Appendix A: Soil Profiles for Field Study Sites	82
Helena Site	83
Leverich Site	84

TABLE OF CONTENTS-CONTINUED

Appendix B: ANOVA Tables referenced in Chapter 2	85
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Soil water content ANOVA p-values for depletion period (DOY 181-262) of both study years at the Helena site.	86
Soil water content ANOVA p-values for depletion period (DOY 181-262) of both study years for the Leverich site	87
ANOVA p-values for cumulative change in soil water storage by depth for species, day of year (DOY) and the interaction of species and DOY effects for Helena	88
ANOVA p-values for cumulative change in soil water storage by depth for species, day of year (DOY) and the interaction of species and DOY effects for Leverich	89
Appendix C: ANOVA Tables Referenced in Chapter 3	90
ANOVA p-values for whole pot effects	91
ANOVA p-values with depth as factor for depth effect and depth interactions	91

LIST OF TABLES

Table

Page

2.1 Monthly and total annual precipitation for study years. Long term average (LTA) is based on 100-year means. Data collected at nearest weather station (WRCC, 2004) 33

LIST OF FIGURES

Figure

Page

2.1 Seasonal changes in θ (mean \pm SE) by soil depth increment for perennial grasses (GRASS), *R. hirta* (RUHI) and *C. maculosa* (CEMA) in 2002 and 2003 at Helena 34

2.2 Seasonal changes in soil water storage (mean \pm SE) by soil depth increment for perennial grasses (GRASS), *R. hirta* (RUHI) and *C. maculosa* (CEMA) in 2002 and 2003 at Helena 35

2.3 Seasonal changes in θ (mean \pm SE) by soil depth increment for perennial grasses (GRASS), *R. hirta* (RUHI) and *C. maculosa* (CEMA) in 2002 and 2003 at Leverich 38

2.4 Seasonal changes in soil water storage (mean \pm SE) by soil depth increment for perennial grasses (GRASS), *R. hirta* (RUHI) and *C. maculosa* (CEMA) for 2002 and 2003 at Leverich 39

3.1 Root mass (mean \pm SE) for *C. maculosa* (CEMA) and *R. hirta* (RUHI) for all soil depth increments. Letters indicate significant differences 56

3.2 Root mass (mean \pm SE) for dry and wet soil water treatments for all soil depth increments. Letters indicate significant differences 56

3.3 Root length (mean \pm SE) for *C. maculosa* (CEMA) and *R. hirta* (RUHI) for all soil depth increments. Letters indicate significant differences 58

3.4 Root length (mean \pm SE) for dry and wet soil water treatments for all soil depth increments. Letters indicate significant differences 58

3.5 Root surface area (mean \pm SE) for *C. maculosa* (CEMA) and *R. hirta* (RUHI) for all soil depth increments. Letters indicate significant differences 60

3.6 Root surface area (mean \pm SE) for dry and wet soil water treatments for all soil depth increments. Letters indicate significant differences 60

3.7 Root volume for *C. maculosa* (CEMA) and *R. hirta* (RUHI) for all soil depth increments. Letters indicate significant differences 61

LIST OF FIGURES-CONTINUED

3.8 Root volume (mean \pm SE) for dry and wet soil water treatments for all soil depth increments. Letters indicate significant differences 61

3.9 Specific root length for *C. maculosa* (CEMA) and *R. hirta* (RUHI) for all soil depth increments. Letters indicate significant differences 62

3.10 Length of root (mean \pm SE) in each diameter class for *C. maculosa* (CEMA) and *R. hirta* (RUHI) 64

3.11 Length of root (mean \pm SE) in each diameter class for wet and dry soil water treatment 64

3.12 Mean length of root (\pm SE) or each diameter class for each soil depth increment 66

ABSTRACT

Centaurea maculosa Lam. (spotted knapweed) is an introduced perennial forb that invades disturbed and undisturbed semiarid grasslands in the western United States. It forms dense monocultures and reduces native and desirable vegetation. We

hypothesized that *C. maculosa* may succeed through a superior ability to access soil water. This study had two objectives, to compare soil water uptake patterns under *C. maculosa*, perennial grass, and *R. hirta* monocultures in the field on semiarid rangeland, and to compare root characteristics of *C. maculosa* and *R. hirta* plants grown under two soil water regimes in the greenhouse. For the first objective, we used two field sites in western Montana. We created four blocks of adjacent monoculture plots of three species (perennial grasses, *C. maculosa* and *R. hirta*) arranged in a randomized complete block design. Soil water dynamics were monitored throughout the growing season at six hour intervals in the upper 0.3 m of the soil profile using TDR, and weekly to biweekly at greater depths using a neutron moisture meter. *Centaurea maculosa* used more water than resident grasses when averaged over the soil profile and had greater water use than grass at greater depths later in the growing season. *R. hirta* displayed similar soil water uptake to *C. maculosa* in the second year of our study. There was no “carry-over effect” of low soil water contents at deep soil depths from the end of the first growing season to the beginning of the next. To address our second objective, we grew 10 individuals of *C. maculosa* and *R. hirta* under either “dry” or “wet” soil water conditions for 3 months in the greenhouse, then extracted, cleaned, stained and scanned their roots to quantify several root characteristics. *Centaurea maculosa* had lower root mass, root length, specific length, root length density and greater average root diameter than *R. hirta* under both soil water regimes. *Centaurea maculosa* had higher root mass ratios than *R. hirta*, but this may have been due to phenological differences at time of harvest or differences in nitrogen utilization. Our results suggest that *C. maculosa* may invade and persist in western rangelands due to its ability to take up soil water unavailable to native grasses which allows it to continue growth and photosynthesis late in the growing season. However, similar water uptake patterns and greater total root system size and efficiency of the non-invasive *R. hirta* indicate that *C. maculosa* does not have unique characteristics indicating superior belowground competitive ability, but may in fact share traits common to other late season tap-rooted forb species.

CHAPTER 1

INTRODUCTION

Invasion of ecosystems by non-native species rivals climate change and habitat loss as the greatest negative effect humans are having on the environment (Vitousek et al. 1996). Once considered an issue only on island habitats, every continent in the world is now subject to invasion by alien species of insects, plants and animals. The percentage of vascular plant species that are present in, but not native to various regions throughout the world ranges from 1% to as much as 64% of total species present (Vitousek et al. 1996).

Alien plant species may be beneficial or relatively innocuous in their effect, but a small percentage become serious pests. These species may reduce native biological diversity, threaten the health of native ecosystems, or contribute to economic losses (Vitousek et al. 1996). Controlling these invasions often proves difficult. There is no set formula to predict “invasiveness”, so generally we identify problematic plant species only after they have become firmly established.

Centaurea maculosa Lam. is a perennial forb native to central Eurasia (Boggs and Story 1987). Since its introduction to North America in the early 1900s as a contaminant in alfalfa seed, *C. maculosa* has successfully invaded 326 counties in the western United States, including every county in Idaho, Washington, Wyoming and Montana (Sheley et al. 1998; Whitson et al. 1996). It forms dense, monotypic stands, reducing native flora and diminishing range quality for livestock and wildlife (Watson and Renney 1974).

Centaurea maculosa is not limited to invasion of disturbed sites, but readily spreads into undisturbed habitats as well (Tyser and Key 1988).

As the cost of agricultural losses attributable to *C. maculosa* have increased (\$4.5 million estimated in 1987; Boggs and Story 1987), so has interest in determining the mechanism(s) by which *C. maculosa* invades and dominates native plant communities. Many resultant studies take the form of observation/description and fail to address the underlying physiology of the species (e.g., Boggs and Story 1987; Jacobs and Sheley 1998; Lacey et al. 1990; Tyser and Key 1988). However, lack of natural predators, above and belowground, prolific seed production, allelopathy and specific germination factors that increase the probability of survival, along with the vague but commonly used phrase “superior competitive ability”, are often cited to explain *C. maculosa*’s success (Bais et al. 2003; Callaway et al. 2004; Eddleman and Romo 1988).

Morphology and phenology differ between *C. maculosa* and the bunchgrasses native to the rangelands that are being invaded. *Centaurea maculosa* is a tap-rooted perennial forb. It has a two stage life cycle, generally remaining a rosette for its first year and bolting in subsequent years. It begins growth early in the spring, and typically flowers from July through September. Native bunchgrasses are fibrous-rooted, with the bulk of root systems distributed in the upper 0.4 m of the soil, although maximal depth is generally much greater under favorable conditions (Coupland and Johnson 1965).

Because belowground competition is the primary form of plant competition in semi-arid environments, and soil water is an important limiting resource in these environments, competitive interactions between these species for soil water warrants

study (Casper and Jackson 1997; Fowler 1986). Differences in soil water uptake due to phenology, plasticity, divergent rooting zones or greater uptake capacity could be responsible for shifting the competitive balance in grassland ecosystems towards *C. maculosa*.

Thesis Objective

The objectives of this thesis were: 1) to determine spatial and temporal soil water uptake patterns of *C. maculosa*, the naturalized forb *Rudbeckia hirta*, L.(black-eyed susan), and perennial grasses under ambient soil moisture conditions; and 2) to compare selected root system attributes of *C. maculosa* and *R. hirta* in response to wet or dry soil water conditions.

Literature Review

Species

Centaurea maculosa (spotted knapweed) is a perennial tap-rooted forb native to Eurasia. Its presence in North America was first noted on Vancouver Island in 1905 (Roche and Talbot 1986). Currently it occurs in every state in the U.S. except Alaska, Georgia, Mississippi, Oklahoma and Texas (<http://invader.dbs.umt.edu>), but is most problematic in the semi-arid grasslands of Montana, Wyoming, Idaho and Washington (Sheley et al. 1998).

Centaurea maculosa is a tap-rooted member of the Asteraceae family. It germinates in late fall or early spring and generally spends its first year as a rosette.

Greatest root development occurs during this stage (Watson and Renney 1974). Plants bolt in mid-May their second year and may have as many as fifteen flowering stalks per plant. Flowers are light purple and generally begin blooming in July (Watson and Renney 1974). Plants produce 5,000 to 40,000 seeds per m² (Sheley et al. 1998). *Centaurea maculosa* plants can live up to nine years, and are capable of producing seed every year (Boggs and Story 1987).

This species tends to form dense, monotypic stands and reduces native plant cover and diversity (Tyser and Key 1988; Watson and Renney 1974). *Centaurea maculosa* is capable of invading disturbed and undisturbed ecosystems and reduces forage for wildlife and livestock (Tyser and Key 1988; Watson and Renney 1974).

Pseudoroegneria spicata (Pursh.) Löve (bluebunch wheatgrass), a cool season grass, was once a dominant species at warm, relatively dry sites throughout the Pacific Northwest (Weaver 1919). This species typically flowers in June, and often becomes quiescent during the hot, dry late-summer period. It is capable of renewed growth in the fall if soil water conditions permit (Weaver 1919). *Pseudoroegneria spicata* possesses a fairly coarse root system and roots are capable of reaching depths as great as 1.2 m, although highest root densities are generally concentrated in the upper portions of the soil profile.

Rudbeckia hirta L. (black-eyed susan), is a short-lived perennial member of the Asteraceae family. Native to the Midwestern United States, it is typically a weedy species, often colonizing disturbed areas and roadsides. Similar to *C. maculosa*, it is a late season, deeply tap-rooted species that typically spends its first year as a rosette, and

flowers during its second year. *Rudbeckia hirta* is not native to Montana, and although its phenology and morphology closely match *C. maculosa*, this species has not become invasive in semi-arid grasslands. *Rudbeckia hirta* was chosen as an additional comparison species to help determine whether *C. maculosa* belowground traits are in fact similar to related forbs. Similar traits would indicate that belowground competition may not be a primary factor in *C. maculosa*'s success in western grasslands.

Belowground Competition

The rooting system of *C. maculosa* differs morphologically from fibrous-rooted native grasses. Morphological differences between co-existing species suggest the species may access different resources, reducing the potential for competition (Pickett and Bazzaz 1976). Different root growth patterns among species are considered an important method of partitioning soil resources, but are usually traits of species that have evolved closely over centuries, and do not lead to competitive exclusions (Parrish and Bazzaz 1976). Furthermore, it is likely that *C. maculosa* and perennial grasses have spatial overlap in soil resources, based on root morphology (Marler et al. 1999), and also because of the negative interference which apparently occurs between these species. *Centaurea maculosa* has roots in the near-surface soil horizons, and perennial grass species likely use subsoil water, despite their apparent dominance in the surface soil layers (Knoop and Walker 1985; Marler et al. 1999).

Resource competition is most generally viewed as negative interference between neighboring plants (Fowler 1986). In their review, Casper and Jackson (1997) stress the importance of studying resource uptake and the associated consequences for the affected

plant when considering competition. Disparities in plant performance or greater ability to exploit a soil resource are not necessarily correlated with competition, but could be linked to other differences such as resource partitioning or use-efficiency (Casper and Jackson 1997; Parrish and Bazzaz 1976). Using their definition, belowground competition occurs when a plant reduces the level of some soil resource, which results in reduced growth, survival or fecundity of neighboring plants (Casper and Jackson 1997). Competition belowground is usually more important than shoot competition in determining competitive balance, especially in arid or semi-arid lands, such as western Montana grasslands (Casper and Jackson 1997; Fowler 1986).

At the most basic level, plants compete belowground through two mechanisms: preemption and depletion. Preemption occurs when one plant exploits soil resources more quickly than another, leaving insufficient amounts of the resource available for the competing plant to complete its life cycle (Tilman 1982). Depletion is the term used when one plant reduces soil resources below the level necessary for its competitor's growth and survival (Tilman 1982). However, these are general mechanisms and the possibilities for indirect or additive interactions between plant roots are limitless (Caldwell 1987).

Soil water is frequently identified as a limiting resource in arid- and semiarid environments, hence, competition for soil water may be an important interaction among plants in these environments (Parrish and Bazzaz 1976). Water has been identified as a critical resource in the dominance of other invasive species (Allen 1982; Harrington 1991; Williams and Hobbs 1989), and it is possible that *C. maculosa* may gain a

competitive edge over grasses by accessing more soil water in time and/or space.

How Plants Use Water

Water uptake in plants is driven primarily by transpiration (only a small amount is growth-induced and osmotically driven) (Sperry et al. 2002). Water moves through the soil to the root via mass flow, is taken up by the roots and pulled through the xylem of the plant due to differences in water potential between the soil, root and atmosphere (Slatyer 1967). The zone of most rapid absorption lies behind the meristematic root tip region (usually 5-10 cm from root tip) (Sperry et al. 2002). The absorbing zone moves through the soil with growth of the root system, and is characterized by development and age more than any other factor. Length of the absorption zone varies depending on root growth rate. Rates of water uptake are affected by root diameter, distance from stem base, age or stage of development, and soil water and temperature conditions (Kramer 1983).

Root hairs may play an important role in water absorption, as they occur most frequently in the zone of absorption, and may dramatically increase surface area for uptake. Secondary growth reduces the root surface area available for absorption, as does suberization. However, suberized roots can still absorb significant quantities of water, and may increase efficiency of transport (Kramer 1983).

In soils near field capacity (0.01 MPa), water moves relatively freely by mass flow towards the root surface, and low density roots can take up sufficient amounts of soil water. However, in dry or drying soils, matric forces become stronger and water movement through the soil becomes limited. In these circumstances, close root contact with individual water-filled soil pores becomes important for gaining adequate water for

transpiration thereby increasing the value of very fine roots. While there are many difficulties in measuring root function in-situ over time, through-time measurement of soil volume water content during drying phases can be used to provide information on plant water uptake (Fernandez et al. 2000).

Plants living in semiarid grasslands use several strategies to gain sufficient soil water. Cool season annuals, such as the invasive *Bromis tectorum* L., concentrate most resources in aboveground biomass and lack deep root systems. However, these species grow and reproduce very quickly, completing their life cycle before the upper regions of the soil dry out (Mulroy 1977). Shallow-rooted grass species, such as *Bouteloua gracilis* H. B. K. Lag., also concentrate roots in upper soil layers. This species is adept at utilizing small rainfall events common in semiarid environments, which wet only the uppermost soil layer, to continue photosynthesis (Sala 1982). Phreaophytic species rely on a deep tap-root to provide access to a relatively stable water source at greater depths in the soil profile.

Changes in Water Use Efficiency

Water use efficiency (WUE) is an important indicator of a plant's adaptation to water limitation and can be defined as the ratio of dry matter produced per unit water uptake (Larcher 2003). While greater WUE in semiarid environments would suggest a competitive advantage, *C. maculosa* did not have greater WUE than native grasses, nor did it generally use more water in a greenhouse study (Blicker et al. 2003); however, this may not be the case during periods of water stress in the field (Judson Hill, pers. communication). Therefore, temporal variations in WUE could be an important factor in

C. maculosa's success.

Centaurea maculosa's WUE was lowest when plants were in the rosette stage, and highest in flowering individuals (Blicker et al. 2003). These changes in WUE coincide with the typical soil water patterns in western grasslands. Rosettes are formed in the early spring or late fall when water availability is high, and bolting occurs in the dry mid- to late summer period (Watson and Renney 1974). High water use when water is most available, followed by continued growth and reproduction during the driest periods of the summer, could allow *C. maculosa* to preempt water from native grasses, which begin growth later in the spring, flower and then become quiescent as water availability decreases in the late summer.

Differences in WUE between two common European pasture crops (*Trifolium repens* L. (white clover) and *Lolium perenne* L. (perennial ryegrass)) have been used to explain the shift in competitive dominance from white clover to perennial rye during periods of drought. Rye is capable of increasing its WUE during dry periods, reducing water uptake while continuing to grow, which causes a shift in pasture composition to rye dominated systems during dry years (Lucero et al. 2000). However, whether the rate of *C. maculosa* invasion increases during dry years has not been documented in the literature. Furthermore, the value of greater WUE during the late summer in determining competitive balance is questionable, since native grasses are typically quiescent during that period, and competitive interactions therefore are at a minimum.

Water Uptake at Critical Periods

Critical periods of water availability are linked to invasion. The survival of the late season annual, *Centaurea solstitialis* L., was tested in plots with total or partial representation of four plant functional groups: annual grasses, perennial grasses, early season forbs and late season forbs (Dukes 2001). *Centaurea solstitialis* invasion was greatest in perennial grass monocultures, and least in monocultures of another late season forb (*Hemizonia congesta*) and plots containing all four functional groups. The availability of soil resources was more important than the presence or absence of functional groups. Plots with high soil water availability in the midsummer were most susceptible to invasion; those containing other summer active, deeply-rooted species were most resistant (Dukes 2001).

Competitive dominance through preemption of soil water early in the spring has been used to explain competitive interactions between desert and crested wheatgrasses (*Agropyron desertorum*, [Fish.] Schult, *A. cristatum* [L.] Gaertn.), two perennial, non-native grass species that are also successful in the semiarid grasslands of the western United States (Eissenstat and Caldwell 1988a). Recruitment of a perennial knapweed species, *Centaurea diffusa* Lam.(diffuse knapweed), was greatly inhibited, almost to the point of exclusion, in plots that had been treated to kill existing diffuse knapweed, and then seeded with crested wheatgrass (Berube and Myers 1982). Presumably crested wheatgrass, which also starts growth very in early spring, was able to preempt soil water at a critical period and thereby prevent knapweed establishment. *Agropyron desertorum*

has a similar inhibitory effect on *Bromus tectorum* survival, further supporting this hypothesis (Eissenstat and Caldwell 1988a).

In western grasslands, the greater availability of soil water during early spring may create conditions favorable for *C. maculosa*. *Centaurea maculosa* develops fine roots earlier in the spring than *Pseudoroegneria spicata* (Marler et al. 1999), and *C. maculosa* rosettes green up earlier than either *Festuca idahoensis* or *P. spicata* (pers. obs.). This may allow *C. maculosa* to preempt soil water, depleting soil water at shallow depths that would otherwise be used by native grasses as they begin to grow in early summer. Similarly, the dominance of *Bromus tectorum* over *P. spicata* can be attributed to early and rapid proliferation of root growth at low temperatures in the early spring by *B. tectorum* (Harris 1967). Competition from *B. tectorum* reduced the rate of elongation of seedling roots of *P. spicata* by depleting soil water reserves ahead of *P. spicata* root apices. *Bromus tectorum* gained a competitive advantage by rapidly elongating its roots and using soil water during the cool winter months, while growth of *P. spicata* was inhibited by the dry soil (Harris 1967).

Alternatively, *C. maculosa* may use more water later in the season, after the native grasses have become quiescent (Sperber 2001). This may result in reduced available soil water for plants at the start of the next growing season in dry years, when complete soil water recharge does not occur (Sperber 2001). This would suggest that *C. maculosa* invasion and population growth might be greater during drought years, but this has not been investigated.

Morphological Root Plasticity

In addition to changes in water use efficiency or exploitation of critical periods of soil water availability, *C. maculosa* could be altering root morphology to access more soil water. Phenotypic plasticity is an individual plant's ability to alter an aspect of its physiology or morphology in response to changes in environmental factors (Schlichting 1986). Plastic individuals are often better competitors in temporally variable environments (Jackson and Caldwell 1989). In the semi-arid rangelands of the intermountain western United States, water status of soils can vary greatly in space and time. Many plants quickly exploit areas of enhanced water availability through plastic growth responses, changing root form in response to changing environmental conditions (Wraith and Wright 1998).

Phenotypic plasticity in spatial positioning of roots is critical to soil resource acquisition (Fitter 1994), and has been intensively studied in response to nutrient heterogeneity (Elberse et al. 2003; Sultan and Bazzaz 1993; Yamauchi and Yamamura 2004). Root morphology is generally more important than physiological factors in nutrient uptake (Silberbush and Barber 1983), and the timing and location of root growth are of particular importance (Eissenstat and Caldwell 1988b). Dicot roots tend to be more plastic than grass roots, and morphological plasticity has been linked to the success of other invasive species (Schweitzer and Larson 1999; Taub and Goldberg 1996).

Plants can change their water uptake patterns through time. The native Australian tree, *Banksia prionotes* Lindl. has a dimorphic approach to soil water uptake (Dawson and Pate 1996). Percent of water in the plant that was absorbed through the tap-root was negatively correlated with the amount of precipitation that fell on the site. This indicates

a change in reliance from tap- to shallower lateral roots dictated by soil water conditions (Dawson and Pate 1996). A similar strategy of plastic root modification, extending shallow roots when surface water is abundant, and relying on its tap-root to reach deep water as the soil surface dries, might give *C. maculosa* a competitive advantage over native species by allowing it to deplete soil water resources to the detriment of the native grass populations.

Root Characteristics

Roots exist to extract water and nutrients from the soil and to anchor the plant. However, roots are incredibly varied structures and the physiological and ecological implications of differences in root system parameters between plants and plant species is not well understood (Atkinson 2000). Recent advances in our abilities to derive quantitative root measures have increased interest in the study of plant roots. In particular, root image analysis systems have vastly reduced the time required to measure morphological root characteristics, and have substantially increased the accuracy of these measurements (Richner et al. 2000).

Certain root characteristics provide direct links to function whereas other characteristics are derived from multiple measurements (Atkinson 2000). Root length, volume, and weight are commonly used to estimate overall root system size (Atkinson 2000). Root length density (length of root per volume of soil) may be used to infer soil exploitation abilities (Atkinson 2000). Specific root length (root length associated with a unit dry weight) is a good indicator of efficiency as it measures possible soil exploited (root length) per unit biomass (Atkinson 2000). Average diameter of roots in a given root

system also indicates rooting efficiency, as smaller diameter roots are able to exploit more soil while requiring less carbon energy input from the plant (Casper and Jackson 1997).

Semiarid rangelands are relatively infertile environments. Water is frequently scarce, therefore nutrient availability likely has a strong temporal element. On western North American semiarid grasslands, soil water and nutrients are more likely to be available in the spring, and in brief pulses after summer rainstorms. Under these conditions, a long-lived, extensive root system would likely have an advantage (Grime 1977).

Knowledge of the rooting patterns of *C. maculosa* is critical to understand the mechanisms through which *C. maculosa* may compete for resources. Although it is commonly classified as a “deeply tap-rooted species” (Jacobs and Sheley 1998; Sheley et al. 1998), actual quantitative descriptions of *C. maculosa* rooting patterns are nearly absent from the literature. Architectural analyses of root systems are becoming more common, and can potentially help elucidate root behavior in response to changing conditions (Fitter and Strickland 1992).

Marler et al. (1999) investigated spatial rooting patterns of *C. maculosa* in the top 60 cm of the soil using a root periscope and found that fine root densities were similar to *Pseudoroegneria spicata* when averaged over 60 cm, but differed significantly with depth within that increment. *Centaurea maculosa* dominated the lower portion, having four

times the root density of bluebunch at 45-60 cm. Both invader and native species' roots responded similarly to environmental fluctuations at shallow depth.

Centaurea maculosa has water uptake patterns similar to fibrous-rooted grasses at shallow depths (5-30 cm) (Sperber 2001). These results are surprising based on the presumed differences in root form between *C. maculosa* and the native bunchgrasses. Tap-roots are usually relatively thick with low specific surface area, and are generally considered to be effective at utilizing soil resources located deep in the profile. However, tap-rooted plants may be limited in the volume of soil they can access, especially at shallower depths. In contrast, many grasses have fibrous, herringbone rooting systems with high specific and total surface areas capable of mining large volumes of soil, but are generally concentrated in the upper layers of the soil (Weaver 1919). That these species would have similar water uptake patterns at shallow soil depths highlights our limited knowledge of the root system of *C. maculosa* in various soil environments, and underscores the need for further study. The ability to alter root system morphology to maintain function and growth when soil resources are limiting may be a key aspect of an individual's adaptive phenotypic plasticity (Grime 1994), and could be important for competitive interactions.

Root plasticity levels are linked to precision in foraging for nutrients. Species differ in timing, precision and magnitude of root responses to nutrient-enriched patches (Eissenstat and Caldwell 1988b; Jackson and Caldwell 1989). Root proliferation of the exotic grass, *Agropyron desertorum* (Fisch.) Schult., allowed it to quickly exploit nutrient patches, but this response varied seasonally. Two native species were also able to

increase relative (root) growth rates in fertile microsites during periods when the plants were actively growing (Eissenstat and Caldwell 1988b).

Maximizing efficiency (energy input per unit soil accessed) of soil exploitation limits potential for exploration of soil resources (Berntson 1993). Large root systems have higher levels of exploitation but lower exploitation efficiency, whereas small systems have lower potential but higher efficiency. This is relevant to competition between *C. maculosa* and perennial grass species. Bunchgrass root systems display a herringbone topology which is considered the ideal in exploitation potential, however, greater branching leads to a reduction in exploitation efficiency (Berntson 1993). While it seems clear that *C. maculosa* root systems are unlikely to have greater uptake potential than the massive root systems many perennial grass species possess (Casper and Jackson 1997), more plasticity in root deployment (greater efficiency) could contribute to greater soil water uptake in the face of spatially and temporally-variable soil water resources.

In contrast to this, competitively subordinate herbaceous species had the highest levels of efficiency in root (and shoot allocation) in one study (Campbell et al. 1991). Those plants with the greatest capacity for dominance had the highest level of dry matter in undepleted sectors, but this was not correlated with plasticity. Instead, competitive dominance depended more on a high growth rate and large amounts of biomass belowground (Campbell et al. 1991) than on levels of plasticity per se. However, this study used nutrient dripping devices which produced enriched patches that remained constant for the entire duration of the study. In western U.S. semiarid grasslands, nutrient availability has a strong temporal component, which was not addressed in this study. In

addition, invasive species may diverge from general trends observed in native plant species, and in fact this might be a factor in their success as community invaders.

Root proliferation responds locally to changes in soil water potential, with roots desiccating and dying in dry strata, and proliferating in wet strata. The strength of the response depends on the differences in the amount of water available. Root patterns of the allophyllous desert tree *Leptadenia pyrotechnica* (Forssk.) Decne. had highest root numbers and greater branching at the wet soil strata throughout its 11.5 m rooting depth (Batanoun and Wahab 1973). Water is generally taken up preferentially from layers where it is abundant, uptake and root growth can continue in layers of low water potential (Borman et al. 1992). This suggests that roots respond to changing soil water availability on a local scale. Under dry conditions with large water potential gradients, often only a small part of a rooting system will be absorbing water depending on the potential gradients near each root, the density of roots, and the extent of suberization (Spurway 1980).

Plastic root responses to soil water differed in two species of *Polygonum* adapted to different environments (Bell and Sultan 1999). Both species had considerable phenotypic plasticity in relative size, structure, and vertical placement of root systems in response to changing soil water environments. Their responses were rapid and precise. However, the species adapted to drier conditions had the fastest root allocation response in drying soils, and was the only one to significantly increase root allocation to lower soil layers in drying soils, suggesting adaptive differences in root plasticity between species (Bell and Sultan 1999).

Small rainfall events are ecologically important in semiarid regions (Sala and Laurenroth 1982). An ability to take up water from small rainfall events before it evaporates allows *Bouteloua gracilis* L. to dominate on the shortgrass steppe of Colorado (Sala and Laurenroth 1982), and could impart a similar advantage to *C. maculosa*. Using small rainfall events to continue growth during periods when soil water is scarce (either through preemption or because perennial grass species do not use these events) might allow *C. maculosa* to out compete native grasses. Soil water contents following a small rainfall event were lower in *C. maculosa* compared with native grass plots, although this may reflect interception by the canopy or litter (Sperber 2001).

Many factors, and likely a complex combinations of factors, may be responsible for *C. maculosa*'s success as an invasive species in western North American grasslands. However, few studies have addressed the general lack of quantitative root system information for *C. maculosa*. We hope that this study provides new insights into whether belowground competition for soil water may be an critical factor to *C. maculosa*'s success in semiarid grasslands.

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CHAPTER 2

SOIL WATER UPTAKE BY *C. MACULOSA*, *R. HIRTA*
AND PERENNIAL GRASSES.Introduction

Centaurea maculosa Lam. (spotted knapweed) is a perennial forb native to central Eurasia (Boggs and Story 1987; Maddox 1979). Introduced to North America in the early 1900's, *C. maculosa* has successfully invaded 326 counties in the western United States, including every county in Idaho, Washington, Wyoming and Montana (Sheley et al. 1998; Whitson et al. 1996). It typically forms dense, monotypic stands, reducing native flora and diminishing range quality for livestock and wildlife (Watson and Renney 1974). Its spread is not limited to disturbed sites, as it readily invades undisturbed habitats as well (Tyser and Key 1988).

Due to *C. maculosa*'s success as an invader and the monetary cost of associated agricultural losses, it is important to determine the mechanism(s) by which *C. maculosa* invades and dominates native plant communities. *Centaurea maculosa*'s success may be related to several factors that have already been investigated: 1) A lack of natural above and belowground predators due to its introduced status (Eddleman and Romo 1988); 2) prolific seed production (Jacobs and Sheley 1998); 3) allelopathy (Bais et al. 2003); and 4) specific germination factors that increase the probability of seedling survival. However these studies have not addressed competitive interactions by which *C. maculosa* may preempt or deplete resources critical to the success of native plants.

Belowground competition is the primary form of competition in semi-arid environments (Casper and Jackson 1997). Soil water is frequently identified as an important limiting resource in these environments (Schwinning and Ehleringer 2001; Yeaton et al. 1977). Therefore competing for soil water may be important to the survival of plants in semi-arid environments (Parrish and Bazzaz 1976). Soil water has been identified as critical in the dominance of other invasive species (Allen 1982; Harrington 1991; Williams and Hobbs 1989), and *C. maculosa* may gain a competitive edge over native species by having greater ability to access soil water in time or space.

The rooting system of *C. maculosa* differs morphologically from fibrous-rooted native grasses. Grasses typically have herringbone root systems with relatively uniform diameters, optimal branching patterns, and highest root densities in upper soil layers, although absolute rooting depth of some grass species exceeds 2 m (Weaver 1919). *Centaurea maculosa* has a tap-root. Although the entire root system of *C. maculosa* has not been described in the literature, tap-roots are thought to enable plants to reach resources located at greater soil depths. Tap-rooted plants are highly variable in the number of laterals and branches that are deployed at shallow depths (Weaver 1919). *Centaurea maculosa* has rooting densities similar to the native grass *Pseudoroegneria spicata* (Pursh) Löve at depths of 0-0.6m (Marler et al. 1999). Rooting patterns in semi-arid environments are depend highly on soil water availability, and specific patterns can play a large role in competitive interactions (Caldwell and Richards 1986; Coupland and Johnson 1965).

Plants compete belowground through preemption and/or depletion. Preemption occurs when one plant exploits soil resources more quickly than another, leaving insufficient amounts of the resource available for the competing plant at a critical period in its life cycle (Tilman 1982). Depletion occurs when one plant draws soil resources down below the level necessary for its competitor's growth and survival (Tilman 1982). However, these are general mechanisms and the possibilities for indirect or additive interactions between plant roots are limitless (Caldwell 1987).

In western Montanan (USA) grasslands, soil water storage has a strong temporal element that can be generally described in two distinct phases. Melting snow and spring storms provide a recharge period; in typical, non-drought years the rooting zone is wetted to or near field capacity (-0.01 MPa) (Wraith et al. 1987). This period is followed by a depletion phase, where inputs of soil water are limited to sporadic convective precipitation events and there is a high evaporative demand for soil water. In the depletion phase, loss of soil water is primarily due to extraction by plants, particularly below about 0.2 m depth. Soil water is extracted from progressively deeper layers over the course of the growing season (Thorgeirsson 1985; Wraith et al. 1987).

Deep roots are an advantage in this environment, allowing a plant to continue to extract soil water and photosynthesize once shallow soil water has been depleted. Fine root densities of *C. maculosa* are similar to the native bunchgrass *Pseudoroegneria spicata* (Pursh) Löve (bluebunch wheatgrass) when averaged over 0.6 cm, but differ significantly with depth (Marler et al. 1999). *Centaurea maculosa* dominated the lower depth, having four times the root density of bluebunch at 0.45-0.6 m. Roots of this

invader and native responded similarly to environmental fluctuations at shallow depth (Marler et al. 1999). In addition, *C. maculosa* had water uptake patterns similar to fibrous-rooted grasses at these shallow depths (0.05-0.3 m) (Sperber 2001).

Thus, *C. maculosa* may use a dimorphic approach to extract soil water in semi-arid ecosystems which favors its persistence over grasses. *Pseudoroegneria spicata* begins root growth later in the spring than many other species (Eissenstat and Caldwell 1987). This delay could allow *C. maculosa* to preempt soil water resources thus reducing reproduction, growth or survival of *P. spicata* and other grasses that may begin growth later in the spring. However, at three sites in Montana, *C. maculosa* did not use more water than native grasses at 0.05, 0.1, 0.15 or 0.3 m depths early in the growing season (Sperber 2001).

Alternatively, *C. maculosa* might deplete soil water contents to a point below which root tips of grass cease to grow. Root tips of *P. spicata* are highly sensitive to dry soil conditions, and roots were rarely found where water potential was below -1.5 MPa, whereas a competing annual grass, *B. tectorum*, had root growth in soils with water potentials as low as -10 MPa (Harris 1967). *Centaurea maculosa* might also draw soil water content down to lower levels than *P. spicata* and perhaps other grasses could endure, thus succeeding through depletion.

The objective of this study was to monitor changes in soil water content under monoculture plots of resident grasses, the invasive forb *C. maculosa*, and a naturalized forb, *Rudbeckia hirta* L. (black-eyed susan). Plots were monitored throughout the growing season to evaluate significant differences in spatio-temporal soil water use

patterns which might influence competitive interactions in a semi-arid ecosystem. We hypothesized that the invasive, non-indigenous forb *C. maculosa* would access more soil water than resident grass species and that *C. maculosa*'s water uptake patterns would differ from those of *R. hirta*, an indigenous (to the US) forb with similar morphology and phenology.

Methods

Study Sites

We selected two field sites in southwestern Montana with long-term *C. maculosa* infestations (more than five years based on aging individual *C. maculosa* plants, and more than ten years based on landowner reports), were similar in topography, and were currently ungrazed and unmanaged (no herbicides).

The Helena site was 23 km southeast of Helena, Montana in Broadwater County. Soils at this site are Rothiemay loams (fine-loamy, mixed, superactive, frigid Aridic Calciustolls) with 1 to 2% slopes (Sperber, 2001; Appendix A). Annual precipitation at Helena averages 304.5 mm (Helena WSO, WRCC, 2003). Dominant vegetation consisted of bluebunch wheatgrass (*Pseudoroegneria spicata* [Pursh] Löve), Kentucky bluegrass (*Poa pratensis*, L.), smooth brome (*Bromus inermis* Leyss.), silver sage (*Artemisia cana* Pursh.), and yellow sweet clover (*Melilotus officinalis* [L.] Lam), as well as spotted knapweed. The site was in a railroad right of way, adjacent to cultivated pasture.

The Leverich site was 8 km south of Bozeman, Montana in Gallatin County. Soils at this site are Farside loams, fine-loamy, mixed frigid Typic Argiustolls with 2% slopes

(Appendix A). Annual precipitation at Bozeman averages 461 mm (Bozeman, Montana State University, WRCC, 2004). Vegetation consists mainly of introduced grasses, including orchardgrass (*Dactylis glomerata* L.), timothy (*Phleum pratense* L.), and smooth brome (*Bromus inermis* Leyss.), with small populations of blue-bunch wheatgrass (*P. spicata*). Forbs present on this site included bladderpod campion (*Silene vulgaris* [Moench] Garcke), and common yarrow (*Achillea millifolium*, L.).

Experimental Design

At each field site, we selected four approximately 25 m² blocks that were similar in topography and vegetative composition. Beginning in mid-May 2002 we established within each block four 2 m² treatment plots separated by a 1 m buffer. The plots were arranged as randomized blocks to minimize the effects of heterogeneity of soil properties, physiography, and other pre-existing differences. Plots were designated *C. maculosa*, perennial grass, *Rudbeckia hirta* L., or *Liatris punctata* L. and treated with glyphosphate herbicide (Roundup^R, Monstanto Corporation, Columbus, OH) to eradicate all other vegetation. *Rudbeckia hirta* and *L. punctata* plots were planted with greenhouse starts (#2 conetainers). *Liatris punctata* failed to establish and it was dropped from the analysis. Plots were visually monitored throughout the study period, and herbicide was applied as necessary to maintain monocultures. Leaf area index was measured (LAI; LAI-2000, LiCor, Lincoln, NE) in each plot in July 2003.

Soil Water Measurements

Each plot was equipped with two vertically installed 0.3 m long time domain reflectometry (TDR) probes (Topp et al. 1980), which were automated and multiplexed (CR10X, TDR100, SDMX50; Campbell Scientific, Inc., Logan, UT) to record soil water content (θ) in the top 0.3 m of soil at 6-h intervals from July 2002 through September 2003. Due to datalogger problems, TDR data collected from the Leverich Site in 2002 was not used. Descriptive statistics (mean and standard error) were calculated for the TDR measurements collected between April and October of both years.

A single neutron moisture meter (503DR, CPN Corp., Martinez, CA; (Fernandez et al. 2000)) access tube was installed in each plot to measure θ at greater depths than the TDR. Neutron access tube depths ranged from 0.4 m to as great as 2 m, depending on the presence of soil obstructions. At the Helena Site, θ was measured to 1 m depth for all species. At the Leverich Site, grass and knapweed measurements reached 1.2 m, but *R. hirta* measurements were limited to 1 m depth. Access holes were lined with 3.8-cm diameter thin-wall PVC pipe and backfilled with fine silica sand to prevent air gaps between tube and soil. Local soil was tamped as fill near the soil surface to prevent channeling of water along the tubes. Soil water content was measured weekly to biweekly throughout the growing season, beginning in mid-May 2002 and April 2003 and ending in mid-September of both years. Soil water content was recorded at 0.2 m depth increments beginning at 0.2 m soil depth. The neutron moisture meter was field-calibrated by collecting volumetric soil cores at both field sites and determining the linear relationships between slow neutron count ratios and volumetric soil water contents obtained by oven drying at 105°C.

Measured θ from the mid- to late growing season drying periods was analyzed

using ANOVA to test for species, time, and depth main effects as well as for interactions. Tests were considered significant at $p < 0.10$ due to the high variability inherent in θ . Each study site and year was analyzed separately. Due to the repeated measurement nature of the neutron moisture meter, ANOVA was run on models fit without correlation factors and models fit with time and depth correlation factors to determine which were most appropriate, whereby $p < 0.10$ indicated significant correlation in the data. No significant correlation between depth or time was evident at the Leverich Site, but Helena required a correlation factor for depth. Additionally, soil water dynamics in the top 0.2 m of the soil profile at the Helena Site were quite different from the rest of the soil profile. A “top effect” factor which divided our data into 0-0.2 m and all other depth increments was added to the Helena models to account for this difference.

Volumetric water contents were converted to soil water storage by multiplying measured θ by the corresponding depth increment of 0.2 m, and subtracting the initial growing season soil water storage. Soil water storage is used to describe soil water content using units of equivalent water depth. Cumulative change in soil water storage provides a convenient measure of vegetation effects on soil water status. Positive values reflect precipitation inputs, and negative values reflect soil water depletion by vegetation, and evaporation near the soil surface. Only data collected during the annual drawdown period were analyzed statistically. Analysis of variance was used to determine species and day of year effects on soil water storage at each depth. P-values less than 0.10 were considered significant.

Results

Helena Site

Site Conditions. In 2002, total annual precipitation at Helena was slightly higher than the long term average. This was mainly due to wetter than usual summer months. Over 11 cm of precipitation fell in June 2002, which is 5.5 cm more than average (Table 2.1). In 2003, particularly May through July, conditions were dry relative to long term average. Air temperatures were close to long term averages for both years (data not shown). At Helena on July 23, 2003 *C. maculosa* had the highest leaf area index, followed by grasses.

Soil Water Content. Mean θ differed among species over the depletion period in 2002 and 2003 ($p < 0.001$, Appendix B). *Centaurea maculosa* had lower mean θ than grass during both study years. *Rudbeckia hirta* had the highest mean θ in 2002 and similar mean θ to *C. maculosa* during 2003. Mean θ increased with depth, and generally decreased over the season.

In 2003, θ of the different depths varied among species (species x depth; $p < 0.001$; Appendix B). Soil water content in the grass plots at 0.6 to 0.8 m depths was greater than θ of *C. maculosa* and *R. hirta* (Figure 2.1). Overall trends in θ over time were similar for all species and both years (Fig. 2.1; Appendix B). The three species did not differ in θ at 0-0.3 m depth, as measured by TDR (data not shown).

Table 2.1. Monthly and total annual precipitation for study years. Long term average

(LTA) is based on 100-year means. Data collected from nearest weather station (WRCC, 2004).

Cumulative changes in soil water storage. In 2002 at Helena, *C. maculosa* and

Year	Precipitation (cm)												Total
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	
Helena													
2002	0.10	0.74	1.32	1.55	4.72	11.07	4.09	3.35	3.10	0.41	1.27	0.13	31.9
2003	1.04	0.74	1.88	5.77	3.18	3.78	0.58	2.62	1.88	0.86	0.51	0.89	23.7
LTA	1.55	1.19	1.83	2.46	4.83	5.36	2.87	2.51	2.82	1.85	1.55	1.50	30.4
Leverich (MSU-Bozeman Weather Station)													
2002	3.07	1.35	0.94	2.44	10.24	12.52	3.68	4.80	4.95	0.48	0.56	0.46	45.5
2003	3.73	6.20	4.27	9.12	6.65	7.18	0.91	1.02	0.38	2.79	1.85	3.99	48.1
LTA	2.24	1.85	3.40	4.60	7.29	7.32	3.43	3.15	4.45	3.78	2.77	2.16	46.4

grasses used more water than *R. hirta* at every depth and *C. maculosa* used more water than grass at all depths except 0.2 m (Fig. 2.2). In 2003, grass used more water at 0.2 m than either *C. maculosa* or *R. hirta*, species did not differ at 0.4 m, and *R. hirta* used more water than *C. maculosa*, which used more than grass, at 0.6, 0.8 and 1 m depths (Fig 2.2).

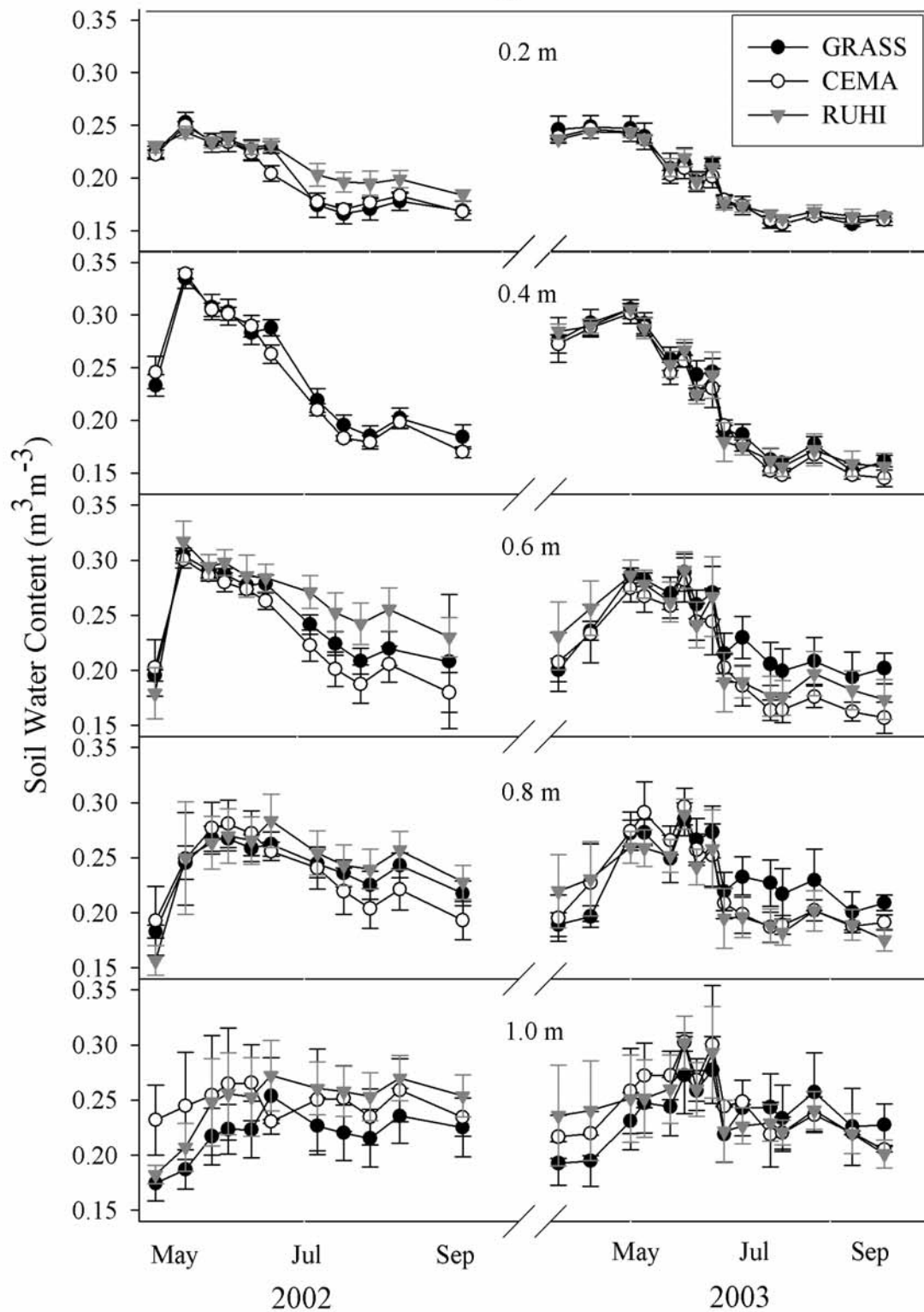


Fig 2.1. Seasonal changes in θ (mean \pm SE) by soil depth increment for perennial grasses (GRASS), *R. hirta*(RUHI), and *C. maculosa* (CEMA) in 2002 and 2003 at Helena.

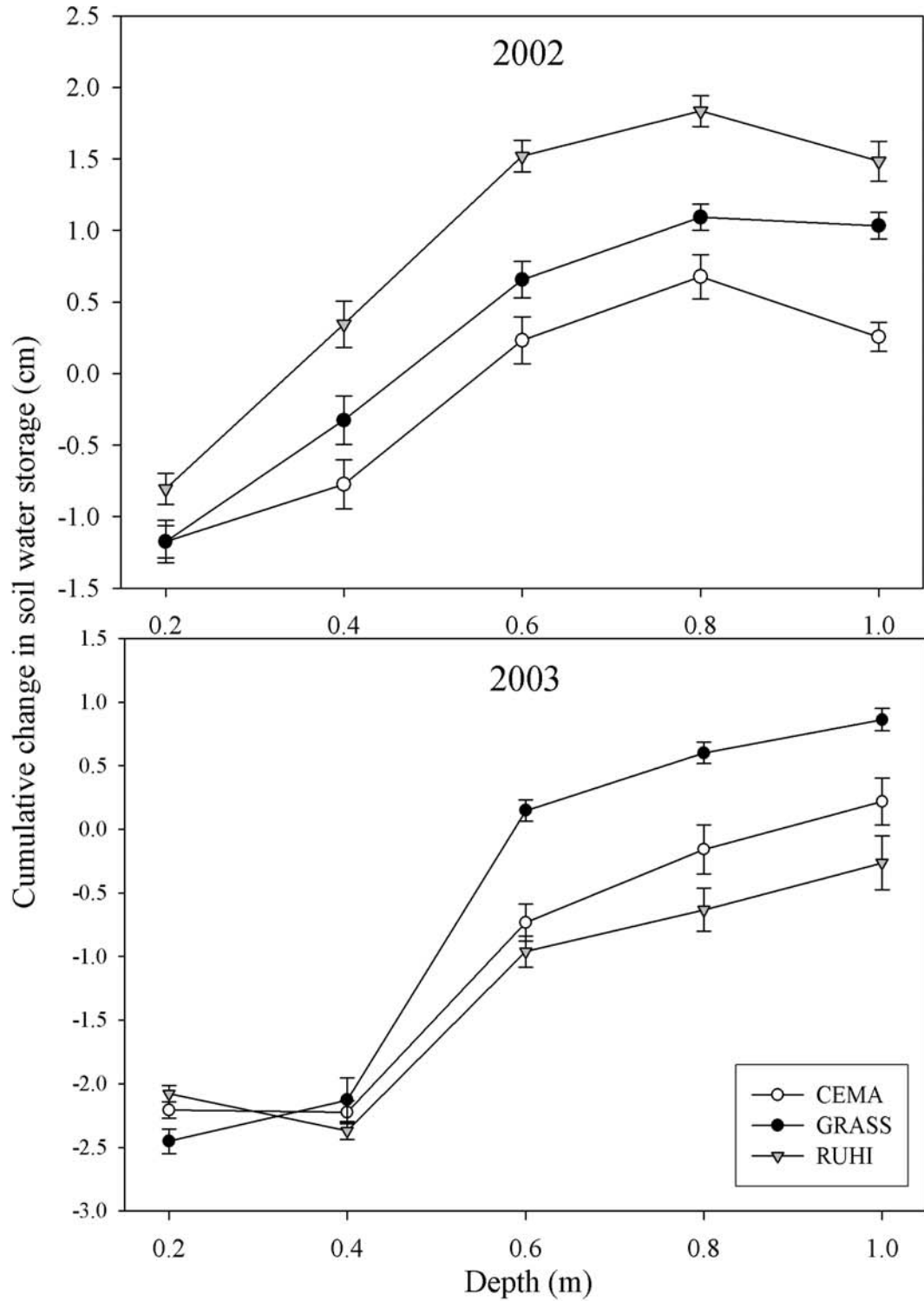


Fig 2.2. Seasonal changes in soil water storage (mean \pm SE) for perennial grasses (GRASS), *C. maculosa* (CEMA) and *R. hirta* (RUHI) by soil depth increment for 2002 and 2003 at Helena.

Leverich Site

Site Conditions. Annual precipitation at the Leverich Site was close to normal in 2002 and 2003 (Table 2.1). However, monthly differences were evident between years. The spring of 2002 (Mar-Apr) was drier than normal and early summer conditions (May-Jun) were wetter, whereas 2003 had a very wet spring (Feb-Apr), and a dry summer (Jul-Sep). Mean air temperatures did not deviate substantially from long term averages in either year (data not shown). On July 23, 2003, *R. hirta* plots had lower leaf areas than the other two species (data not shown).

Soil Water Contents. Initial soil water contents were similar for all species across depths, except at 1 m. *Centaurea maculosa* had higher initial θ content at 1 m in 2002 and 2003 at Leverich, although θ in *C. maculosa* plots were equivalent to the other species at this depth by the end of the sampling periods (Fig. 2.3). Mean θ in grass and *C. maculosa* plots was similar in 2002, though both had lower overall mean θ than establishing *R. hirta* plants at the shallow to intermediate depths (0.2 to 0.6 m; data not shown). *Centaurea maculosa* had the highest mean seasonal θ at 1 m, whereas *R. hirta* had the lowest, which reflected that *C. maculosa* had a higher initial θ than the two other species at 100 cm depth.

In 2003, grass plots had higher mean seasonal θ than *C. maculosa* and *R. hirta*, which did not differ from each other. By depth, *C. maculosa* had lower θ than grass at 0.6 and 0.8 m (Figure 2.3). *Rudbeckia hirta* had lower θ than *C. maculosa* and grass plots at 1 m depth (Fig 2.3). Similar to 2002, *C. maculosa* had a large mean θ at 1 m, such that θ at this depth was significantly higher than for *R. hirta*. At 1.2 m, water contents in *C. maculosa* plots were significantly lower than for grasses.

Cumulative changes in soil water storage. At the 0.2-0.6 m in 2002, grass and *C. maculosa* plots had similar water losses that were greater than for *R. hirta* ($p < 0.001$; Fig. 2.4; Appendix B) . At 0.8 and 1 m in 2002, *C. maculosa* used more water than grass and *R. hirta*, which did not differ. Grass had greater change in soil water at 1.2 m than *C. maculosa* ($p = 0.002$).

In 2003, water losses were similar among species at 0.2 and 0.4 m depths. *R. hirta* had greater loss of soil water than grass at 0.6 m ($p = 0.0082$) and *C. maculosa* lost more water than grass at 0.80 m ($p = 0.020$), 1 m ($p < 0.001$) and 1.2 m ($p = 0.0034$), and more than *R. hirta* at 1 m ($p < 0.001$). Similar to results at Helena, the net decrease in soil water storage increased over the course of the growing season.

Discussion

Site Conditions

Leaf area indices (LAI) of *C. maculosa* and grass plots were lower at the Helena than at the Leverich Site. Annual precipitation is usually lower at the Helena Site, which has less vegetative cover than Leverich (pers. obs). *Rudbeckia hirta* LAI was lower at the Leverich site; this was likely the result of apparent intensive grazing by deer during fall of 2002 (J. Wraith, Pers. Comm.). *Centaurea maculosa* had higher LAI than grass and *R. hirta* at both study sites. In part, this reflects measurements taken in late July, when *C. maculosa* plants had bolted and were flowering, but this may also reflect differences in plant densities within monoculture plots.

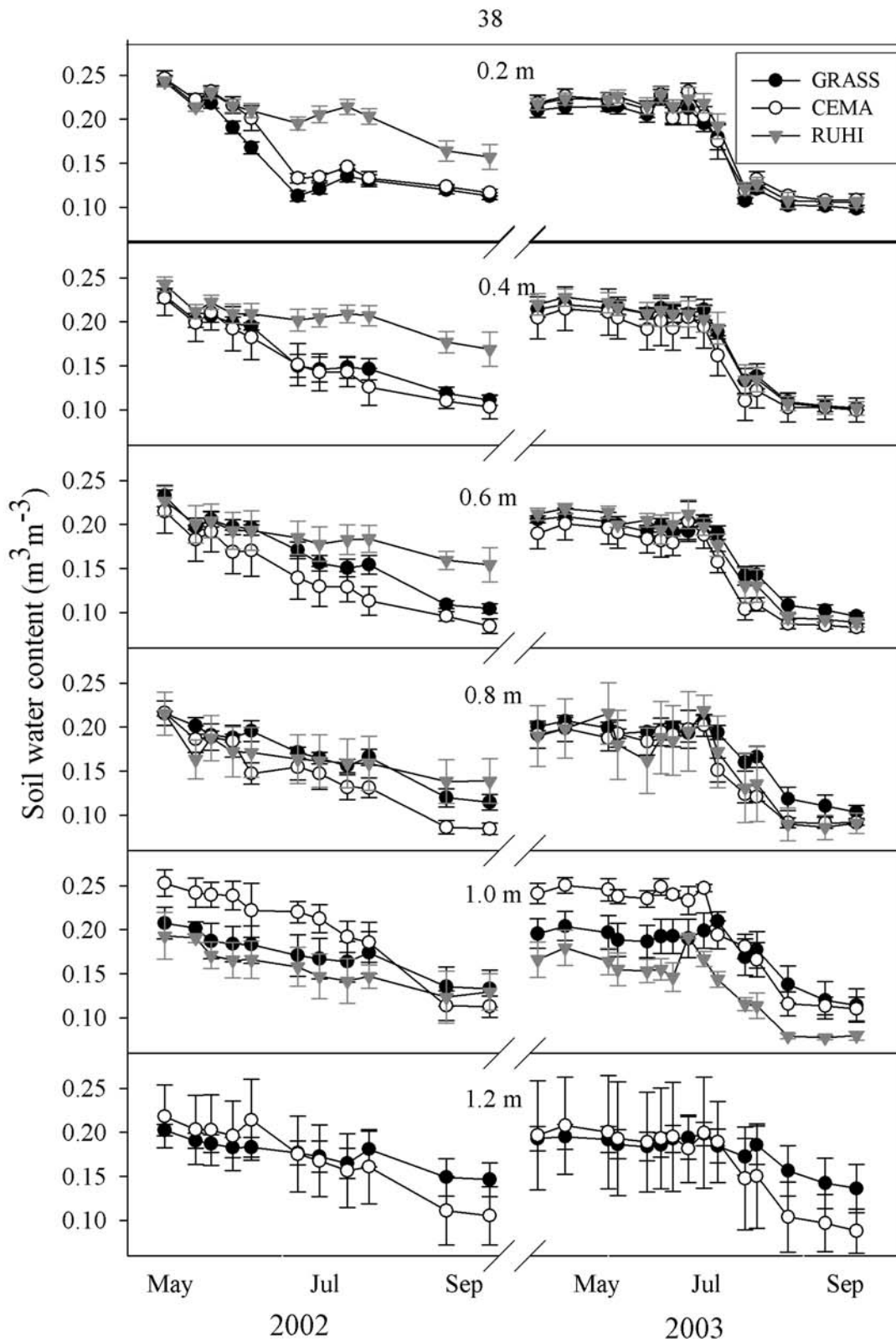


Fig 2.3. Seasonal changes in θ (mean \pm SE) for perennial grasses (GRASS), *R. hirta* (RUHI), and *C. maculosa* (CEMA) by soil depth increment for 2002 and 2003 at Leverich.

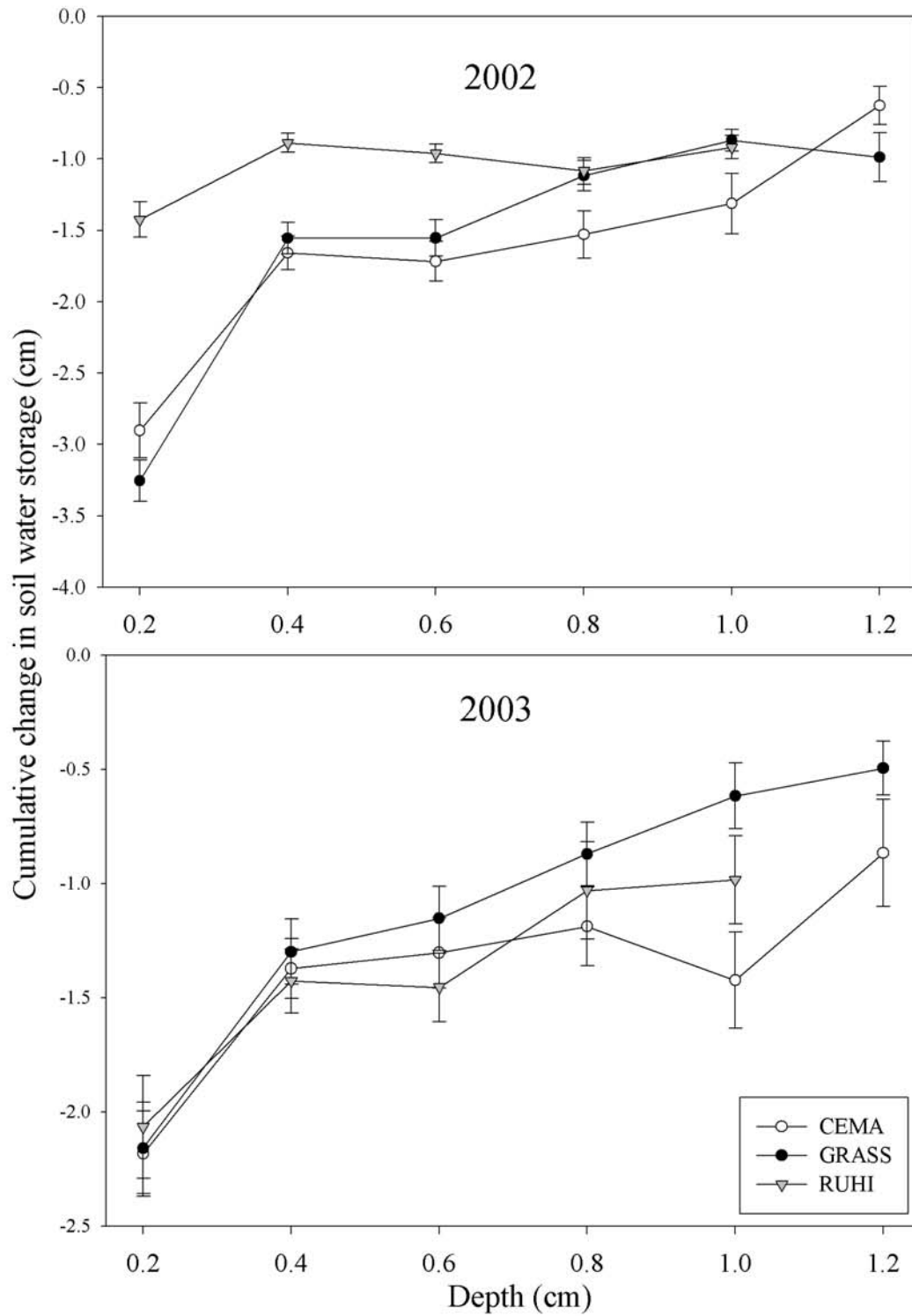


Fig 2.5. Seasonal changes in soil water storage (mean \pm SE) for perennial grasses (GRASS), *C. maculosa* (CEMA) and *R. hirta* (RUHI) by soil depth increment for 2002 and 2003 at Leverich.

Soil types differed between sites. The Leverich Site had a deep silt loam A horizon over a thin, highly gravelly silt loam layer, over a gravelly silt loam. The Helena Site had a thin silt loam A horizon over a more clayey subsoil (Appendix A). Mean soil water contents were higher at the Helena Site during both years of the study, and this corresponds with the higher proportion of clay at this site, resulting in greater water-holding capacity. The silt loam of the Leverich Site would hold less soil water at field capacity, but should have greater plant-available water holding capacity than the Helena soil, though this was moderated by gravel content (Or and Wraith 1999).

Soil Water Content

Competition for soil water is an important interaction between plants in semi-arid environments (Allen 1982; Grime and Curtis 1976; Harrington 1991; Yeaton et al. 1977). Ability to acquire more soil water than co-existing plants can lead to increased growth and survival (Allen 1982; Eissenstat and Caldwell 1988; Robberecht et al. 1983). We hypothesized that the invasive, non-indigenous forb *C. maculosa* would access more soil water than resident grass species. We further hypothesized that *C. maculosa*'s water uptake patterns would differ from *R. hirta*, a naturalized forb with a similar morphology and phenology.

Averaged over depth and depletion period, *C. maculosa* generally used more soil water than resident grasses at both sites. *Rudbeckia hirta* had different water use patterns in 2002 and 2003. Low soil water use in 2002 reflects the fact that this was an establishment year for these plants, and they were much younger and smaller than the established, resident plants which populated the grass and *C. maculosa* plots. *Rudbeckia*

hirta plant height did not exceed 0.05 m and most plants had less than 10 leaves when they were transplanted into the sites in May, 2002. However, in 2003, *R. hirta* plants had similar water uptake patterns to *C. maculosa*.

Initial θ s were similar among all species and years, except for *C. maculosa* at 100 cm where θ was higher than for the other species in 2002 and 2003 at the Leverich Site, and 2002 at the Helena Site. Similar initial θ s indicate that low θ in *C. maculosa*-infested plots at the end of the prior growing season did not carry-over to the following spring, although this was previously observed after a very dry year (Sperber 2001). Soil water recharged sufficiently both years such that all species began the growing season on similarly wet soils. This may not always be the case when winter and spring conditions are very dry. That *C. maculosa* had higher initial θ at 1 m depth was unexpected. One *C. maculosa* plot at Leverich had consistently higher θ throughout the early growing season, which might be related to a soil anomaly such as perched soil water over a gravel lens.

Mean θ by depth remained similar among species prior to about July 1. This was consistent for both years of our study, despite dissimilar precipitation patterns. Early spring conditions (Mar-Apr) in 2002 were dry relative to long term averages, and wet in 2003. The trend in late summer (Jul-Sep) was the opposite; these months received more than twice the amount of precipitation in 2002 than 2003. Dry, summer-like conditions with warmer air temperatures generally begin in early July in southwestern Montana, and continue through August.

All species had similar changes in θ through time. This provides evidence that preemption of soil water by earlier growth and water uptake is not a strategy important to *C. maculosa*'s success in these grasslands, although it has been shown to be important in competitive interactions between other species (Berube and Myers 1982; Eissenstat and Caldwell 1988). Possibly, species differences in soil water uptake at shallow depths could be masked by early season precipitation events and show up later as reduced percolation to deeper depths. This seems unlikely for our study considering the large differences in spring precipitation inputs between years, as well as the lack of significant species effects in the high temporal resolution TDR data that continuously measured water content in the upper 30 cm of the field plots.

Cumulative Changes in Soil Water Storage

Differences in soil water uptake were most readily apparent when considered as cumulative changes in soil water storage. These data were analyzed differently than soil water content; they were compared for each depth increment, instead of over the whole profile. Changes in cumulative soil water storage for our study indicate that *C. maculosa* uses more water than resident grasses at greater depths later in the growing season. During its second year of growth, *R. hirta* used similar to greater amounts of soil water than *C. maculosa* at these deeper depths, and had similar water uptake patterns over time.

Competitive Effects

Direct competitive effects between *C. maculosa* and perennial grasses were not investigated in this study. To have a competitive advantage, a species must not only use

more of a potential resource supply, but its greater use must have a deleterious effect on co-existing species (Casper and Jackson 1997). *Centaurea maculosa*'s greater water use occurred in the late part of the growing season, when grasses are typically approaching quiescence. Additionally, low soil water content at greater depths under *C. maculosa* did not result in lower soil water contents in the spring of the following year, unlike the results of Sperber (2001) at the Helena site.

Native grasses that use the C3 photosynthetic pathway in semiarid environments generally begin growth early in the spring (or the previous fall if sufficient moisture exists) and typically become quiescent in late July. In southwestern Montana, most precipitation falls during the spring and early summer. Thereafter soil water is steadily depleted as it evaporates and is taken up by plants. Potential evapotranspiration in semiarid environments often exceeds precipitation inputs, therefore the cost of photosynthesis is high (Sala et al. 1992; Singh et al. 1998).

Centaurea maculosa also uses the C3 photosynthetic pathway. However, consistent with water use patterns, *C. maculosa* continued to photosynthesize longer into the late summer than sympatric grasses at these sites (Judson Hill, unpublished data). The exact mechanism behind this ability is unknown but may be related to higher water-use efficiency during the late summer stage of its life cycle (mature plant) than in its rosette form (Blicker et al. 2003).

Pseudoroegneria spicata was present to varying degrees on both study sites. This native bunchgrass has relatively coarse, heavily suberized roots that penetrate up to 2 m (Harris 1967; Weaver 1919). Therefore this species presumably has access to deep soil

water resources that *C. maculosa* and *R. hirta* use later in the season. It may not be using this resource due to phenological constraints (quiescent period) or because of low rooting density at deeper depths (Dobrowolski et al. 1990; Weaver 1919). In soils close to field capacity (-0.01 MPa), root density should have little effect on acquiring soil water, which moves from the soil to the root via mass flow. In dry soils, where strong matric forces limit water flow, close proximity of the root surface to water filled soil water pores becomes crucial therefore high rooting densities are more important in dry soils (Harper et al. 1991). In our study, the rate of water extraction at greater depths in plots dominated by *P. spicata* did not change significantly as soil water content decreased. A change in rate of water extraction would be expected if water uptake was strongly influenced by rooting density. However, low water contents at depth did not occur until late in the growing season, when much of the perennial grass population was quiescent, and thus not extracting soil water.

Pseudoroegneria spicata ceases root growth in soils at a higher matric potential than *Bromus tectorum* L., a competing annual grass (Harris 1967). Our grass species may have likewise ceased water uptake in soils at higher matric potentials than *C. maculosa* (and *R. hirta*) in this study. Species such as *C. maculosa* and *R. hirta* may be able to osmoregulate root tissues to continue water uptake in drying soils (Slatyer 1967). Continued root growth by these forbs could result in steadily increasing dominance over belowground physical space by having a longer root growth period. Encroachment by neighboring roots can have negative effects on a plant due to overlapping nutrient (and soil water) depletion zones, and the fragmentation of physical space (McConnaughay and

Bazzaz 1992; Nye and Tinker 1977). *Centaurea maculosa* exudes allelopathic chemicals from its roots, which might further reduce grass rooting volumes (Bais et al. 2003).

Rudbeckia hirta is indigenous to the midwestern U.S., has been naturalized in Montana for at least 30 years, and often colonizes disturbed sites (Lackschewitz 1991). However, *R. hirta* has not been invasive in western Montanan grasslands. Once it had become established during the second year of our study, *R. hirta* had similar water use patterns to *C. maculosa*. Similar water use patterns indicate that *C. maculosa*'s invasive success may not derive (solely) from its ability to differentially access soil water relative to grasses. That is, its water uptake patterns are similar to *R. hirta*, and may be common to all tap-rooted late season forb species. Therefore *Centaurea maculosa* does not appear to be a uniquely more competitive in terms of its water use patterns.

Our results demonstrate differences in late-season soil water uptake between *C. maculosa* and resident grasses in semiarid grasslands of southwest Montana. Although direct competitive effects were not measured in this study, a longer season of growth and water uptake could allow *C. maculosa* to exert competitive pressure on grass species which have phenologic constraints on seasonal growth; thereby resulting in reduced fitness or grass mortality. Furthermore, comparisons of soil water dynamics under monocultures of *C. maculosa* and the naturalized forb *R. hirta* indicate that this pattern of late season soil water uptake is not specific to the invasive forb, but may in fact be a trait common to other late season, tap-rooted forb species.

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CHAPTER 3

ROOT SYSTEM CHARACTERISTICS OF *C. MACULOSA*
AND *R. HIRTA* IN RESPONSE TO TWO SOIL WATER REGIMESIntroduction

Despite early efforts to generalize traits of “weedy species”, we remain unable to predict potential invasiveness of introduced plant species (Baker 1974), perhaps because environmental factors play such a large role in a species’ success. Comparison of traits between morphologically or phenologically similar invasive and non-invasive species are becoming more common in the literature as the pressure exerted by invasive species on ecosystems steadily increases (Radford and Cousens 2000; Rejmanek and Richardson 1996; Thebaud et al. 1996). Such studies may help to identify general traits that may be important to invasive ability, as well as identify specific factors responsible for an invasive species’ success in a particular ecosystem.

Centaurea maculosa Lam. is a perennial forb native to central Eurasia (Boggs and Story 1987). Since its introduction to North America in the early 1900s as a contaminant in alfalfa seed, *C. maculosa* has spread prolifically through disturbed and pristine habitat (Sheley et al. 1998; Whitson et al. 1996). It forms dense, monotypic stands which reduce native flora and degrade range quality for livestock and wildlife (Watson and Renney 1974; Tyser and Key 1988). *Centaurea maculosa* can be controlled through herbicide application, but this type of control has limited feasibility considering the extent of its invaded range (Griffith and Lacey 1991; Rice et al. 1997; Sheley et al. 1998). A greater

understanding of the mechanisms responsible for *C. maculosa*'s success may improve our ability to manage this species.

In semiarid environments such as western Montanan (USA) grasslands, competition for belowground resources comprises the bulk of competitive interactions between plants (Casper and Jackson 1997). Perennial plants invest heavily in root systems to acquire sufficient water and nutrients. In particular, competition for soil water is an important factor mediating plant populations in xeric environments (Yeaton et al. 1977). The success of several non-indigenous plants has been linked to competition for soil water (Allen 1982; Eissenstat and Caldwell 1988; Harris 1967; Thorgeirsson 1985; Williams and Hobbs 1989).

Centaurea maculosa may have a greater ability to take up soil water than native grasses. *Centaurea maculosa* accesses greater amounts of soil water later in the growing season than native grasses (Sperber 2001). However, we found only Marler et al. (1999) examined the root systems of *C. maculosa* and native plants in detail, and addressed only fine root dynamics in the upper 60 cm of the soil profile. It is therefore unclear whether *C. maculosa* has morphological root characteristics that make it a unique or superior belowground competitor, or whether its belowground habit might share common characteristics of other tap-rooted forbs.

We conducted a greenhouse study to compare root systems of *Rudbeckia hirta* L. and *C. maculosa*. Although these species are not closely related taxonomically, they share phenological and morphological characteristics. Both are late season, tap-rooted, C3 members of the Asteraceae family and are non-indigenous to Western Montana. Both

species have been present in Montana for more than 30 years, but *R. hirta* has remained localized in small patches while *C. maculosa* has invaded millions of hectares (Lackschewitz 1991). We compared root system characteristics in response to wet and dry soil water conditions to identify rooting patterns or root characteristics which may enhance resource uptake and increase *C. maculosa*'s success as an invader of western North American grasslands.

Methods

Species

Twenty plants each of *C. maculosa* and *R. hirta* were grown from seed in 15 liter pots (Treepot[®], Stuewe and Sons, Inc. Corvallis OR, USA) containing 2 parts sand and 1 part pasteurized greenhouse soil mix. The pots were 45 cm tall and tapered from 20 cm diameter at the top to 15 cm at the bottom. A thin (~1 cm) layer of soil from a spotted knapweed-infested field site was added approximately 5 cm below the surface to provide a source of mycorrhizal inoculum. Ten seeds were planted per pot in early August 2003, thinned to three seedlings at 2 weeks, and to one plant at one month.

Treatments

After obtaining a soil water retention relationship (Or and Wraith 1999) for our soil mix, we fitted the measured values to a non-parametric water retention model which describes the relationship between soil water content and matric potential (van Genuchten 1980). We then selected two matric potentials, the first near field capacity water content

(-10 kPa) and the other moderately dry (-400 kPa), to use as our water treatments. For the sand:soil mix these corresponded to 0.16 and 0.059 (kg:kg) water contents respectively. Soil water treatments were initiated when 80% of the plants of a species reached the 5-leaf stage. Thereafter, pots were weighed every three days and water was added as necessary, alternately from the top and bottom, to maintain the target water status.

Pots were arranged in a completely randomized design in the greenhouse. Every two weeks, plant height was measured, number of leaves counted and location was re-randomized. Beginning in mid-September, plants were given supplemented light (GE multivapor MVR1000/C/U, General Electric Company, Cleveland, OH) from 0600 to 1000 and from 1800 to 2000 hours to maintain a 14-h photoperiod. In late October we began using supplemented light throughout the 14-h photoperiod due to decreasing ambient light conditions. Temperature in the greenhouse was 24 °C during the day and 15 °C at night for the duration of the experiment.

Harvest

Plants were harvested in mid-November after 12 weeks of growth. Plants were measured and leaves counted a final time, then aboveground biomass was removed at the soil surface and dried and weighed. Pots containing soil and roots were placed in 4 °C storage. For root harvest, each pot was cut in half to facilitate removal of contents, and soil divided into four 11-cm depth increments. Roots were removed from soil, cleaned of large organic debris, placed in labeled ziploc bags with distilled, deionized (DI) water, and returned to 4 °C storage until harvesting was complete. Roots were then closely inspected and all remaining soil debris removed by hand.

Roots were prepared for image analysis following a modified method of Kasper and Ewing (1997). Cleaned roots were stained to provide enhanced resolution for image analysis by placing them for 30 min in a 1:200 (g:ml) solution of crystal violet and DI water. Following staining, roots were thoroughly rinsed with DI water, rebagged in a 5:1 (vol:vol) mixture of DI water and ethanol, and returned to 4 °C storage.

Immediately prior to scanning, roots were removed from bags and rinsed with DI water. We used a modified flatbed scanner (Epson Expression 1680, Epson America, Long Beach, CA) equipped with supplemental top lighting to obtain digital images of root samples. For scanning, roots were divided into 0.5-1.5 g (wet weight; depending on type and thickness) aliquots and placed in transparent plastic root trays (20 x 30 x 2 cm, Regent Instruments, Quebec, Canada). Approximately 4 mm of water was added to trays to facilitate root separation. Roots were manually dispersed to minimize overlap and clumping, and digital images obtained. Images were saved as TIFF files for subsequent analysis (WinRhizoPro software, Regent Instruments).

We analyzed whole pot root data for total biomass, root mass, root mass ratio (root mass:total mass), root length, root length density, surface area, specific length, root volume, average diameter, and length within 20 diameter classes ranging from 0-0.1 mm to >1.9 mm using two-way ANOVA and a significance level of $p < 0.1$. In addition, we used a compound symmetric correlation model and ANOVA to analyze measurements by soil depth increment for root biomass, length, root length density, surface area, specific length, volume, average diameter, and length within each diameter class. Tukey's comparisons were used to identify significant differences.

Results

Total Biomass and Root Mass Ratio

Total (whole plant) biomass was higher for *R. hirta* than for *C. maculosa* when averaged over soil water treatment ($p < 0.001$; Appendix C). *Rudbeckia hirta* total weight averaged 11.7 g across treatments, whereas *C. maculosa* was 5.3 g. In addition, plants subjected to the wet treatment had 57% greater total biomass than those in the dry treatment ($p < 0.001$; Appendix C). Root mass ratios were slightly higher for *C. maculosa* than for *R. hirta* ($p < 0.001$). Both species had greater root mass ratios in response to the dry treatment ($p = 0.0062$; Appendix C).

Root Mass

Rudbeckia hirta had 77% greater total root biomass ($p = 0.013$) when analyzed for whole pot effects (Appendix C). When analyzed by depth, species varied by depth ($p = 0.018$), and treatment response varied by depth (0.0060; Appendix C). Root mass was greatest for the 0-11 cm depth increment, generally decreased over depth, and was greater for *R. hirta* than *C. maculosa* (Fig. 3.1). Plants in the dry treatment had lower whole pot root mass values than those of the wet treatment ($p < 0.001$) and lower root mass than the wet treatment at all but the shallowest (0-11cm) depth increment when averaged over species (Fig. 3.2). Species did not vary by treatment on a whole pot basis for root mass or any other response variable (data not shown).

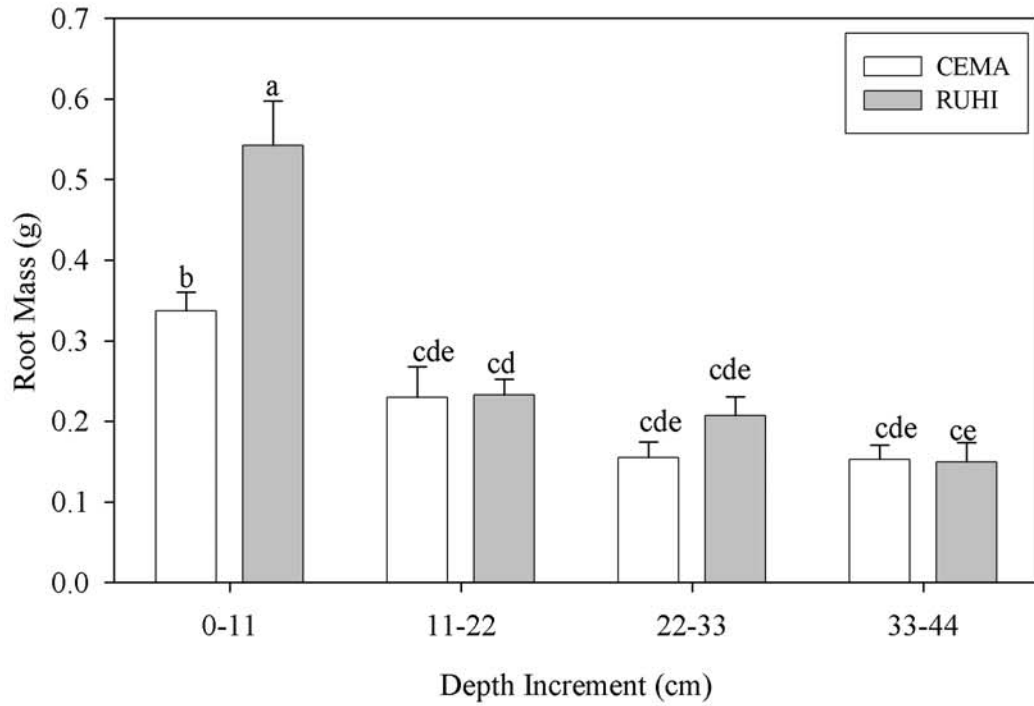


Fig 3.1. Root mass (mean \pm SE) for *C. maculosa* (CEMA) and *R. hirta* (RUHI) for all soil depth increments. Letters indicate significant differences.

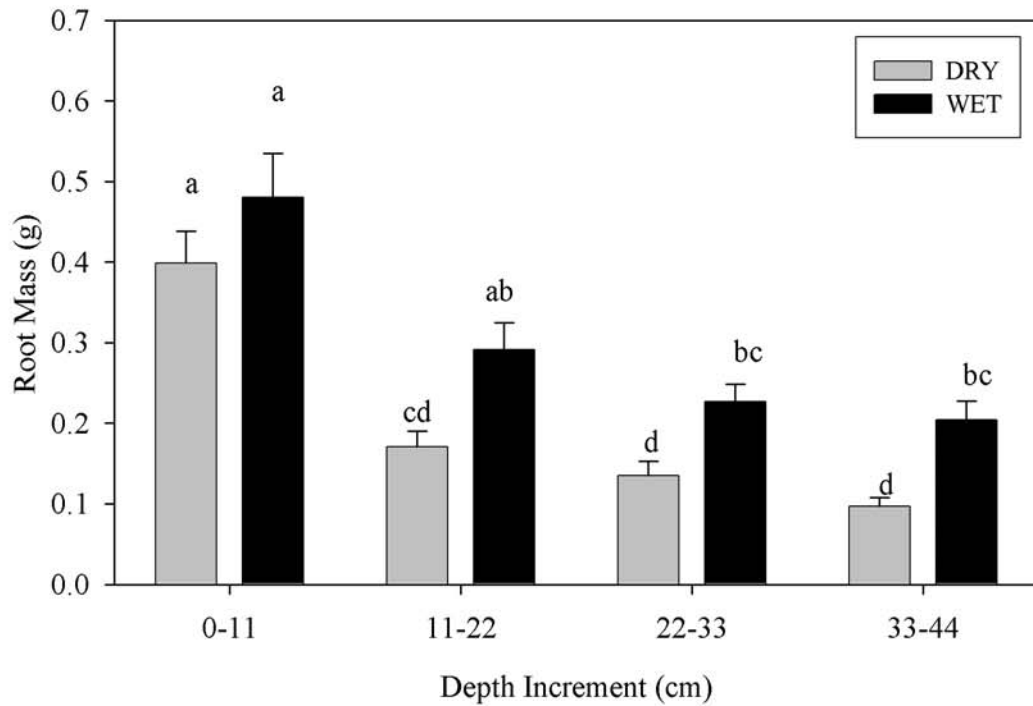


Fig 3.2. Root mass (mean \pm SE) for dry and wet soil water treatments for all depth increments. Letters indicate significant differences.

Root Length

Rudbeckia hirta generally had greater root length than *C. maculosa* ($p < 0.001$; Appendix C). Length of *R. hirta* roots was 395 m when averaged over both treatments, *C. maculosa* was 214 m. The lower depth increments had the greatest variation in root length by species (Fig. 3.3). Root length for *R. hirta* was greater than *C. maculosa* at 11-22, 22-33 and 33-44 cm ($p = 0.0060$; Fig 3.4; Appendix C). Root length decreased by depth in pots subjected to the dry treatment (Fig. 3.4) and root length was lower in dry treatment plants than wet treatment plants at 22-33 and 33-44 cm depths ($p = 0.015$; Appendix C).

Root Length Density

Root length density (RLD), the length of root per unit soil volume, was nearly 50% greater for *R. hirta* than for *C. maculosa* ($p < 0.001$) and was 25% higher for plants of the wet treatment than the dry ($p < 0.001$; Appendix C). Root length density also varied by depth densities were highest at the shallowest depth increment (0-11 cm) and lowest at the greatest soil depth increment (33-44 cm) ($p < 0.001$; Appendix C).

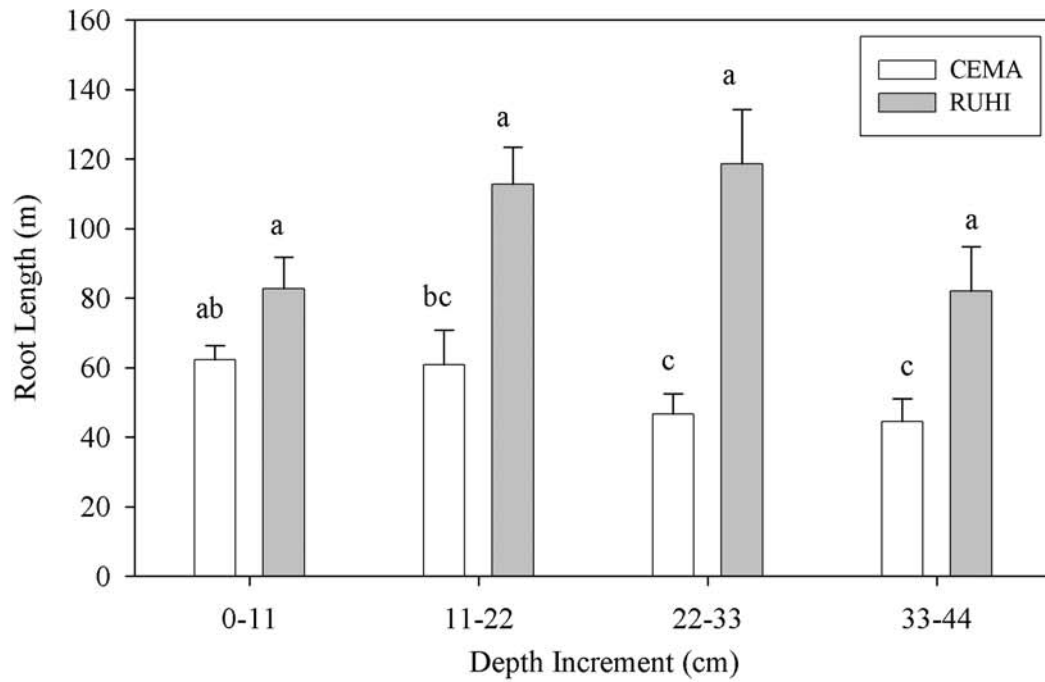


Fig. 3.3. Root length (mean ± SE) for *C. maculosa* (CEMA) and *R. hirta* (RUHI) for all soil depth increments. Letters indicate significant differences.

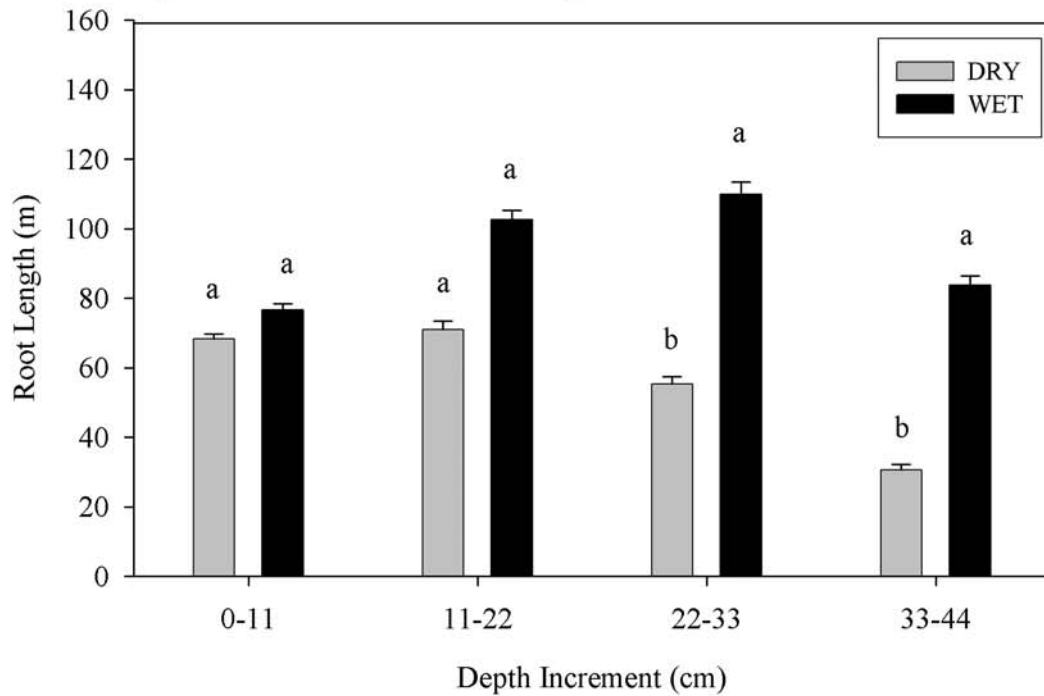


Fig. 3.4. Root length (mean ± SE) for dry and wet soil water treatments for all depth increments. Letters indicate significant differences.

Surface Area

Averaged over depths, *R. hirta* had higher root surface area than *C. maculosa* ($p < 0.001$; Appendix C). Surface areas in the upper depths (0-11 cm) were greater than for the 22-33 and 33-44 depth increments. *Rudbeckia hirta* had greater root surface area than *C. maculosa* in all but the shallowest depth increment ($p = 0.014$; Fig. 3.5; Appendix C).

Root surface areas generally decreased with depth for *C. maculosa* but did not change for *R. hirta* (Fig 3.5). Surface areas were higher in plants of the wet treatment ($p < 0.001$; Appendix C), with the largest differences at deeper (22-33, 33-44 cm) depth increments ($p = 0.023$; Fig 3.6).

Root Volume

Total volume of root varied by species and by treatment ($p < 0.001$; Appendix C). *Rudbeckia hirta* had greater overall root volume on a whole pot basis ($p < 0.001$), and had greater volume than *C. maculosa* in the 22-33 cm depth increment ($p = 0.038$; Fig. 3.7; Appendix C). Pots in the wet treatment had a greater volume of roots than dry treatment ($p > 0.001$; Appendix C), and this was especially true at the greater depths (22-33 and 33-44 cm) ($p = 0.034$; Fig 3.8). Averaged over species and treatment, root volume was greater in the 0-11 cm depth increment than the 22-33 cm increment ($p < 0.001$; Appendix C).

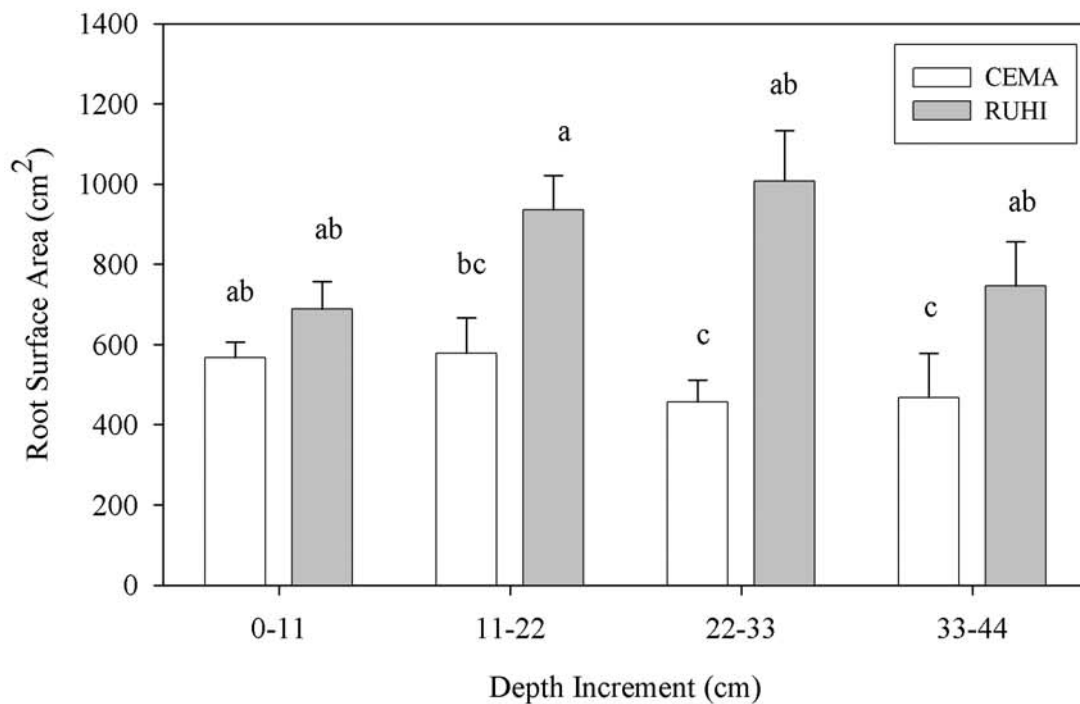


Fig 3.5. Root surface area (mean ± SE) for *C. maculosa* (CEMA) and *R. hirta* (RUHI) for all soil depth increments. Letters indicate significant differences.

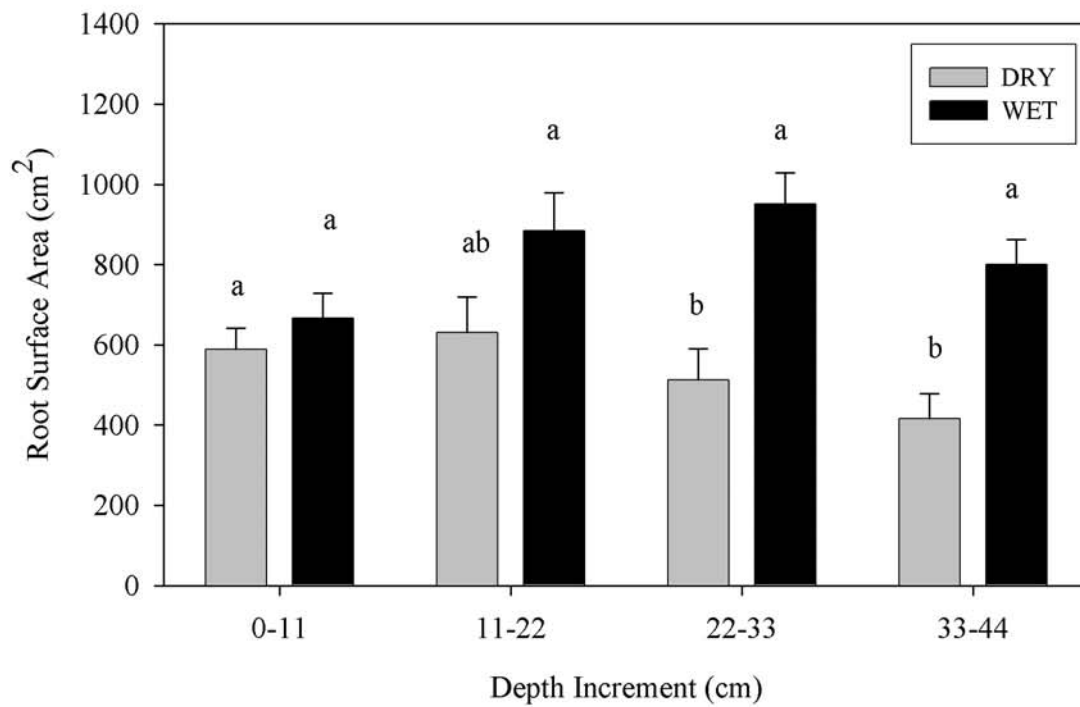


Fig 3.6. Root surface area (mean ± SE) for dry and wet soil water treatments for all depth increments. Letters indicate significant differences.

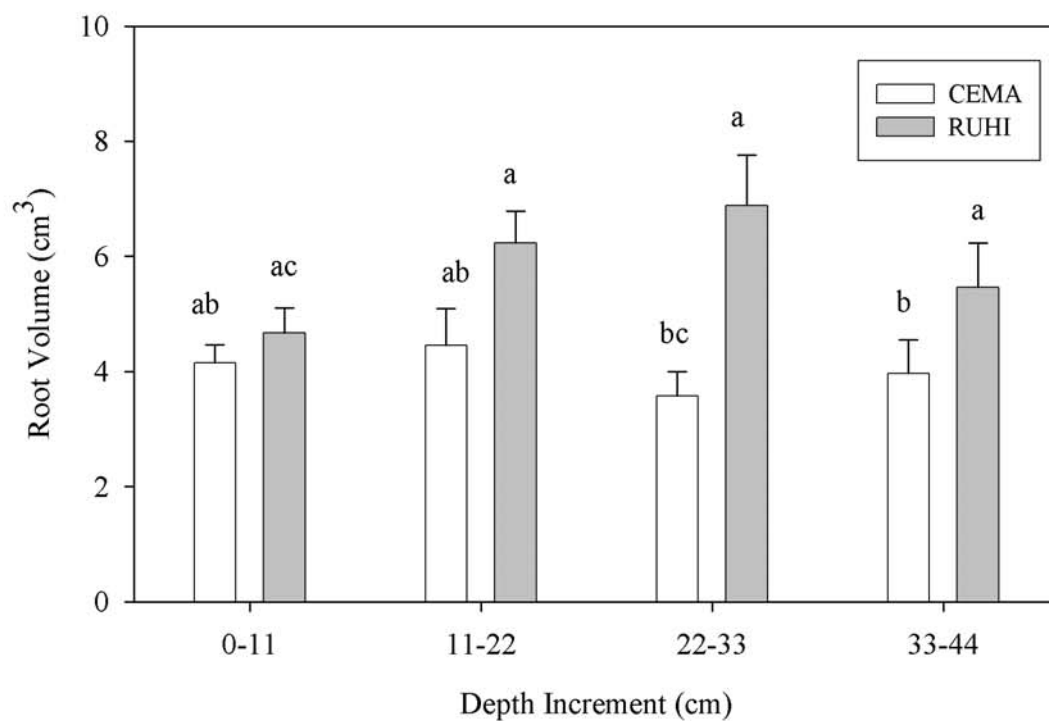


Fig 3.7. Root volume (mean \pm SE) for *C. maculosa* (CEMA) and *R. hirta* (RUHI) for all soil depth increments. Letters indicate significant differences.

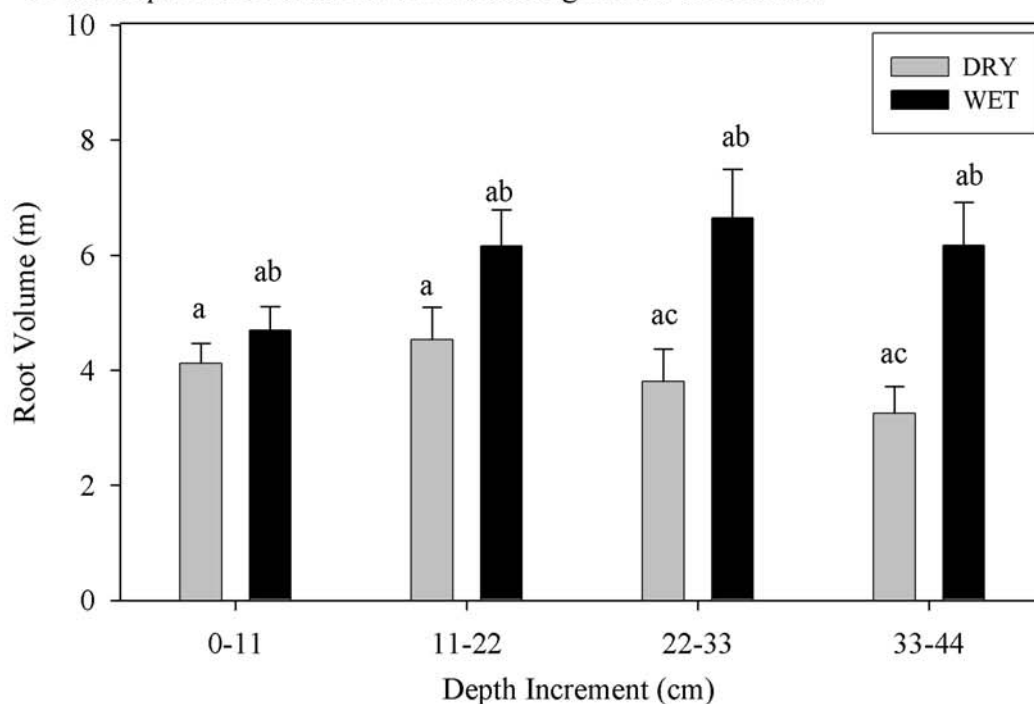


Fig 3.8. Root volume (mean \pm SE) for dry and wet soil water treatments for all depth increments. Letters indicate significant differences.

Specific Length

On a whole pot basis, specific root length varied only by species ($p < 0.001$; Appendix C). Specific length also varied by depth ($p < 0.001$) and by depth for species (spp x depth interaction $p < 0.001$; Fig 3.7; Appendix C). Lowest specific root lengths were in the upper depth increment (0-11 cm) for *R. hirta*, and specific length at this depth did not vary between species. At all other depths *R. hirta* had higher specific length than

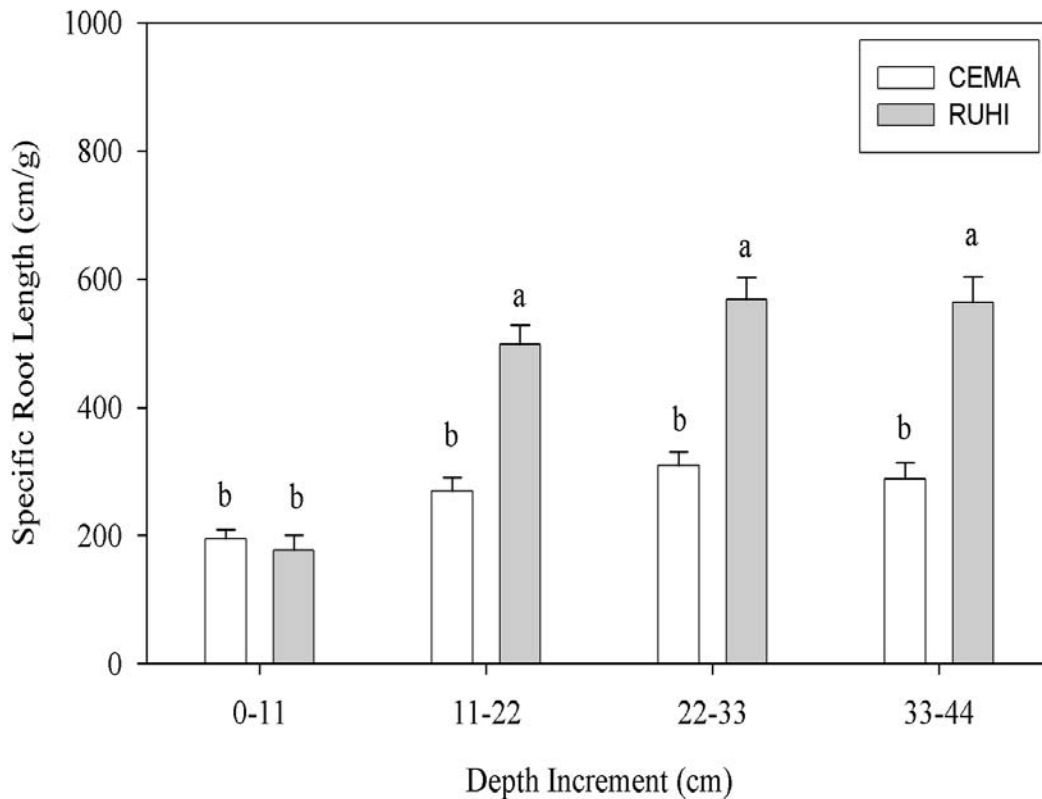


Fig 3.9. Specific root length (mean \pm SE) for *C. maculosa* (CEMA) and *R. hirta* (RUHI) for all soil depth increments. Letters indicate significant differences.

C. maculosa, and specific lengths were relatively constant at the greater depths (Fig. 3.7).

Root Diameter

Centaurea maculosa generally had larger diameter roots than *R. hirta*. This relationship did not change with depth or soil water treatment. For roots of the smallest diameter class (0.0-0.1 mm), *R. hirta* had nearly 3 times greater length than *C. maculosa*. Length of root in this class was also greater for plants in the wet treatment ($p < 0.001$, Appendix C). Similar whole pot patterns were seen for all diameter classes (0.0->1.9 mm, in 0.1 mm increments) except the 0.8-0.9 mm class. Length of root in the 0.8-0.9 mm class was similar between the two species when subjected to wet treatments, but was much greater for *R. hirta* than *C. maculosa* in plants of the dry treatment. *Rudbeckia hirta* had the greatest total length of roots in the 0.1-0.2 mm class whereas *C. maculosa*'s greatest root length was derived from roots in the 0.2-0.3 mm class (Fig. 3.10). In both species, the bulk of total root length consisted of roots less than 0.5 mm in diameter (Fig 3.10). Similar patterns were observed for root length by soil water treatment.

Each of the 20 root diameter classes varied by depth. Smallest diameter roots (0-0.1 and 0.1-0.2 mm) had the greatest root length at the surface (0-11 cm) depth, and the least in the two lowest depths (22-33 and 33-44 cm) (Fig. 3.11). Pots of the dry treatment had smaller mean length of roots in 0 to 0.2 mm diameter classes than wet treatment at every depth but the surface.

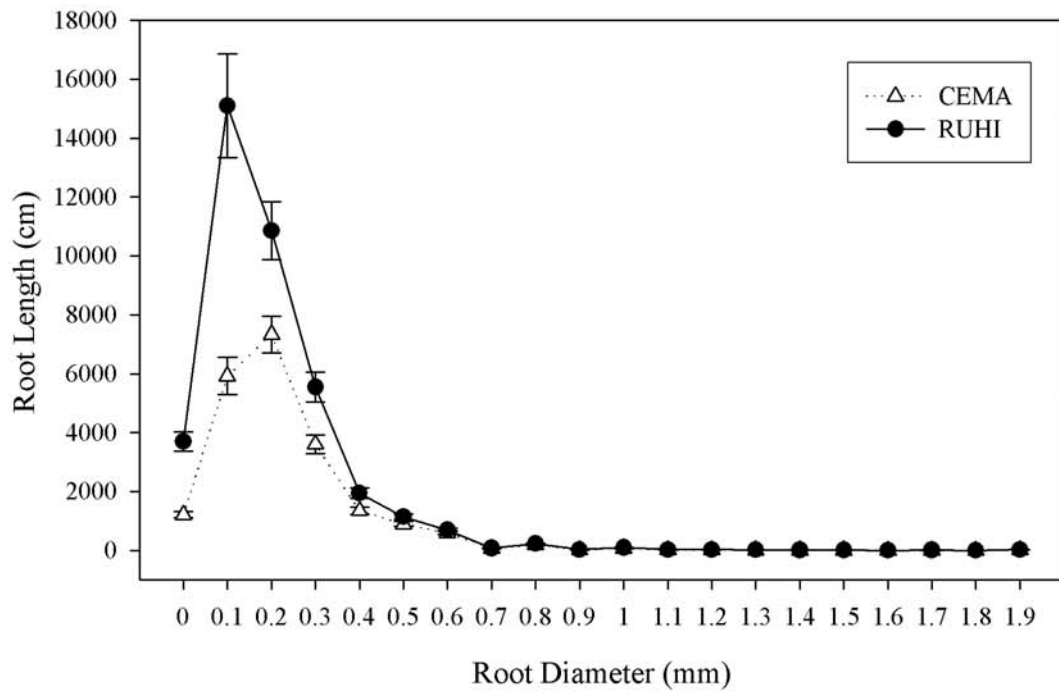


Fig 3.10. Length of root (mean \pm SE) in each diameter class for *C. maculosa* (CEMA) and *R. hirta* (RUHI).

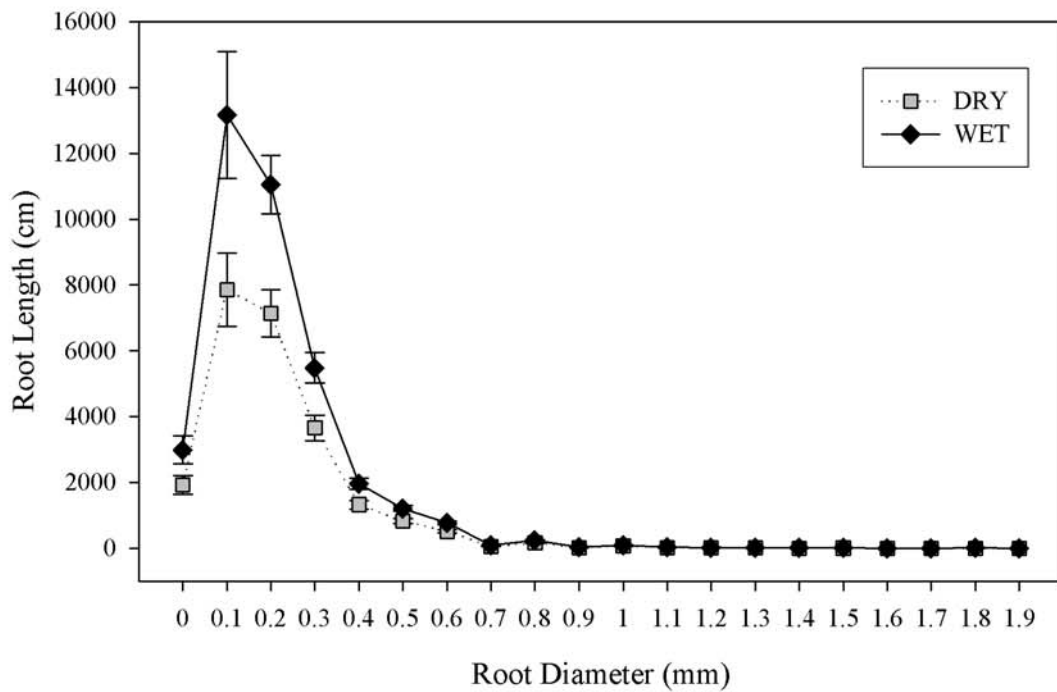


Fig 3.11. Length of root (mean \pm SE) in each diameter class for wet and dry soil water treatment (mean \pm SE).

Roots with diameters of 0.2-0.3 mm did not vary between the upper two soil depths (0-11 and 11-22 cm), but the surface depth increment had more roots of this diameter than the deeper depths (22-33 and 33-44 cm). *Rudbeckia hirta* plants had more roots of this diameter than *C. maculosa* at all depths but the surface (0-11 cm). Root length of 0.2-0.3 mm roots decreased more strongly with pot depth in the dry treatment than for wet. The wet treatment had more roots of this diameter than for the dry at the two lower depth increments (22-33 and 33-44 cm). Similar patterns were seen in the 0.3-0.4 mm diameter root class, although the differences between the upper and lower depth increments became less pronounced as root diameter increased.

In the 0.4-0.5 mm range, root lengths were less for *C. maculosa* than *R. hirta* at 22-33 cm and less for plants subjected to the wet treatment than the dry at the two greatest depths. Root length of this diameter was greater in the 33-44 depth than the 0-11 depth in wet treatment plants. The same pattern was seen in slightly larger diameter roots (0.5 to 0.8 mm), and peaked in 0.5-0.6 mm diameter roots, which had greater length at the deepest depth increment than any other depth increment in wet treatment pots. Length of roots with diameters from 0.7 to 0.9 mm differed between species and treatment. The two forbs had similar lengths of these roots for the wet treatment, whereas *R. hirta* had much greater length of this diameter than *C. maculosa* in dry treatment plants.

Roots ranging from 0.9 to 1.4 mm, only differed by depth, and generally had decreasing root length with increasing soil depth (Fig 3.12). Roots greater than 1.4 mm were more frequent in the surface soil depth (0-11 cm) for *R. hirta*, and the 11-22 cm depth for *C. maculosa*, and were nearly absent from the deeper depths (22-33 and 33-44

cm). The largest diameter roots (>1.9 mm) were more common in the surface (0-11 cm) depth than any other depth increment for both species.

Discussion

Biomass

Rudbeckia hirta had much higher total biomass than *C. maculosa*. This likely reflects, in part, the fact that all *R. hirta* individuals bolted during our study, whereas none of the *C. maculosa* plants did. Weedy species are often characterized by relatively high rates of growth (Grime 1977; Huston and Smith 1987), and minimizing time to reach maturity may be an important trait of invasive species (Rejmanek and Richardson 1996). However, it seems unlikely that high growth rate is a significant factor for *Centaurea maculosa*'s invasive success. It did not grow faster than two late-seral native grasses in a previous study (Blicker et al. 2003), nor faster than *R. hirta* in this study. This difference in plant growth rate may be further related to the fact that *C. maculosa*, which is often found on disturbed sites, regularly invades and dominates intact ecosystems, whereas (in Montana, USA) *R. hirta* seems to be more limited to recently disturbed sites (Lackschewitz 1991; Tyser and Key 1988). Additionally, our soil mix contained high levels of NO₃-N (~20 ppm) which may have been used differently by the two species.

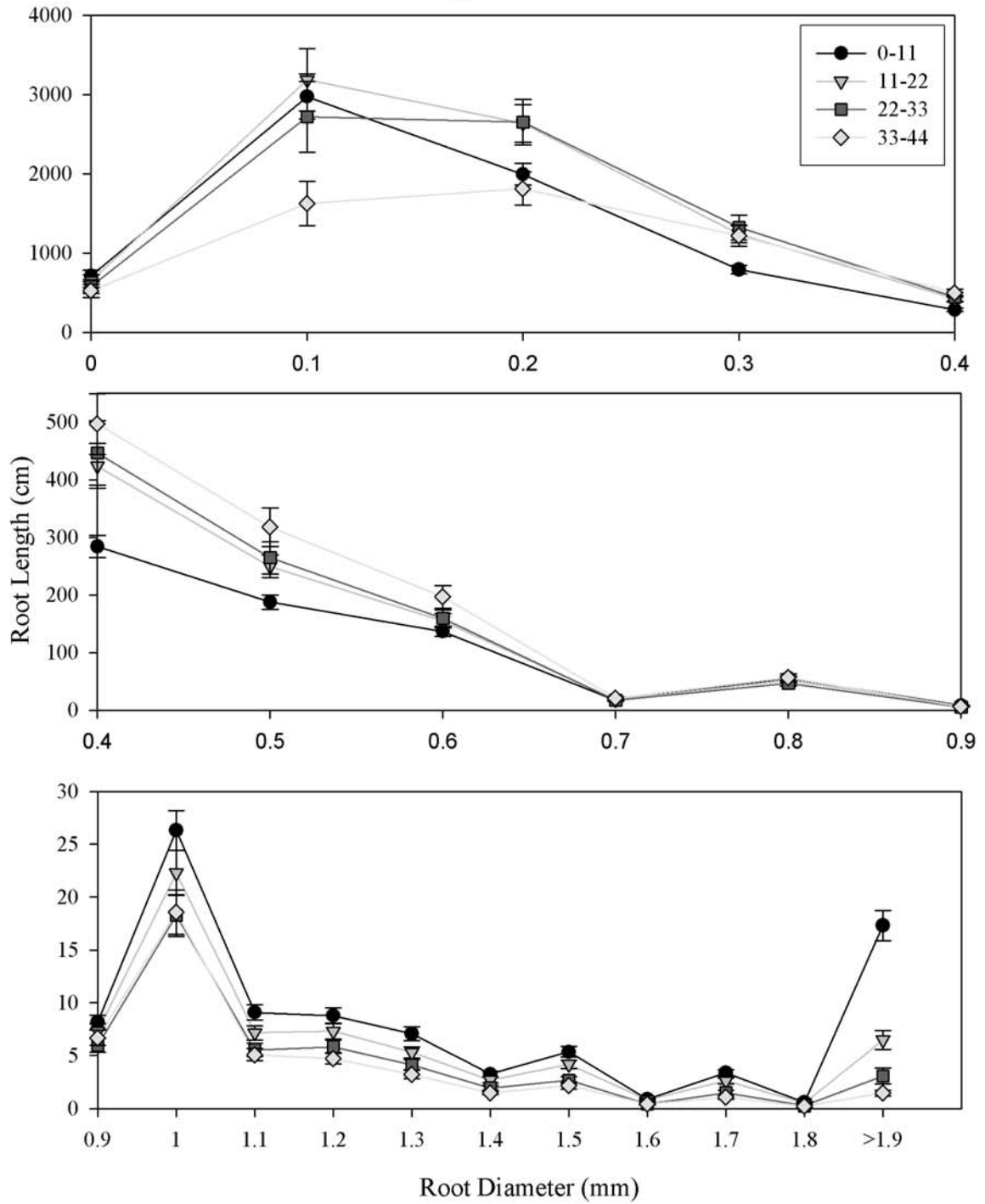


Fig 3.12. Mean length of root (\pm SE) in each diameter class for each soil depth increment.

Root mass ratio

Root mass ratio (RMR) indicates how a plant partitions resources between roots and shoots with a higher RMR meaning increased root system allocation relative to overall plant biomass. For this study, RMR was higher in *C. maculosa* than *R. hirta*. However, RMR was determined while the two species were at different phenologic stages. *Rudbeckia hirta* plants were all flowering at time of harvest, while *C. maculosa* plants remained rosettes. Bolting increases the amount of biomass aboveground which reduces RMR.

RMR was also higher for plants in the dry treatment. This likely indicates greater allocation to roots in response to drier soils (Navas and Garnier 2002; Tomlinson and Anderson 1998). However, RMR is not always strongly affected by variable soil water availability (McConnaughay and Coleman 1999; Pooter and Nagel 2000). Overall, RMRs were much lower than expected, perhaps due to very high NO₃-N levels in the topsoil used for our study (Brouwer and de Witt 1969).

Root System Size

Greater root system size is linked to increased competitive ability (Aerts et al. 1991; Berntson and Wayne 2000; Ennik and Hofman 1983) as larger root systems take up more physical space belowground and provide the plant access to a greater portion of soil resources (McConnaughay and Bazzaz 1991). Generally, greater occupation of soil space is considered the principle determinant of belowground competitive success (Casper and Jackson 1997). However, massive root systems require significant inputs from the plant to build and maintain, and may experience high levels of intra-plant

competition which could decrease total uptake (Kramer and Boyer 1995). Smaller root systems require less carbon inputs for structure and respiration, are often more plastic in morphology, and depending on their ability to selectively exploit nutrient and water-rich patches within the soil matrix, can be very competitive (Berntson 1993; Jackson and Caldwell 1989).

All measurements of root system size (e.g., root mass, length, volume, surface area) responded consistently between species and treatments. *Centaurea maculosa* had smaller root systems than *R. hirta* in response to both soil water treatments. Allocation differed somewhat between the two species; generally *C. maculosa* had less roots in the deeper soil layers, especially in response to the dry soil water treatment.

Centaurea maculosa had fewer roots in greater soil depths when subject to the dry soil treatment than *R. hirta*, which is unexpected based on habitat differences between the two species. *Rudbeckia hirta* is native to the more mesic midwestern United States, whereas *C. maculosa* invasions tend to be more dense in drier systems (Lackschewitz 1991; Mooers and Willard 1984). Greater root allocation at greater depths in response to dry soils is a strategy common to many drought-tolerant species (Carrow 1996; Hurd 1974). Furthermore, previous studies have shown that *C. maculosa* uses more water at deeper depths than perennial grasses during the late, driest part of the growing season (Sperber 2001; Swan 2004).

As mentioned previously, *C. maculosa* may have a lower relative growth rate (RGR) than *R. hirta* and that our study did not allow sufficient time for the bulk of *C. maculosa*'s roots to reach greater depths. Alternatively, *C. maculosa* plants in the field

may increase rooting depth early in the season when the soil profile is near field capacity and may not be able to effectively increase root depth in dry soils. Our dry soil column water treatment was applied early in plant development, and remained uniform throughout the study period.

Root System Efficiency

In relation to measurements of root system efficiency (specific length, average diameter, root length density), *C. maculosa* again lagged behind *R. hirta*. Although fine roots comprised the bulk of both species root systems, *C. maculosa* had fewer fine roots than *R. hirta*, and fewer roots of all diameters that reached the deeper soil depth increments, especially in the dry treatment. *Centaurea maculosa* also had lower specific root length (length/mass) and root length density (root length/soil volume), both of which are functionally related to root diameter (large root diameters lead to low specific length, low relative root length) (Atkinson 2000). The length of roots of every diameter class decreased in response to the dry soil treatment, as did the RLD. However, specific length did not vary in response to soil water treatment, indicating that all diameters decreased in a similar fashion in response to the low water treatment. In general, efficiency responses varied according to soil depth, with the greatest values at the shallowest depth increments, indicating higher amounts of fine roots at these depths.

Root Diameter Classes

A tradeoff exists between fine and coarse roots. Fine roots have high surface area and lower cost, but are typically short-lived. Coarse roots grow quickly and are able to be

maintained over longer periods of time, but have lower surface areas and therefore lower uptake potentials for immobile nutrients and mobile nutrients in dry soils (Fitter 1994). Most of the root system of both our plant species was comprised of fine (>0.5 mm) roots. There were small differences in the diameter of maximal root length. *R. hirta* had greatest length in the 0.1-0.2 mm diameter increment, whereas *C. maculosa* had more roots in the slightly higher diameter class (0.2-0.3 mm). To our knowledge, there is no information in the literature regarding possible effects of small differences in root diameter, but such a small difference in root diameter of greatest length seems unlikely to be a defining character influencing competitive interactions.

Root length of the finest diameter classes was less in dry treatment pots for both species. Dry soils reduce root growth due to increasing forces holding soil particles together (Westgate and Boyer 1985 as presented in Chen and Leith 1993). Optimality theory predicts growth of fine roots may not be as cost effective in dry environments because water availability affects the availability of soil nutrients including nitrogen and phosphorous (Bloom et al. 1985; Harper et al. 1991; Nye and Tinker 1977). In resource-limited sites, larger diameter, longer lived roots may be favored due to the high cost associated with fine root turnover (Eissenstat and Yanai 1997; Espeleta and Donovan 2002). Alternatively, long lived roots may be favored in high nutrient environments because of the benefits (nutrients) associated with keeping them alive (Burton et al. 2000). Patterns of fine root demography in different environments is still unclear (Eissenstat and Yanai 1997).

Overall, lengths associated with the root diameter classes varied more by soil depth than they did by species. Fine roots, and the large diameter roots associated with tap-root structures, were more common in the upper soil depths, whereas roots of mid-range diameters (0.4 to 0.8 mm) had greater lengths in deeper depth increments. High nitrogen levels in our soil mix may have influenced root distribution.

Based on the results of our study, root characteristics of the invasive forb, *C. maculosa*, differed significantly from the naturalized, non-invasive forb, *R. hirta*. We had hypothesized that the invasive *C. maculosa* would exhibit greater biomass, root system size and efficiency characteristics than the non-invasive *R. hirta*. However, for our study, the opposite proved true. *Rudbeckia hirta* had higher total and root biomass for both soil water treatments, as well as consistently greater characteristics of root system size and efficiency than *C. maculosa*. Although this study focused on general morphological traits and did not address other root characteristics that might influence competitive interactions such as root hairs and root architecture (Fitter 1987; Fitter and Strickland 1991; Harper et al. 1991), mycorrhizal associations (Landhausser et al. 2002; RuizLozano and Azcon 1995), or plasticity (Berntson 1993; Jackson and Caldwell 1989), our results indicate that characteristics other than those related to root system morphology may be responsible for the success of the invasive *C. maculosa* in the semi-arid rangelands of Montana (USA).

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CHAPTER 4

SUMMARY

The objectives of this study were to 1) determine spatial and temporal soil water uptake patterns of *C. maculosa* Lam., the naturalized forb *Rudbeckia hirta*, L., and resident perennial grasses under ambient soil moisture conditions in the field; and 2) compare selected root system attributes of *C. maculosa* and *R. hirta* in response to variable soil water conditions. We predicted that *C. maculosa* might have a superior ability to access soil water or might possess a rooting systems of greater size and efficiency which might contribute to its invasive success in semiarid grasslands.

We measured temporal changes in soil water content during two growing seasons under monocultures of *C. maculosa*, perennial grasses and the naturalized forb *R. hirta*, to determine whether *C. maculosa*'s water uptake patterns differed significantly from the other species. We found that *C. maculosa* generally used more water than grass when averaged over the growing season and all depths. Furthermore, *C. maculosa* used more water than perennial grasses later in the growing season and at depths greater than 0.4 m. The non-invasive tap-rooted forb *R. hirta* had soil water use patterns similar to *C. maculosa*.

Because periods of greater water use by *C. maculosa* occurred later in the growing season when native bunchgrasses are typically quiescent, direct competition between *C. maculosa* and perennial grasses is unlikely to be occurring. However, *C. maculosa* continued active growth longer than the perennial grasses at our study site. A

longer period of growth could lead to increased nutrient depletion and greater root system size, both of which could negatively impact perennial grass communities. In addition, the similar water uptake patterns of the two tap-rooted forbs suggest that *C. maculosa*'s water uptake pattern may not be unique to this species, but may be common to many late-season tap-rooted forbs, many of which are not invasive to semiarid grasslands. Therefore *C. maculosa*'s invasive success cannot be explained by its soil water uptake patterns alone.

We also grew *C. maculosa* and *R. hirta* in pots under wet and dry soil water conditions to compare the root characteristics of these two plants in detail. We harvested roots in four depth increments and analyzed them for selected root attributes of size and efficiency. For our study, *R. hirta* generally had greater root system size and efficiency than *C. maculosa* in response to both soil water treatments. Species did not vary in response to soil water treatment on a whole pot basis, but there were differences between species root systems when analyzed by depth increment. Generally, *C. maculosa* had fewer roots at the greatest depth increments, and a stronger decrease in roots at the greatest depth increments in response to the dry soil water treatment. In addition, *C. maculosa* had lower total biomass than *R. hirta* in response to both soil water treatments.

The results of our greenhouse study indicate that *C. maculosa* does not possess root systems of greater size or efficiency than the non-invasive forb *R. hirta* for the root characteristics that we measured. This result was surprising since root systems of greater size often show greater competitive ability, and furthermore high root system efficiency is important to plant competition in patchy or low resource environments such as western

Montanan grasslands.

The results of our study suggest that *C. maculosa*'s invasive success cannot be attributed entirely to greater competition for soil water or to root systems of greater size or efficiency. Overall, *C. maculosa* did not appear to have enhanced root system characteristics when compared to a non-invasive tap-rooted forb of similar phenology. It is possible that other characteristics, such as greater plasticity in water use efficiency, or prolific seed production and high germination success, may be responsible for *C. maculosa*'s success on western Montanan grasslands.

APPENDICES

APPENDIX A

SOIL PROFILES FOR FIELD STUDY SITES

Site Name: Helena
Site Location: 21 km southeast of East Helena, Montana in Broadwater County
Latitude: 46° 33' 1"
Longitude: 111° 45' 38"
Elevation: approximately 1285 m
Slope: approximately 0.7%
Aspect: Northeast
Vegetation: *Centaurea maculosa*, *Pseudoroegneria spicata*, *Artemisia cana*, *Poa pratensis*,
Melilotus officinalis, *Bromus inermis*
Depth of Soil Pit: 112 cm

Depth (cm)	Horizon Classification	Horizon Description
0 - 5	A	7.5YR 4/2 dry, 7.5YR 2.5/2 moist silt loam; granular structure; 2 % rocks; pH 7
5 - 14	Bw	7.5YR 4/3 dry, 7.5YR 2.5/2 moist clay loam/silt loam; blocky structure; 5% rocks; pH 7, slight HCl reaction
14 - 31	Bk1	7.5YR 7/2 dry, 7.5YR 6/3 moist clay; massive structure, powdery; pH 8, violent HCl reaction
31 - 120	Bk2	5YR 8/1 dry, 5YR 7/2 moist clay; massive structure, powdery; pH 8.7, violent HCl reaction

Series: Rothiemay
 Fine-loamy, mixed, superactive, frigid Aridic Calcistolls
Taxonomic Class:

Site Name: Leverich
Site Location: 8 km S of Bozeman, Montana in Gallatin County
Latitude: 45° 36' 5"
Longitude: 111° 2' 45"
Elevation: 1615 m
Slope: 2%
Aspect: North
Vegetation: *Centaurea maculosa*, *Phleum pratense*, *Dactylis glomerata*, *Bromus inermis*,
Pseudoroegneria spicata, *Silene vulgaris*
Depth of Soil Pit: 112 cm

Depth (cm)	Horizon Classification	Horizon Description
0-22	A	10YR 3/2 dry, 10YR 2/2 moist silt loam; fine granular structure; 5% coarse fragments; pH 6.5-7.0
22-38	Bt1	10YR 4/6 dry, 10YR 3/3 moist gravelly loam; subangular blocky structure; 25% coarse fragments; pH 6.5
38-104	Bt2	10YR 3/6 dry, 10YR 3/4 moist gravelly loam; subangular blocky; 10% coarse fragments; pH 7.0
104+	BC	10YR 5/6 dry, 10YR 3/4 moist gravelly sandy loam; massive; 35% coarse fragments; pH 7.0-8.0

Series: Farside
Taxonomic Class: Fine-loamy,mixed, frigid Typic Argiustoll

APPENDIX B

ANOVA TABLES REFERENCED IN CHAPTER 2

Soil water content ANOVA p-values for depletion period (DOY 181-262) of both study years at the Helena site.

Factor	df	p-value
2002		
Depth	1	<0.0001
Top	1	<0.0001
DOY	6	<0.0001
Species	2	<0.0001
Depth*DOY	6	<0.0001
Depth*Species	2	0.63
DOY*Species	12	0.65
Depth*DOY*Species	12	0.89
2003		
Depth	1	<0.0001
Top	1	<0.0001
DOY	6	<0.0001
Species	2	<0.0001
Depth*DOY	6	0.06
Depth*Species	2	0.0002
DOY*Species	12	0.65
Depth*DOY*Species	12	1.0

Soil water content ANOVA p-values for depletion period (DOY 181-262) of both study years for the Leverich site.

Factor	df	p-value
2002		
Depth	5	0.02
DOY	7	<0.0001
Species	2	<0.0001
Depth*DOY	35	1.0
Depth*Species	10	<0.0001
DOY*Species	14	0.006
Depth*DOY*Species	62	1.0
2003		
Depth	5	<0.0001
DOY	6	<0.0001
Species	2	<0.0001
Depth*DOY	30	0.67
Depth*Species	10	<0.0001
DOY*Species	12	0.84
Depth*DOY*Species	53	1.0

ANOVA p-values for cumulative change in soil water storage by depth for species, day of year (DOY) and the interaction of species and DOY effects for Helena.

	p-values			
	Depth	Species	DOY	Species*DOY
2002				
	20	<0.001	<0.001	0.25
	40	<0.001	<0.001	0.33
	60	<0.001	<0.001	0.43
	80	0.0095	<0.001	0.90
	100	0.0033	<0.001	0.74
2003				
	20	0.44	<0.001	0.22
	40	0.18	<0.001	0.88
	60	<0.001	0.0039	0.64
	80	<0.001	0.012	0.54
	100	0.26	0.21	0.97

ANOVA p-values for cumulative change in soil water storage by depth for species, day of year (DOY) and the interaction of species and DOY effects for Leverich.

	p-values			
	Depth	Species	DOY	Species*DOY
2002				
	20	<0.001	<0.001	<0.001
	40	<0.001	<0.001	<0.001
	60	<0.001	<0.001	0.22
	80	<0.001	<0.001	0.020
	100	<0.001	<0.001	<0.001
	120	0.020	<0.001	0.16
2003				
	20	0.35	<0.001	0.62
	40	0.24	<0.001	0.76
	60	0.0081	<0.001	0.98
	80	0.020	<0.001	0.71
	100	<0.001	<0.001	0.49
	120	<0.0038	<0.001	0.029

APPENDIX C

ANOVA TABLES REFERENCED IN CHAPTER 3

ANOVA p-values for whole pot effects

Response	Species	Treatment	Species*Treatment
Total biomass	<0.001	<0.001	0.34
Root mass ratio	<0.001	0.0062	0.72
Root mass	0.013	0.0018	0.46
Root length	<0.001	<0.001	0.97
Root length density	<0.001	<0.001	0.27
Surface Area	<0.001	<0.001	0.75
Specific length	<0.001	0.366	0.99

ANOVA p-values with depth as factor for depth effect and depth interactions.

Response	Depth	Depth*Species	Depth*Treatment	Species*Depth*Treatment
Root mass	<0.001	0.018	0.0060	0.84
Root length	<0.001	0.060	0.015	0.71
Root length density	<0.001	0.86	0.21	0.41
Surface Area	<0.001	0.014	0.023	0.46
Specific length	<0.001	<0.001	0.14	0.93
Root volume	<0.001	0.038	0.034	0.76
Average diameter	<0.001	0.27	0.24	0.21