



AN ABSTRACT OF THE DISSERTATION OF

Eric J. Dinger for the degree of Doctor of Philosophy in Forest Resources presented on May 17, 2012.

Title: Characterizing Early-Seral Competitive Mechanisms Influencing Douglas-fir Seedling Growth, Vegetation Community Development, and Physiology of Selected Weedy Plant Species

Abstract approved: \_\_\_\_\_

Robin Rose

Three studies were conducted to characterize and present early-seral competition between Douglas-fir seedlings and the surrounding vegetation communities during Pacific Northwest forest establishment. The first experiment served as the foundation for this dissertation and was designed to quantify tradeoffs associated with delaying forest establishment activities by introducing a fallow year in order to provide longer-term management of competing vegetation. A range of six operationally relevant treatments were applied over two growing seasons that included in the first (1) a no-action control, (2) a spring release only, (3) a fall site preparation without sulfometuron methyl followed by a spring release, as well as (4) a fall site preparation with sulfometuron methyl and a spring release. In the second year, there was (5) a fall site preparation without sulfometuron methyl followed by a spring release and also in the second year (6) a fall site preparation with sulfometuron methyl and a spring release. Treatments 5 and 6 were left fallow without planting during the first year. These treatments were applied in two replicated experiments within the Oregon Coast Range.

After adjusting for initial seedling size, year-3 results indicated that plantation establishment and competition control immediately after harvest (i.e. no fallow

period) enabled seedlings to be physically larger than those planted after a one year delay. At the Boot study site, limiting vegetation below 20% for the first growing season improved year-3 Douglas-fir seedling stem volume over 273 cm<sup>3</sup>. Delaying establishment activities one year and reducing competing vegetation below 11% enabled seedling volume after two years to be statistically the same as three year old seedlings in the no-action control, a volume range of between 148 to 166 cm<sup>3</sup>. Delaying forest establishment at Jackson Mast improved seedling survivorship over 88% when a spring heat event reduced survivorship of trees planted a year earlier to less than 69%. The combined effect of applying a fall site preparation and spring release was necessary to reduce competitive cover below 10% in the year following treatment and provided longer-lasting control of woody/semi-woody plants. Less intense control measures (i.e. no-action control and treatment 2) were not able to restrain woody/semi-woody plant cover which grew to nearly 40% at Boot and over 24% at Jackson Mast in three years. No treatment regime provided multi-year control of herbaceous species. Including sulfometuron methyl in the fall site preparation tank-mix did not have a negative effect on seedling growth or provide significant reductions in plant community abundance in the year following application when compared to similar regimes that did not include the chemical. Delaying establishment lengthened the amount of time associated with forest regeneration except on a site that accentuated a spring heat event.

In the second study, horizontal distance and azimuth readings provided by a ground-based laser were used to stem map seedling locations and experimental unit features at Boot. These data were used to create a relative Cartesian coordinate system that defined spatially explicit polygons enabling, for the first time, the ability to collect positional data on competing forest vegetation within an entire experimental unit. Deemed “vixels” or vegetation pixels, these polygons were assessed for measures of total cover and cover of the top three most abundance species during the initial three years of establishment. An alternate validity check of research protocols was provided when total cover resulting from this vixel technique was compared to a

more traditional survey of four randomly located subplots. The resulting linear regression equation had an adjusted  $R^2$  of 0.90 between these two techniques of assessing total cover. When compared within a treatment and year, total cover differed by less than 12 percentage points between the two techniques. Analysis of year-3 woody/semi-woody plant cover produced by the techniques led to identical treatment differences. Two treatments resulted in woody/semi-woody cover of approximately 1500 ft<sup>2</sup> by the vixel method and nearly 40% cover by the subplot method while the remaining four treatments were grouped below 600 ft<sup>2</sup> or 20% cover, respectively. With continued refinement, these techniques could visually present forest development through all phases and provide long-term information used to bolster growth and yield models, measures of site productivity, as well as community ecology research.

The third study evaluated the season-long gas exchange and biomass partitioning of four weedy plant species capable of rapidly colonizing Pacific Northwest regenerating forests. *Cirsium arvense*, *Cirsium vulgare*, *Rubus ursinus* and *Senecio sylvaticus* were studied at two sites. A greenhouse was used to introduce two levels of irrigation (well-watered and droughty). These species were also studied while growing among a larger vegetation community at a field site. Irrigation treatments had little impact on gas exchange rates. Species achieved maximum photosynthetic rates of 30, 20, 15 and 25  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  (respectively) prior to mid-July coinciding with an active phase of vegetative growth. As the season progressed, photosynthetic rates declined in spite of well-watered conditions while transpiration rates remained relatively consistent even when soil water decreased below 0.25 m<sup>3</sup> H<sub>2</sub>O/m<sup>3</sup> soil. Water use efficiency was high until late-July for all study species, after which time it decreased below 5  $\mu\text{mol CO}_2 \cdot \text{mmol H}_2\text{O}^{-1}$ . Multi-leaf gas exchange measurements as well as biomass data provided a holistic view of plant-level mechanisms used to shunt activity toward developing tissues. Herbaceous species had assimilation rates that differed vertically (within each species) by as much as 10 to 20  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  from July to September as lower leaves senesced in

favor of those higher on study plants. Specific leaf area was greatest in June for all species then declined indicating species placed little effort into sacrificial early season leaves when compared to those higher on the plant that could continue to support flowering or vegetative growth. The study of seasonal gas exchange in the presence of declining water availability has helped to describe competitive mechanisms at work during forest regeneration as well as provide physiologic support for the application of vegetation management regimes.

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Characterizing Early-Seral Competitive Mechanisms Influencing Douglas-fir  
Seedling Growth, Vegetation Community Development, and Physiology of Selected  
Weedy Plant Species

by

Eric J. Dinger

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APPROVED:

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I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

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Eric J. Dinger, Author

## ACKNOWLEDGEMENTS

My process of becoming a reforestation scientist began as a child. When I look back and see what it has taken to get here, at the culmination of this degree and the continuation of a life-long academic adventure, I can not help but feel that this is an incredible gift. None of this would have been possible without God's faithful provision as well as the support of family, friends, and mentors.

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I have written this dissertation in the hopes that it can be a kind of encouragement for you, the unknown reader, as I share the passion I have been given for reforestation, discovery, and service to the people in my life.

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## **CHAPTER 1.0**

### **INTRODUCTION AND LITERATURE REVIEW**

#### **1.1 Succession and Silviculture**

The physical act of harvesting a Pacific Northwest (PNW) conifer forest dramatically increases the availability and quality of light, soil water, and has the potential to scarify the upper soil horizon (Bazzaz 1979). These activities introduce a disturbance that can provide the stimulus and growing conditions necessary for the development of early-seral plant species (Bazzaz 1979; Dyrness 1973; Radosevich and Osteryoung 1987). West of the Cascade crest in Oregon, this developing plant community can be a diverse and complex assemblage of species representing a variety of taxa and growth habits (Chen 2004; Dinger and Rose 2009; Halpern 1989).

In this open disturbed environment, plant community development follows various secondary successional trajectories as residual sprouting species, those germinating from the existing seed bank, and seed dispersed to the site begin to simultaneously colonize (Bazzaz 1990; Clements 1916; Duke 1985; Schoonmaker and McKee 1988). As this plant community grows and exploits these open conditions, roots begin to overlap and canopies begin to shade one another creating intense competition for limited site resources (Antos and Halpern 1997; Balandier et al. 2006; Goldberg 1990). The PNW has a Mediterranean climate marked by a pronounced summer drought period making soil water a primary limiting resource during the initial years of forest establishment (UW 2007; Petersen et al. 1988; Harrington and Tappeiner 1991). Researchers have reported that herbaceous vegetation is able to rapidly exploit open conditions creating short-term competition for soil water where as woody perennial species are slightly slower to respond but have longer-lasting competitive effects (Wagner 1989; Miller et al 1991; Cain 1999; Rose and Rosner 2005).

Vegetation management prescriptions are often used to restrict plant community growth during stand initiation (Nyland 2002) where planted tree seedlings with limited root systems, are susceptible to the influences of interspecific competition for soil water (Casper and Jackson 1997; Morris et al. 1993; Zutter et al. 1986). Commonly these prescriptions either prepare the site for the arrival of the seedlings or release them from competition (Walstad and Kuch 1987) through the planned interruption of successional processes. Connell and Slayter (1977) illustrated this concept with alternate developmental pathways that permitted additional disturbances to change the course of succession. These interruptions are not permanent as open areas are again colonized by various plant species (Comeau and Harper 2009; Miller et al. 1999; Boateng et al. 2000). When appropriately applied and specific thresholds reached, these treatments have the potential to improve tree seedling survivorship and growth (Cousens 1987; Wagner et al. 1989) allowing the process of reforestation to proceed rapidly.

The length of time vegetation control is necessary to avoid growth losses is deemed the critical period (Nieto 1968; Wagner et al. 1996) and is relatively short for long-lived tree species. Occurring within the first five years of establishment, this period of time is marked by activities designed to slow or temporarily halt normal successional development and favor crop tree acquisition of site resources. As the crop trees grow, a notable shift occurs where additional competition release treatments are not required (Wagner et al. 1996). This shift is principally due to the desired tree species developing root systems capable of exploiting deeper or more contiguous soil resources with above-ground portions tall enough to limit the negative effect of reduced light interception (Sands and Nambiar 1984; Chan and Walstad 1987). The application of these principles has been shown to improve seedling access to site resources enabling well-documented improvements in growth (Rosner and Rose 2006; Miller et al. 1995; Dinger and Rose 2009; Newton and Preest 1988) and increasing the probability of the seedlings growing to become dominant life form on the site, a

characteristic mandated by Oregon law (Adams and Storm 2011, Oregon Department of Forestry [ODF] 2010).

## **1.2 Operational Forestry on Private Land**

Forests managed for the sustainable production of timber resources use various means of vegetation management to reduce competition, improve seedling access to limited site resources, and direct successional processes. While there are multiple non-chemical methods that can be used on private lands in the PNW, the application of herbicides is a common means of reducing competitive influences due to the lower cost, higher efficacy, and reduced risk of injury to personnel (Newton 2008; Wagner 1993). These compounds, labeled for use in forestry, are applied in season-specific treatments as either a fall site preparation or spring release(s). A fall site preparation is applied prior to planting tree seedlings using chemicals which are effective at controlling a broad range of perennial and annual species. Spring release treatments are applied prior to tree bud break. They are designed to provide continued control of forb and grass species during the year following the fall site preparation or release trees from competition at some future point in time when the treatment is deemed necessary. The need for additional spring release treatments is often based on application cost and an inverse relationship between the level of vegetation remaining on the site in the late-winter/early-spring and crop tree growth. Multiple herbicide applications can be required due to the breakdown of these chemicals according to soil-specific half-lives (Ahrens et al. 1994) enabling plants to recolonize these areas.

In the PNW, these fall site preparation and spring release treatments typically involve one to four applications of herbicides during the early years of establishment. These treatments are often tank-mixes of two or more different chemicals applied as a single solution. The choice between the constituent parts of these applications is based on site specific measures of target species to be controlled, training, and experience. These assessments include abundance estimates of herbaceous and woody/semi-woody vegetation as well as the species comprising these groups.

Selectivity and seedling safety is achieved through differences that exist between weedy plants and conifer seedlings including metabolic pathways blocked with different chemical choices, application prior to planting, or application during a period of seedling dormancy prior to bud break in the late-spring (Ahrens et al. 1994).

This cycle of fall site preparation and spring release may be unnecessary or ineffective if the regenerating unit of land and its vegetation community are out of phase with these regimes. While forest managers successfully apply the discussed scientific principles of vegetation management in the PNW, the specific tailoring of these principles to a situation that does not fit into the normal cycle of early establishment activities can raise considerable debate. Exacerbating this debate is the paucity of regionally relevant information available on vegetation community effects within these management situations.

A challenge to traditional early establishment regimes is illustrated by the management of a unit of land harvested during the late-spring or summer. At the typical time of fall site preparation in late-summer, plant abundance may be low because of the combined effects of harvest disturbance and summer drought. It is possible that, due to the limited development of leaf area, herbicidal chemicals may only be absorbed through root contact and that sprouting residual plants with roots below the influence of the chemicals (Ketchum et al. 1999) may be unhindered. This could make the treatment an ineffective and costly mistake.

One option is to delay forest establishment activities through the introduction of a one-year fallow period with special care to remain legally compliant with the Oregon Forest Practices Act (ODF 2010). This fallow year with no management activities would allow the vegetation community to grow and increase leaf surface area while providing the time needed to return the unit of land to common management cycles. Researchers have tested this idea of matching management cycles with the establishment of a crop and control of weedy plant species. Whether it is to accommodate the logistics of heavy mechanized equipment on wet soil conditions in the spring (Shaw 1996) or investigating the timing of management

activities to balance different rates of germination and establishment (Buhler 1997; Nielsen et al. 2002; Lauer and Quicke 2006), there are situations within a single year that require the adjustment of management techniques. Research suggests that depending on the crop and measure of merchantable yield, time dependent tradeoffs are associated with delaying activities (Helms et al. 1990).

The concept of delaying establishment a few weeks or months to achieve a particular objective appears to be sound, but it is difficult to apply this to a tree crop and establishment activities that span two different years. One obvious tradeoff is that the strategy automatically adds one year to rotation length and may not take advantage of any natural lag in growth of competing vegetation following harvest disturbance. The best techniques for adapting management practices to this situation are not understood. In fact, no forestry study has been found reporting the benefits and drawbacks of this strategy with respect to Douglas-fir seedling growth or response of the competing herbaceous and woody/semi-woody species. It may be possible to adapt current management regimes, but a side by side test of these options in a replicated experiment is needed to better understand ecologic consequences as well as inform forest establishment decisions.

### **1.3 Characterizing and Presenting Early Stand Conditions**

Assessing vegetation communities at the species level for measures of abundance and distribution require on-site visual estimates. While technology is being developed and used successfully to detect various plant communities through remotely sensed data (Omasa et al. 2007; Hopkinson et al. 2004; Tarp-Johansen 2002), there are no known computerized or photographic methods that can characterize individual species with varying abundances that overlap in a three dimensional matrix associated with complex early-seral vegetation community. Despite potential sources of human error, a trained botanist can easily distinguish these characteristics. Botanists conducting vegetation surveys can identify individual species at various stages of

development as well as locate plants within a sampling frame that would otherwise be unobserved due to small stature, low individual numbers, or visual obstructions.

Researchers have reported strong positive correlations between measures of plant cover abundance and levels of competition as forests develop (Greig-Smith 1983; Rose et al. 1999; Cole and Newton 1986). Vegetation surveys are typically conducted on a smaller number of randomly selected subsamples within experimental units (Comeau and Harper 2009; Dinger and Rose 2009; Halpern 1989; Maguire et al. 2007; Rosner and Rose 2006, Zutter et al 1986). Experimental units can be relatively large and permanent plot subsamples of the plant community generally occur on smaller areas. Spatial and temporal sources of variation exist within plant communities, even on relatively small scales. Alternate vegetation assessment methods could permit a better understanding of how well small subsamples of the plant community characterize the overall variation within experimental units.

Graphical images of this complexity in response to spatial distribution and growth development have not been found in the forest vegetation management literature. Methods for presenting the results of early-seral forest plant community development commonly include graphical trend lines, tables of means, or long species lists. Ecological studies have illustrated concepts of colonization and expansion using Cartesian coordinate data in scatter and contour plots of experimental units (Rossi et al 1992; Greig-Smith 1983; Cromack and Ord 1979). As an example, Kooijman (1976) studied the colonization of acorn barnacles on the side of a ship hull in the Netherlands producing figures that incorporate spatial data. These data were used to show a key component of interpreting the randomness associated with spatial distribution patterns and the size of individuals inherent in ecological data (Cromack 1979; Rossi et al 1992; Kooijman 1976). Techniques need to be developed to characterize and present this spatial complexity to further the understanding of early-seral forest plant communities.

Programs such as the Stand Visualization System were designed as an educational aid to present images of forests under different silvicultural regimes

(McGaughey 1998; Roth and Finley 2007). Mapping procedures presented by Ek (1969) have the ability to create this kind of information, but these visualization and data collection techniques have not been applied to a regenerating forest. Commonly, these stem mapping efforts focus on older more mature stands as illustrated by Panandeh (1974) who used Ek's work in conjunction with additional data to present the spatial patterns of forest stands that would be managed using mechanized equipment. Spatial analytic procedures were used to present the distribution patterns (nearest neighbor and Ripley's  $K(d)$  analysis) of trees in a northern Idaho old growth forest by Moeur (1993). The analysis focused solely on the interactions of trees or groups of trees and helped define how past stand development could result in the patterns currently observed. These techniques and the resulting data have not been adapted to the establishment phase of forest development or included methods for assessing plant community development and species turnover associated with successional processes affected by silvicultural regimes.

Techniques that accurately portray early seral conditions as well as the dynamic changes of the vegetation community are needed. Beyond demonstration purposes of silvicultural regimes, these methods could be used to challenge assumptions of how well subplots portray conditions in studies with experimental designs. Presenting graphical images of these plant communities will require the integration of field data with forest visualization software and other graphical programs. Finally, there is room to develop techniques to understand how plant communities develop and individual species respond to silvicultural regimes.

#### **1.4 Weedy Plant Physiology**

Measures of species abundance and expansion are typically used to quantify levels of competition in forested systems and hence the need for silvicultural intervention. While these abundance data are closely associated with competition in early forest environments, they represent a coarser-scale measure of much finer physiological processes leading to observed growth responses. The physiologic

attributes of gas exchange and morphologic development that make certain species successful at colonizing disturbed sites remains a fundamental unknown in the PNW forest establishment literature.

*Cirsium arvense* (Canada thistle), *Cirsium vulgare* (bull thistle), *Senecio sylvaticus* (woodland groundsel), and *Rubus ursinus* (trailing blackberry) are commonly associated with regenerating forest plant communities in the PNW (Halpern 1989; Dyrness 1973; Schoonmaker and McKee 1988; West and Chilcote 1968; Rose and Ketchum 2002). These species represent a range of life history strategies (Grime 2002; Grace and Tilman 1990) including annual to perennial life spans, herbaceous and semi-woody growth habits, reproductive methods, and to a forest manager, different tactics that can be used to control their influence on planted trees. Studies have defined certain physiologic aspects of reproduction (McDowell and Turner 2002; Lalonde and Roitberg 1994), competitive characteristics (Randall and Rejmánek 1993; Nkurunziza et al. 2010), responses to rises in atmospheric CO<sub>2</sub> (Ziska 2002, Ziska et al. 2004), and general autecology of these species (Michaux 1989; Heimann and Cussans 1996). A degree of physiologic knowledge is missing despite this amount of information. *S. sylvaticus*, for example, has been studied since the late-1960's and various aspects of physiology and dispersion are reported (Halpern et al. 1997; West and Chilcote 1968; van Andel and Vera 1977; Fioretto and Alfani 1988). However, there are no published gas exchange rates for this species that would aid in characterizing the link between the photosynthetic process and the growth responses observed during forest establishment.

Research has generally defined traits that confer competitive advantages in disturbed habitats including high carbon fixation rates, high specific leaf area, rapid growth, large effort in seed production, and rapid utilization of site resources and/or ability to use resources at lower levels than other plants (Huston and Smith 1987; Bazazz 1999; Larcher 2003). Researchers have reported comparative results on plant species utilization of light, water, or nutrient availability in an attempt to understand how co-occurring or congener plants may compete in particular environments (Brock

and Galen 2005; Feng et al. 2007; Patterson and Flint 1983). The majority of these gas exchange measurements occur within a relatively short period of time using methods that can not account for seasonal developmental patterns. Longer time frames that include an entire season of growth have been studied on shrub and tree species (McAlpine 2008; Limousin et al. 2010; Ow et al. 2010). While this research has produced a significant amount of knowledge regarding the competitiveness of certain species, the work has been done in settings that are different from that of a developing forest in the PNW, making it difficult to extrapolate results. The PNW has a pronounced summer drought period and it is of keen interest to understand how species competing with crop trees regulate the gas exchange process, utilize soil water, and grow on a season-long timescale.

## **1.5 Summary of Dissertation**

Wagner (1993) proposed that vegetation management studies need to be years ahead of current forest management practices in order to develop research that can be used to meet silvicultural challenges. The three studies reported in this dissertation are focused on one primary aspect of Wagner's overall goal, defining competitive mechanisms between Douglas-fir seedlings and the vegetation community during the initial three years of establishment. Operationally relevant management practices may be adapted to particular management situations, but this process requires the coordinated study of Douglas-fir seedling response as well as the surrounding plant communities. Within the PNW forest establishment literature, new techniques that challenge traditional research methods of presenting stand conditions need to be developed. Little work has been done to assess the accuracy of vegetation survey results, and spatially explicit graphical data on plant communities in the early-seral environment do not exist. While there is evidence to suggest weedy plants do not compete equally, a lack of data exists that could aid in defining the physiology of how species of interest grow and utilize limited site resources. This dissertation was

designed to begin answering some of these questions and provide information that can lead to the continued refinement of best management practices.

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## CHAPTER 2.0

### DELAYING DOUGLAS-FIR (*Pseudotsuga menziesii*) SEEDLING ESTABLISHMENT TO IMPROVE THE EFFICACY OF VEGETATION MANAGEMENT REGIMES

#### 2.1 Introduction

This study was designed to test strategies that can be adapted to deal with a Pacific Northwest (PNW) forest regeneration scenario. Production schedules as well as state reforestation law (Adams and Storm 2011, ODF 2010) dictate that stand establishment begins within one year of harvesting operations through the careful application of silvicultural prescriptions. On managed timber lands, a site harvested in the late-spring or summer may be relatively devoid of vegetation at the time a chemical site preparation treatment would normally be applied. The effectiveness of herbicides depends upon maximizing the potential for contact and absorption into plant tissues (Colquhoun 2001). It has been shown that if sufficient leaf area does not exist, it may be difficult to control certain PNW plants, particularly those residual sprouting species that have deep well-established root systems (Ketchum et al. 1999). In this context, applying a chemical site preparation may be an unjustifiable expense as difficult-to-control residual plants may not absorb enough of the herbicidal chemicals to reduce their abundance and competitive effect.

In an attempt to improve the efficacy of herbicidal chemicals, foresters may choose to create a fallow period where no management activities occur. After allowing the vegetation community to grow unhindered for one growing season, a chemical site preparation is applied in the second fall after harvest with seedlings planted that winter in the hopes of providing a better start to the establishment of the next stand. It is possible that this strategy may result in more lasting control of residual plant species, but the decision automatically lengthens the time associated with the regeneration period. The decision may also fail to take advantage of the

harvesting disturbance and the natural lag in competition from the developing vegetation community. In the end, this decision could represent a significant financial mistake.

The concept of delaying establishment activities has been studied in the agricultural literature. Crop production systems have required the evaluation of techniques that delay seed sowing to accommodate the logistics of heavy mechanized equipment in the presence of wet spring soil conditions (Shaw 1996). Weed management considerations also include matching the biology of the plants to be controlled (Buhler et al. 1997) with the physiologic needs of the crop during establishment and growth (Buhler and Gunsolus 1996; Nielsen et al. 2002). Results from these studies indicate that delaying crop establishment can decrease weed abundance across a growing season through more complete control. However, this delay may reduce crop yield and potential profits (Helms et al. 1990) depending on the cultivar, final merchantable product, and weather patterns associated with the growing season.

Silvicultural research has investigated the timing of particular management activities. Forest nursery studies have reported the optimum timing for seed sowing in order to maximize germination and growth of seedlings (Jinks and Jones 1996; Morris et al. 2000). Forest scientists in the Southeastern United States have conducted research to understand the timing of establishment regimes necessary to maximize early pine plantation growth through adequate vegetation control. Lauer and Quicke (2006) reported the optimum time to spray imazapyr in order to provide long-term control of woody vegetation was from July to September. Associated with this study, various combinations of the timing and frequency of mechanical and herbicidal control measures were applied over the course of a single year (Lauer and Zutter 2001; Zhao et al. 2008). In these systems, where both woody and herbaceous vegetation competition can be intense, effective mechanical control (e.g. bedding) as well as both pre- and post-plant vegetation control were required to significantly improve pine growth (Lauer and Zutter 2001; Zhao et al. 2008).

One Pacific Northwest study incorporated delaying seedling establishment of two different crop tree species across multiple years. Radosevich et al. (2006) and Grotta et al. (2004) investigated the potential for timber production benefits using mixed stands of Douglas-fir and red alder, a nitrogen fixing symbiont. Given the rapid juvenile growth rate of red alder, one treatment included delaying alder establishment for five years in order to limit the competitive interaction with Douglas-fir. These studies utilized a common experiment that was focused on assessing future stand characteristics associated with species composition (Radosevich et al. 2006) and the effects on stem and wood quality (Grotta et al. 2004).

The challenge common to all of these studies is that any management delay falls within a single year or does not incorporate establishment regimes that can be adapted to PNW managed forests. Traditionally forest managers use a combination of a fall site preparation and spring release treatment(s) to reduce competing vegetation. Fall site preparations are applied from July to October, three to six months prior to the planting of tree seedlings and are broadcast applied tank-mixes utilizing herbicides and rates that will maximize the potential for control of difficult species (Newton 2008; Ferrell et al. 2009). The potential for damage to trees is minimized due to chemical breakdown over the fall and winter but, in certain cases (e.g. sulfometuron methyl), short-term negative impacts have been reported (Burney and Jacobs 2009). Spring release treatments are designed to maintain low cover values during the growing season of application and allow seedlings unhindered access to site resources. While this treatment is broadcast applied over planted Douglas-fir seedlings, selectivity is achieved through a combined effect of timing, chemical choices, and lower use rates. Choosing between these regimes and the chemicals that will be used is based on a forest manager's training and experience with the vegetation community, soils, and the chemicals themselves.

While certain aspects of delaying management activities and vegetation control options are known, considerable debate exists on how to translate these findings to a regenerating PNW forest that is out of phase with common management cycles. This

study was designed to 1) compare Douglas-fir seedling morphology and growth responses to a range of operationally relevant vegetation management treatment regimes including a one year fallow period 2) compare the ability of these regimes to reduce competitive cover 3) quantify treatment efficacy for continued herbaceous and woody/semi-woody vegetation control and 4) investigate if the addition of sulfometuron methyl to a tank-mix of chemicals applied in the fall site preparation negatively affects seedling growth and plant community abundance.

## **2.2 Materials and Methods**

### **2.2.1 Site Descriptions**

Boot is 10 km (6 miles) south of Falls City, Oregon (44° 46.6' N, 123° 27.9' W) on lands managed by Forest Capital Partners, LLC (Appendix 1a). It has deep well-developed soils classified as fine, mixed, active, mesic Typic Haplohumults (NRCS 2011) and a Douglas-fir site index of 41 m (135 feet) at 50 years. The site is at an elevation of 152 m (500 feet) and faces north with slopes ranging from 0 to 5 degrees. Jackson Mast is 22 km (14 miles) south of Cottage Grove, Oregon (43° 37.3' N, 123° 12.4' W) on lands managed by Lone Rock Timber Company (Appendix 1b). Soils on the site are classified as fine, mixed, mesic, Ultic Palexeralfs (NRCS 2011). The site is at 205 m (675 feet) in elevation and supports a Douglas-fir site index of 35 m (115 feet) at 50 years. This site has a W/SW facing aspect and a slope of 10 to 25 degrees.

According to research plans, both sites were to be harvested as late in the season as possible in order to truly represent the management scenario. A market spike for Douglas-fir logs in the spring of 2007 dictated that the sites be harvested earlier than anticipated. Jackson Mast was harvested from late-April to mid-May 2007 and Boot was harvested from late-May to early-June 2007.

### 2.2.2 Experimental Design

The study sites were established in the summer of 2007. Each site consisted of twenty-four plots 18.3 x 18.3 m (60 x 60 ft) at Boot and 24.4 x 24.4 (80 x 80ft) at Jackson Mast using a complete randomized block design. The six treatment regimes (Table 2.1) were randomly assigned within each of four blocks (replicates). The no-action control (treatment 1) served as a reference where trees were planted but no vegetation control was done. The remaining regimes were designed to represent five potential management options that are available with a late-harvested unit. If there was very little vegetation at the time of fall site preparation, a forester may choose to do only a spring release (treatment 2) taking advantage of the low cover and potential cost savings (i.e. no fall site preparation). Treatments 3 and 4 included the standard fall site preparations (with and without sulfometuron in the tank mix) followed by a spring release in the first year. Treatments 5 and 6 delayed the chemical applications by one year introducing a fallow period. All treatment regimes were broadcast applied with a backpack sprayer. The date, herbicides included, and rates of application are included in Tables 2.2 and 2.3 (Boot and Jackson Mast, respectively). These chemical mixes and the rates of application were chosen by the forester managing these lands and represented operation treatments applied in other areas with similar plant communities.

In order to avoid localized and intense competition from sprouting *Acer macrophyllum* (bigleaf maple) stumps, all treatment plots were chemically treated. At Boot, a hatchet was used to open the bark down to the cambial tissue every 8 cm (3 inches) circumferentially around the stump. Undiluted imazapyr (Chopper<sup>®</sup>) was then squirted into the cut using a spray bottle. This treatment (called a “hack and squirt”) was done on the same day as the fall site preparation in 2007. The *Acer macrophyllum* stumps at Jackson Mast were treated using a solution of 20% triclopyr (Element 4<sup>®</sup>) in petroleum oil (Brush and Basal Oil<sup>®</sup>) on 24 January 2008. This solution was applied directly to the basal bark of the sprouts growing from the stumps. No herbicide drift

Table 2.1: Description of the six treatment regimes used in the study. Subscript “o” signifies a fall site preparation with sulfometuron methyl included in the tank-mix (treatments 4 and 6). Treatments 1-4 were planted February 2008 while treatments 5 and 6 were planted in January 2009.

	Treatment	Plantation establishment immediately following harvest		Delay plantation establishment one year	
		Fall Site Preparation	Spring Release	Fall Site Preparation	Spring Release
Planted 2008	1 - OO/OO	no	no	no	no
	2 - OS/OO	no	yes	no	no
	3 - FS/OO	yes	yes	no	no
	4 - F <sub>o</sub> S/OO	yes	yes	no	no
Planted 2009	5 - OO/FS	no	no	yes	yes
	6 - OO/F <sub>o</sub> S	no	no	yes	yes

Table 2.2: Herbicides applied according to the treatment regimes at Boot. Individual treatments (additionally marked by bolded larger font) were tank mixes and broadcast applied using backpack sprayers and a waving-wand technique within treatment plots. Sulfometuron was only added to the tank mixes applied in treatments 4 and 6.

<b>Boot</b>				Active Ingredient Rate Applied
Application	Regime	Trade Name (product use rate)	Chemical Name	
20 September 2007	Fall Site Preparation	Treatment 3 <b>F</b> S/OO	Accord <sup>®</sup> (7 L/ha or 3 qts/ac)	Glyphosate 3.77 L/ha
			Escort <sup>®</sup> (70 g/ha or 1 oz/ac)	Metsulfuron methyl 42 g/ha
			Chopper <sup>®</sup> (0.58 L/ha or 8 oz/ac)	Imazapyr 0.16 L/ha
			Induce <sup>®</sup> (0.58 L/ha or 8 oz/ac)	Adjuvent 0.52 L/ha
		Treatment 4 <b>F<sub>o</sub></b> S/OO	Accord <sup>®</sup> (7 L/ha or 3 qts/ac)	Glyphosate 3.77 L/ha
			Escort <sup>®</sup> (70 g/ha or 1 oz/ac)	Metsulfuron methyl 42 g/ha
			Chopper <sup>®</sup> (0.58 L/ha or 8 oz/ac)	Imazapyr 0.16 L/ha
			Induce <sup>®</sup> (0.58 L/ha or 8 oz/ac)	Adjuvent 0.52 L/ha
			Oust <sup>®</sup> (210 g/ha or 3 oz/ac)	Sulfometuron methyl 158 g/ha
26 April 2008	Spring Release	Treatments 2, 3, and 4 <b>O</b> S/OO	Velpar <sup>®</sup> DF (2.24 kg/ha or 2.0 lbs/ac)	Hexazinone 1.68 kg/ha
		<b>F</b> S/OO	Weedone <sup>®</sup> LV6 (1.75 L/ha or 0.75 qts/ac)	2, 4-D 1.52 L/ha
		<b>F<sub>o</sub></b> S/OO		
3 September 2008	Fall Site Preparation	Treatment 5 OO/ <b>F</b> S	Accord <sup>®</sup> (7 L/ha or 3 qts/ac)	Glyphosate 3.77 L/ha
			Escort <sup>®</sup> (70 g/ha or 1 oz/ac)	Metsulfuron methyl 42 g/ha
			Chopper <sup>®</sup> (0.58 L/ha or 8 oz/ac)	Imazapyr 0.16 L/ha
			Induce <sup>®</sup> (0.58 L/ha or 8 oz/ac)	Adjuvent 0.52 L/ha
		Treatment 6 OO/ <b>F<sub>o</sub></b> S	Accord <sup>®</sup> (7 L/ha or 3 qts/ac)	Glyphosate 3.77 L/ha
			Escort <sup>®</sup> (70 g/ha or 1 oz/ac)	Metsulfuron methyl 42 g/ha
			Chopper <sup>®</sup> (0.58 L/ha or 8 oz/ac)	Imazapyr 0.16 L/ha
			Induce <sup>®</sup> (0.58 L/ha or 8 oz/ac)	Adjuvent 0.52 L/ha
			Oust <sup>®</sup> (210 g/ha or 3 oz/ac)	Sulfometuron methyl 158 g/ha
15 April 2009	Spring Release	Treatments 5 and 6 OO/ <b>F</b> S	Velpar <sup>®</sup> DF (2.24 kg/ha or 2.0 lbs/ac)	Hexazinone 1.68 kg/ha
		OO/ <b>F<sub>o</sub></b> S	Weedone <sup>®</sup> LV6 (1.75 L/ha or 0.75 qts/ac)	2, 4-D 1.52 L/ha

Note: All sprouting *Acer macrophyllum* stumps were directly treated. A “hack and squirt” treatment was completed on 20 September 2007 using undiluted Chopper<sup>®</sup> (imazapyr) applied to fresh cuts.

Table 2.3: Herbicides applied according to the treatment regimes at Jackson Mast. Individual treatments (additionally marked by bolded larger font) were tank mixes and broadcast applied using backpack sprayers and a waving-wand technique within treatment plots. Oust Extra<sup>®</sup> (sulfometuron at 56.25% and metsulfuron at 15% by weight) was added to treatments 4 and 6.

<b>Jackson Mast</b>				Active Ingredient Rate Applied
Application	Regime	Trade Name (product use rate)	Common Name	
25 September 2007	Fall Site Preparation	Treatment 3	Foresters <sup>®</sup> (4.67 L/ha or 2 qts/ac)	Glyphosate
		<b>F</b> S/OO	Escort <sup>®</sup> (70 g/ha or 1 oz/ac)	Metsulfuron methyl
				2.51 L/ha
				42 g/ha
		Treatment 4	Foresters <sup>®</sup> (4.67 L/ha or 2 qts/ac)	Glyphosate
			<b>F<sub>o</sub></b> S/OO	Metsulfuron methyl
			Oust Extra <sup>®</sup> (210 g/ha or 3 oz/ac)	32 g/ha
				Sulfometuron methyl
				118 g/ha
11 April 2008	Spring Release	Treatments 2, 3, and 4	Velpar <sup>®</sup> L (7.0 L/ha or 3 qts/ac)	Hexazinone
		<b>O</b> S/OO		
		<b>F</b> S/OO	Transline <sup>®</sup> (0.73 L/ha or 10 oz/ac)	Clopyralid
		<b>F<sub>o</sub></b> S/OO		0.30 L/ha
30 September 2008	Fall Site Preparation	Treatment 5	Mad Dog <sup>®</sup> (9.34 L/ha or 4 qts/ac)	Glyphosate
			Escort XP <sup>®</sup> (70 g/ha or 1 oz/ac)	Metsulfuron methyl
			Sylgard <sup>®</sup> 309 (70 g/ha or 1 oz/ac)	Silicone Surfactant
				3.8 L/ha
		Treatment 6	Mad Dog <sup>®</sup> (9.34 L/ha or 4 qts/ac)	Glyphosate
			<b>OO/F</b> S	Metsulfuron methyl
			Oust Extra <sup>®</sup> (210 g/ha or 3 oz/ac)	32 g/ha
				Sulfometuron methyl
				118 g/ha
			Sylgard <sup>®</sup> 309 (70 g/ha or 1 oz/ac)	Silicone Surfactant
				~
20 April 2009	Spring Release	Treatments 5 and 6	Velpar <sup>®</sup> DF (2.24 kg/ha or 2.0 lbs/ac)	Hexazinone
		<b>OO/F</b> S		
		<b>OO/F<sub>o</sub></b> S	Transline <sup>®</sup> (0.58 L/ha or 8 oz/ac)	Clopyralid
				0.24 L/ha

Note: A solution of 20% Element 4<sup>®</sup> (triclopyr) and 80% Bark and Basal Oil<sup>®</sup> (petroleum oil) was applied to the *Acer macrophyllum* stump sprouts on 24 January 2008.

was observed in any treatment plot and over the three years of stand establishment reported here, control of these sprouting stumps was near complete.

### 2.2.3 Seedlings

Seedlings were grown at a nursery unique to each company but utilized the same nursery and seed source for the seedlings planted both years (Silver Mountain Nursery, Sublimity, OR, at Boot and IFA Humboldt, Humboldt, CA, at Jackson Mast). Douglas-fir bareroot 1+1 seedlings were planted by a professional crew at each site on a pre-marked 3.05 x 3.05 m (10 x 10 ft) grid. Buffer rows were included at both sites. At Boot, the buffer row was between plots and at Jackson Mast the buffer row was inside each plot. Treatments 1-4 were planted on 5 and 8 February 2008 at Boot and Jackson Mast, respectively. Treatments 5 and 6 were planted on 8 and 28 January 2009 at Jackson Mast and Boot, respectively. Vexar<sup>®</sup> tubes (Pacforest Supply Company, Springfield Oregon) and bamboo stakes were used to protect seedlings from ungulate browse damage.

At each planting date, one randomly selected bag of seedlings (approximately 100) were brought back to Oregon State University and tested by Seedling Quality and Evaluation Services (SQES). Shoot and root volumes were assessed by displacement on approximately 40 trees. Shoot to root ratio was calculated as the shoot volume divided by the root volume. The remaining 60 trees were divided into four groups of 15 and potted into 1 gallon containers. Each group of 15 trees was then subjected to a freeze test according to a pre-programmed regime with a unique low temperature for each of the four groups (Burr et al. 2001; Duryea 1985). These trees were then placed in a nearby heated greenhouse and maintained in a well-watered status for 6 or 7 days. After this period, SQES personnel used standardized grading criteria to check each seedling for freeze damage to buds, needles, stems, and branches calculating the lethal temperature to 50% of a tissue or plant (abbreviated LT50) for each of the four groups.

Seedling height to the nearest centimeter and diameter at ground line to the nearest millimeter were measured one month after planting (initial) and again each fall

(September or October) for the first three years. Stem volume was calculated using the formula  $V = (\pi \cdot \text{dia}^2 \cdot \text{ht}) / 12$  where “dia” is the diameter at ground line and “ht” is the height. Seedling growth during the 2010 season was the difference between the 2009 and 2010 measurements for height, diameter, and stem volume. Survivorship percentage in 2010 was calculated as the number of living trees within each plot divided by the total number planted (n=25 at Boot and n=36 at Jackson Mast) and multiplied by 100.

#### 2.2.4 Vegetation Community

Four permanent vegetation subplots were randomly located in each treatment plot (n=96 on each site) and were positioned equidistant between measurement trees. A 1 meter radius PVC (polyvinyl chloride) sampling frame was used to aid in determining percent cover of vascular plants in 1% increments up to 15% then in 5% increments up to 100%. Plants were identified to species level, but when plants did not have the necessary parts to accurately determine the correct species, genus or family level identifications were used. Occasionally a cotyledon could not be identified and was included in the survey as an “unknown forb.” Nomenclature follows Hitchcock and Cronquist (1973) but Pojar and MacKinnon (1994) and Gilkey and Dennis (2001) were used as plant identification references. Vegetation subplots were measured in September 2007 prior to herbicide application and in July 2008, 2009, and 2010. Summed cover values were derived by adding the cover percentages of each species found in the subplots. This technique allowed cover values to exceed 100% as species often overlapped. For the purposes of simplicity, summed cover will henceforward be referred to as cover.

Management of vegetation survey data required a unique process in order to compare surveys with various species which may or may not be common to all plots. This data management process is outlined in Appendix 2. The technique allowed the inclusion of additional plant information such as growth habit (forb, fern, graminoid, shrub, tree, and vine/shrub) or whether a plant was deemed “herbaceous” or

“woody/semi-woody.” Descriptive information such as these enabled the cover values to be divided into distinct groupings. Herbaceous vegetation included all forb, fern, and graminoid species whose above-ground portions typically die back during the winter months. It is recognized that there are notable exceptions (e.g. overwintering biennials, fern species like *Polystichum munitum*, etc.) but, in the broadest sense, this botanical generalization is correct. Woody/semi-woody plants are those whose above-ground portions do not die back and have perenniating tissue that would be classified as woody or semi-woody (shrub, tree, and vine/shrub species).

#### 2.2.5 Environmental Characteristics

Environmental data was collected every four hours at both sites since the fall of 2007. A centrally-located Hobo Microstation (model # H21-002, Onset Computer Corporation, Bourne, MA) was connected to a tipping bucket rain gage (model #S-RGA-M002, Onset Computer Corporation, Bourne, MA) and a temperature and relative humidity sensor (model #S-THA-M002, Onset Computer Corporation, Bourne, MA). Data were continuous at Jackson Mast, but an elk chewing sensor cables at Boot caused two disruptions. During the periods of 7 May to 13 June and 14 September to 31 December 2010, precipitation data were estimated using two weather stations to form an averaged single daily precipitation total. One station was located 11 miles due west of Boot while the second was 17 miles to the south/southeast. Temperature data during these time periods came from the station 17 miles to the south/southeast.

Monthly soil cores were collected during the summer of 2008 and 2009 at both sites using an AMS core sampler with a slide hammer (AMS Inc., American Falls, ID). One randomly located position was used in each of 12 plots that represented two replicates of each treatment regime. Initially a small hole was dug and at each subsequent sampling, fresh soil was exposed. The core was taken horizontally centered at 10 cm depth, labeled, and taken to laboratory facilities at Oregon State University. The sample was taken from the sleeve, weighed, dried for 48 hours at

45°C (113°F), and reweighed. Volumetric soil moisture of the sample was calculated by multiplying the gravimetric water content by the bulk density (see Brady and Weil 2002).

## **2.3 Statistical Analysis**

Means for each response variable were calculated by experimental unit and these values (n=24; 6 treatments x 4 replicates) were analyzed using Statistical Analysis Software version 9.1 (SAS Institute, Inc., Cary, NC). Analysis of covariance was carried out using mixed model approaches (PROC MIXED) and analysis of variance was conducted with generalized linear models (PROC GLM). Blocks (replicates) were considered random effects in the models while treatments were fixed effects. Assumptions of normality, linearity, and constant variance were examined on the residuals for each response variable tested in the following analyses. No transformations were required to meet model assumptions. Fisher's protected least significant difference t-tests were used to compare treatment means. SAS software "pdmix800.sas" was used to assign letters for treatment when multiple comparisons were made in PROC MIXED (Saxton 1998). Unless otherwise stated, an alpha level of 0.05 was used to determine statistically significant results.

### **2.3.1 Seedling Data**

Analyzing seedling growth responses with plantings that occurred in two different years required careful consideration of statistical procedures. Trees planted on the site were grown over different years and slight changes to cultural practices at the nursery can influence seedling morphology sent to outplanting sites (Burdett 1990; Nyland 2002). At the nursery, bareroot 1+1 seedlings were grown for one year, lifted, replanted at a lower density, and grown for a second year. This meant that seedlings in treatments 1-4 were grown in 2006 and 2007 making them available for planting in February 2008. Seedlings in treatments 5 and 6 were germinated and grown in 2007 and 2008 making them available for outplanting in January 2009.

Accounting for the potential for variability in initial seedling size was done using analysis of covariance which blends regression techniques with standard ANOVA procedures. Initial measurements (height, diameter at ground-line and stem volume) taken in March serve as the covariate. Following procedures outlined in Littell et al. 1996, an interaction term was included in the full model between each initial measurement and the 2010 response variable of interest. No interactions were significant indicating that common slope model was appropriate. Dropping this interaction term allowed the adjustment of means and the comparison of seedling height, diameter at ground line, and volume treatment responses in October 2010.

The first season of growth for most seedlings is minor when compared to future years. This period is marked by a seedling's need to establish intimate contact with the soil (Burdett 1990; van den Driessche 1987). Once seedlings have established contact and extended roots to deeper and more continuous moisture reserves, growth is dictated by the conditions of the site. The growth during 2010 represented the first common year after the initial season of establishment for seedlings in all of the treatment regimes. This growth data (height, diameter, and stem volume) along with the survivorship in 2010 were compared using ANOVA procedures.

### 2.3.2 Vegetation Community Data

Cover of the vegetation community was analyzed individually by site and year using ANOVA procedures. Only the July surveys in 2008, 2009, and 2010 were analyzed. The September 2007 survey was conducted prior to the application of the treatment regimes. These data were not analyzed for treatment differences, but are reported to illustrate the observed level of competitive vegetation in the first fall post-harvest. Delaying establishment to gain longer-lasting control can be assessed by looking at the composition of the vegetation community beyond the time when regimes are finished. Cover values in July 2010 were separated into two categories, herbaceous plants (forbs, ferns, graminoids) and woody/semi-woody plants (shrubs, trees, vine/shrubs). These values were analyzed individually by site for the July 2010

survey to understand if the treatments had significantly affected the longer-term abundance of these two vegetation community components.

Orthogonal contrasts were constructed to test for specific preplanned comparisons on the herbaceous and woody/semi-woody components of the vegetation community in July 2010. The first compared the no-action control with the remaining treatments to test for a herbicide effect (treatment 1 vs treatments 2, 3, 4, 5, and 6). Contrast two compared the spring release only treatment (2-OS/OO) with those treatments which received a fall site preparation and spring release application (treatments 3, 4, 5, and 6). Contrast three compared the two treatment regimes that did not include sulfometuron methyl in the fall site preparation (3-FS/OO and 5-OO/FS) with the two treatments that included the chemical in the fall site preparation (4-F<sub>o</sub>S/OO and 6-OO/F<sub>o</sub>S). Contrasts four and five compared similar treatment regimes applied one year apart (3-FS/OO vs 5-OO/FS and 4-F<sub>o</sub>S/OO vs 6-OO/F<sub>o</sub>S, respectively).

## 2.4 Results

*Summary: After accounting for the initial size of seedlings, treatment regimes which reduced competitive cover improved seedling growth relative to the no-action control. In October 2010, trees planted after a fallow year were indeed smaller in height, diameter, and volume than those planted the year before. Seedling growth in the delayed treatments was not enough to surpass trees planted the prior year. A spring release only was capable of restraining vegetation community growth below 20% during the initial year. Whether vegetation was controlled by a fall site preparation and spring release in year one or after a delay, both resulted in a vegetation community below 11%. Dynamic changes occurred to the herbaceous and woody/semi-woody components of the vegetation community in response to the chemicals included in the different treatment regimes. Including sulfometuron methyl in the fall site preparation did not improve seedling growth or competition control when compared to a fall site preparation without the chemical.*

#### 2.4.1 Seedling morphology and growth 2010

Data provided by SQES is presented in Figure 2.1. Shoot to root ratio lines of 4:1, 2:1 and 1:1 have been included to illustrate general trends in seedling morphology planted across the two years on each site. Seedling shoot to root ratio at Boot noticeably decreased from 2008 to 2009. Seedlings at Jackson Mast were similar across the two years. Cold hardiness testing by SQES revealed that seedlings planted in February 2008 had LT50's of  $-9.2^{\circ}\text{C}$  and  $-12.9^{\circ}\text{C}$  at Boot and Jackson Mast, respectively. Seedlings planted in January 2009 had LT50's below  $-17^{\circ}\text{C}$  at both sites. Cold hardiness to temperatures below  $-9^{\circ}\text{C}$  suggest, at least by this measure of stress resistance, seedlings were well-prepared for planting (Glerum 1985; Richie 1986).

After accounting for initial seedling size, treatment regimes significantly affected height, diameter, and volume measurements taken in October 2010 (Table 2.4). The adjusted means presented in Table 2.5 demonstrate the effect these regimes had on 2010 seedling morphology. Height at Boot was modestly improved by competition control ranging from 121 cm in the no-action control to 149 cm in treatment 2. At Jackson Mast, height was also different and ranged from 96 to 123 cm in the no-control and treatment 3, respectively. Seedling height in the delayed treatments (5 and 6) was not different from the no-action control at either site. Seedling diameter at Boot fell into two distinct groups, herbicidal control during the first year increased diameter to 2.5 to 2.7 cm, while diameter in the no-action control and the two delayed treatments (5 and 6) were 2.1, 2.0, and 2.0 cm, respectively. Jackson Mast seedling diameter was less defined among the treatments ranging from 1.5 in the no-action control to 2.0 in treatment 3.

At Boot, 2010 stem volume (Table 2.5) in the delayed treatments (5 and 6) was not different from the no-action control and ranged from  $148\text{ cm}^3$  in treatment 6 to  $166\text{ cm}^3$  in the no-action control. Treatments that controlled competing vegetation and established seedlings immediately after harvest (treatments 2, 3, and 4) improved stem volume by over 64% when compared to the no-action control. At Jackson Mast, stem volume in treatments 2, 3, and 4 was between 95% to 144% larger than the

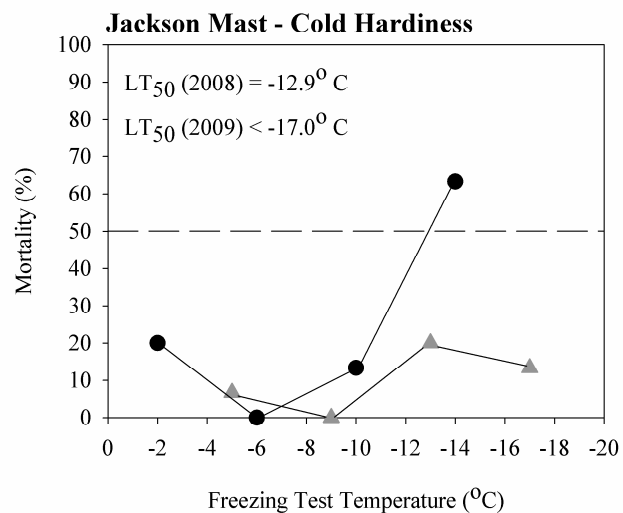
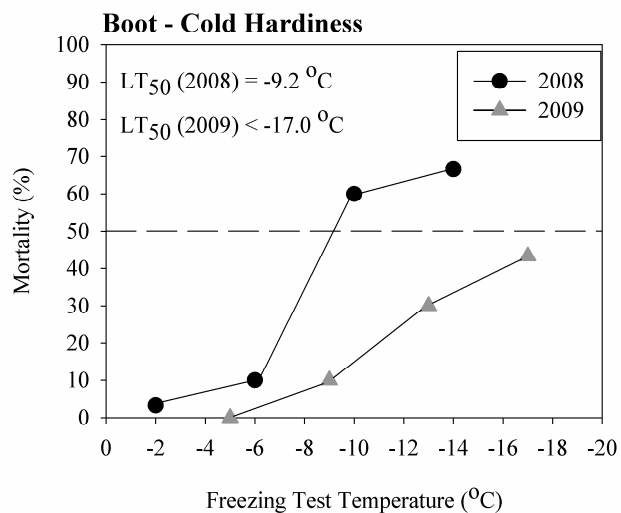
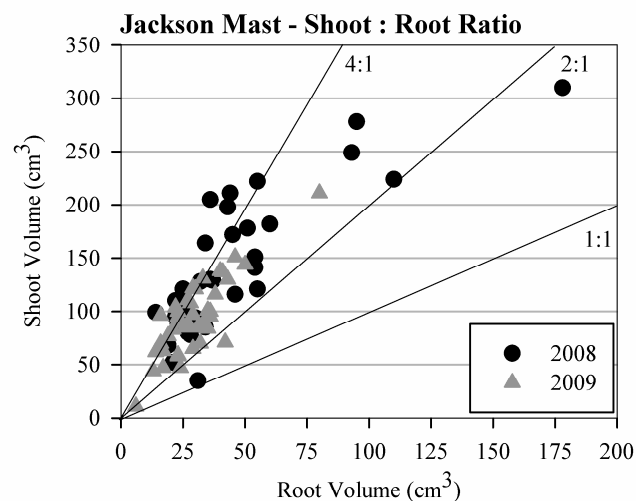
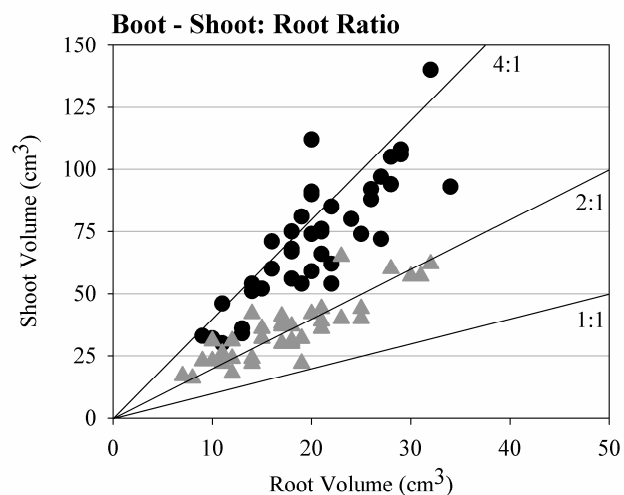


Figure 2.1: Seedling shoot: root ratio (upper) and cold hardiness (lower) presented by site and by year. Note the different scales used on the shoot: root ratio figures.

Table 2.4: ANCOVA tables presenting Type III effects for seedling height, diameter at ground-line, and stem volume in the fall of 2010 as well as the survivorship and growth of each parameter during the 2010 season. Growth was calculated as the difference between the 2009 and 2010 measurements. Significant treatment effects are indicated by bolded P-values ( $\alpha=0.05$ ).

					Site			
					Boot		Jackson Mast	
	Parameter	Effect	Num. df	Den. df	F Stat.	P-value	F Stat.	P-value
October 2010	Height	Treatment	5	14	3.10	<b>0.0433</b>	6.98	<b>0.0018</b>
		Initial Ht.	1	14	2.17	0.1629	1.59	0.2282
	Diameter	Treatment	5	14	4.72	<b>0.0098</b>	6.65	<b>0.0023</b>
		Initial Dia.	1	14	0.98	0.3390	1.06	0.3212
	Stem Volume	Treatment	5	14	6.48	<b>0.0026</b>	5.33	<b>0.0060</b>
		Initial Vol.	1	14	3.04	0.1033	1.57	0.2311
	Parameter	Effect	Num. df	Den. df	F Stat.	P-value	F Stat.	P-value
Growth during 2010	2010 Survivorship	Treatment	5	15	1.81	0.1705	23.30	<b>&lt;0.0001</b>
	Height	Treatment	5	15	1.72	0.1906	1.36	0.2951
	Diameter	Treatment	5	15	2.44	0.0824	1.45	0.2643
	Stem Volume	Treatment	5	15	12.62	<b>&lt;0.0001</b>	1.99	0.1394

Table 2.5: ANCOVA (adjusted) means for treatment effects on seedling height (cm), diameter at ground-line (cm), and stem volume (cm<sup>3</sup>) in the fall of 2010. Means within a column that have the same letter are not statistically different at  $\alpha=0.05$ .

<b>Boot</b>					
		Treatment	Height	Diameter	Stem Volume
Planted	2008	1 - OO/OO	121 b	2.1 b	166 b
		2 - OS/OO	149 a	2.5 a	297 a
		3 - FS/OO	136 ab	2.5 a	273 a
		4 - F <sub>o</sub> S/OO	126 b	2.7 a	297 a
	2009	5 - OO/FS	116 ab	2.0 b	155 b
		6 - OO/F <sub>o</sub> S	109 ab	2.0 b	148 b

<b>Jackson Mast</b>					
		Treatment	Height	Diameter	Stem Volume
Planted	2008	1 - OO/OO	96 c	1.5 c	61 c
		2 - OS/OO	117 ab	1.9 ab	119 ab
		3 - FS/OO	123 a	2.0 a	149 a
		4 - F <sub>o</sub> S/OO	108 bc	1.9 ab	125 ab
	2009	5 - OO/FS	103 c	1.9 ab	108 b
		6 - OO/F <sub>o</sub> S	100 c	1.7 b	90 bc

no-action control. Delayed treatments (5 and 6) at Jackson Mast were numerically greater in stem volume at 90 and 108 cm<sup>3</sup> (respectively) when compared to 61 cm<sup>3</sup> in the no-action control.

Height and diameter growth that accrued during the 2010 growing season was not affected by the treatment regimes at either site (Table 2.4, Figure 2.2, and Figure 2.3). Only stem volume growth in 2010 at Boot was significantly affected by the vegetation management regimes (Table 2.4). Seedlings in treatments 2, 3, and 4 at Boot grew between 202 and 234 cm<sup>3</sup> in 2010 (Figure 2.2). The no-action control grew 141 cm<sup>3</sup> and delayed treatments 5 and 6 increased 81 and 79 cm<sup>3</sup>, respectively. The inclusion of sulfometuron methyl in the fall site preparation tank-mix (treatments 4 and 6) did not statistically improve height, diameter, and shoot volume growth in 2010 when compared to the companion treatment without the chemical (treatments 3 and 5). Survivorship was not different among the treatments at Boot (Figure 2.2) ranging from 79% in treatment 3 to 93% in treatment 5. At xeric Jackson Mast, survivorship was affected by the treatment regimes (Table 2.4 and Figure 2.3). The no-action control had the lowest survival at 44%. Treatments 2, 3, and 4 had greater seedling survivorship at 63 and 69%. Seedlings planted in the delayed treatments in 2009 had the highest survivorship found at Jackson Mast with 90 and 88% in treatments 5 and 6, respectively.

#### 2.4.2 Vegetation Community and Composition

When compared to the no-action control, chemical treatment regimes employed in the study significantly affected summed cover during the year of application (Table 2.6). In 2008, the vegetation communities unrestrained by herbicidal application (treatments 1, 5, and 6) ranged from 39% to 43% at Boot and 51% to 59% at Jackson Mast (Figure 2.4 and Table 2.7). Applying a spring release in 2008 according to treatment 2 limited the vegetation community to less than 20% cover at both sites. At Boot, treatments 3 and 4 had less than 6% cover and were lower than the 19% cover found in treatment 2. The same comparison at Jackson

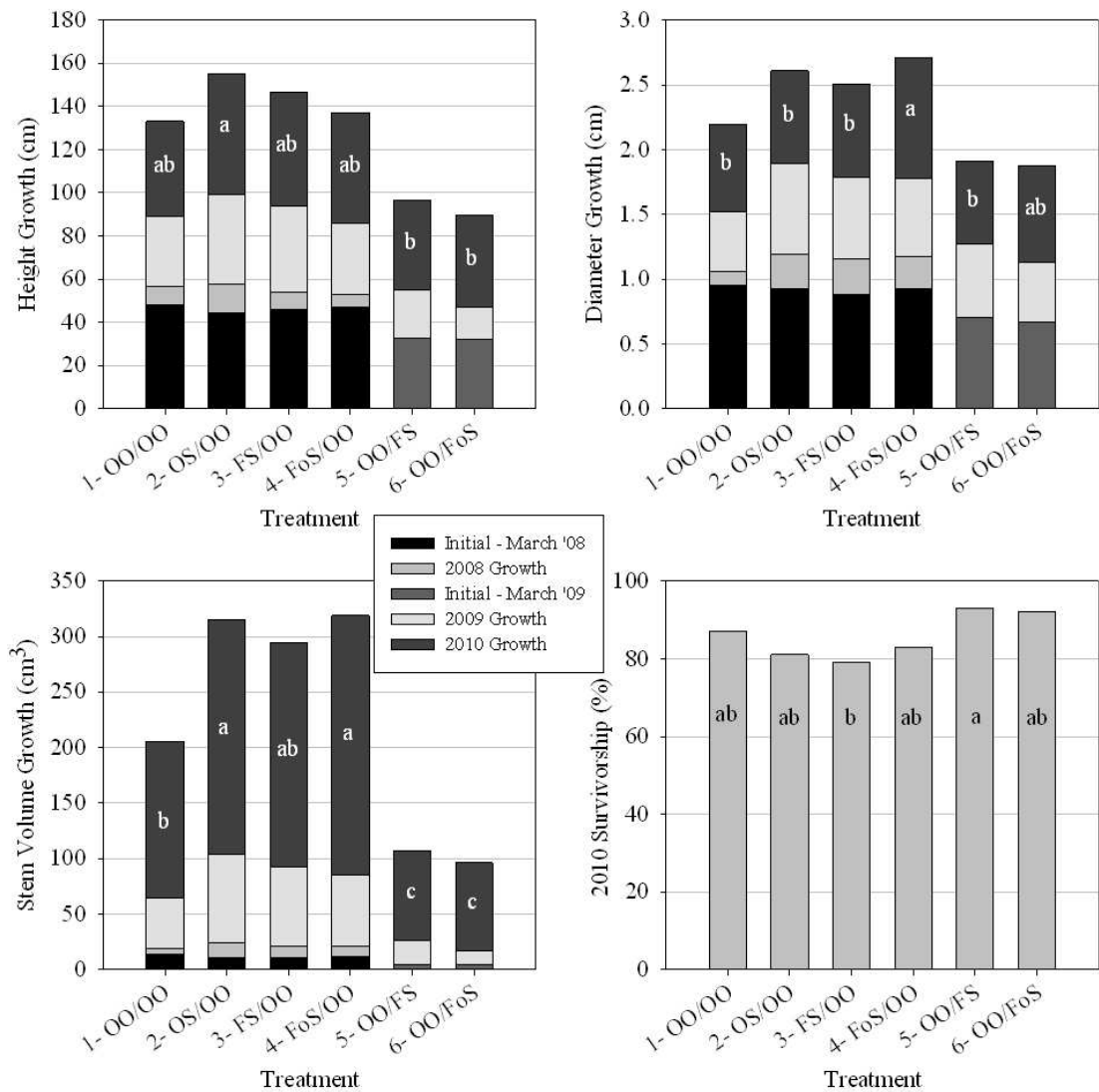


Figure 2.2: Boot seedling height, diameter, stem volume growth during the 2010 season as well as survivorship in October 2010. Treatment means with the same letters are not statistically different at  $\alpha=0.05$ .

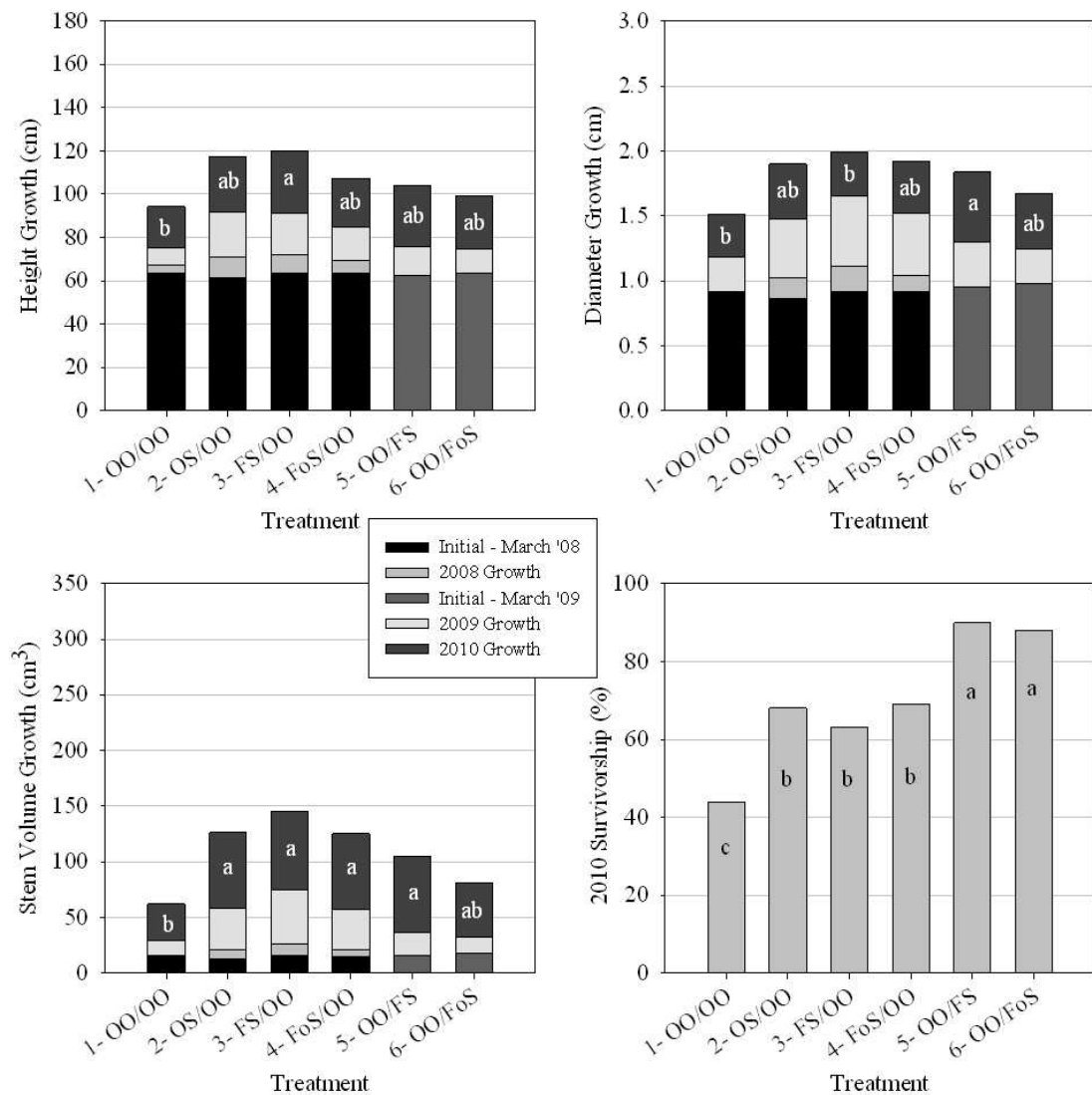


Figure 2.3: Jackson Mast seedling height, diameter, stem volume growth during the 2010 season as well as survivorship in October 2010. Treatment means with the same letters are not statistically different at  $\alpha=0.05$ .

Table 2.6: ANOVA tables for vegetation cover by year as well as ANOVA tables for herbaceous and woody/semi-woody vegetation in 2010. Significant treatment effects are indicated by bolded P-values ( $\alpha=0.05$ ).

Boot	Parameter	Source	DF	Type III SS	Mean Square	F value	Pr>F
	Cover	Block	3	312.8925	104.2975	2.66	0.0862
	July 2008	Treatment	5	6406.7396	1281.3479	32.62	<b>&lt;0.0001</b>
	Cover	Block	3	763.7708	254.5903	1.67	0.2154
	July 2009	Treatment	5	16296.0833	3259.2167	21.41	<b>&lt;0.0001</b>
	Cover	Block	3	254.3125	84.7708	1.20	0.3447
	July 2010	Treatment	5	20428.0313	4085.6063	57.67	<b>&lt;0.0001</b>
	Parameter	Source	DF	Type III SS	Mean Square	F value	Pr>F
	Herbaceous	Block	3	736.2188	245.4063	1.98	0.1610
		Treatment	5	6778.7708	1355.7542	10.91	<b>0.0001</b>
Jackson Mast	Woody/Semi-woody	Block	3	468.7813	156.2604	1.46	0.2662
		Treatment	5	7310.9271	1462.1854	13.63	<b>&lt;0.0001</b>
	Parameter	Source	DF	Type III SS	Mean Square	F value	Pr>F
	Cover	Block	3	263.2787	87.7596	0.72	0.5558
	July 2008	Treatment	5	12189.8255	2437.9651	19.98	<b>&lt;0.0001</b>
	Cover	Block	3	612.5104	204.1701	2.11	0.1424
	July 2009	Treatment	5	11149.8021	2229.9604	23.00	<b>&lt;0.0001</b>
	Cover	Block	3	988.3125	329.4375	2.09	0.1442
	July 2010	Treatment	5	10774.0208	2154.8042	13.68	<b>&lt;0.0001</b>
	Parameter	Source	DF	Type III SS	Mean Square	F value	Pr>F
	Herbaceous	Block	3	831.7578	277.2526	2.73	0.0805
		Treatment	5	2318.0130	463.6026	4.57	<b>0.0099</b>
	Woody/Semi-woody	Block	3	237.1745	79.0582	0.68	0.5789
		Treatment	5	3627.3672	725.4734	6.22	<b>0.0026</b>

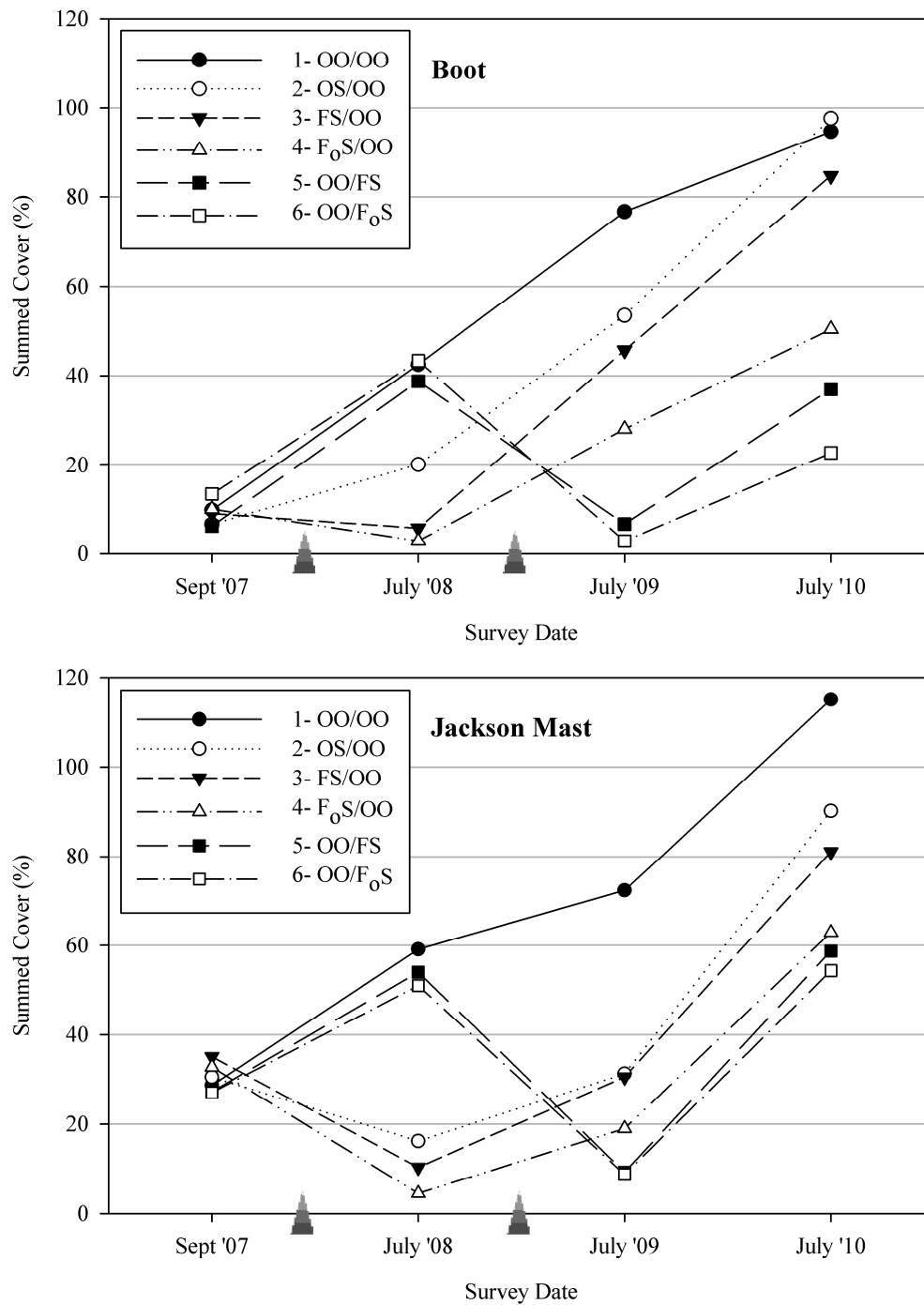


Figure 2.4: Summed cover development for Boot and Jackson Mast presented by survey date. Triangles represent approximate times of herbicidal application according to the treatment regimes.

Table 2.7: Mean summed cover of the vegetation community by site, year, and treatment regime. Analysis was conducted individually by site and year. Significant treatment responses were found each year. Means within a column that have the same letter are not statistically different at  $\alpha=0.05$ .

		<b>Boot</b>			
Planted		Treatment	2008	2009	2010
	2008	1 - OO/OO	43 a	77 a	95 ab
		2 - OS/OO	19 b	54 b	98 a
		3 - FS/OO	6 c	46 bc	85 b
		4 - F <sub>o</sub> S/OO	3 c	28 c	50 c
	2009	5 - OO/FS	39 a	7 d	37 d
		6 - OO/F <sub>o</sub> S	39 a	3 d	23 e

		<b>Jackson Mast</b>			
Planted		Treatment	2008	2009	2010
	2008	1 - OO/OO	59 a	72 a	115 a
		2 - OS/OO	16 b	31 b	90 b
		3 - FS/OO	10 b	31 b	81 bc
		4 - F <sub>o</sub> S/OO	5 b	19 bc	63 cd
	2009	5 - OO/FS	54 a	9 c	59 d
		6 - OO/F <sub>o</sub> S	51 a	9 c	54 d

Mast was not statistically different and ranged between 5% and 16%. Including sulfometuron methyl in the fall site preparation (treatment 4) did not significantly reduce the vegetation at either site when compared to the regime that did not incorporate the chemical into the tank-mix (treatment 3).

Without continued herbicidal control in 2009, treatments 2, 3, and 4 gained 35, 40, and 25 percentage points (respectively) in their cover values at Boot and 15, 21, and 14 percentage points (respectively) at Jackson Mast (Figure 2.4 and Table 2.7). Cover in the no-action control treatments at Boot and Jackson Mast increased to 77% and 72%, respectively. According to the delayed treatment regimes (5 and 6) vegetation communities that grew unhindered in 2008, were reduced to less than 10% cover at both sites in 2009 (Figure 2.4 and Table 2.7). No statistical differences were found between these two treatments at either site.

By the time of the July 2010 vegetation survey, two years had passed since herbicide application in treatments 2, 3, and 4 and one year had elapsed in treatments 5 and 6. Despite this passage of time, summed cover in 2010 was different among the treatments (Figure 2.4 and Table 2.7). Treatments 1, 2, and 3 at Boot had grown to over 85% cover. Treatment 4 was lower at 50% cover. At Jackson Mast, cover in treatment 2 was 90% and was less than the 115% observed in the no-action control. Treatments 5 and 6 at Boot increased to 37% and 23% cover in 2010 and were only 9 and 5 percentage points lower than the levels found in treatments 3 and 4 (respectively) the prior year. This comparison at Jackson Mast did not respond in a similar manner. In 2010, the delayed treatments (5 and 6) increased to 59% and 54%, respectively. This increase, in the year following herbicide application, was between 28 and 35 percentage points higher than the 31% and 19% cover found in treatments 3 and 4 (respectively) the previous year.

Treatment regimes significantly affected the composition of the vegetation community in July 2010 (Table 2.6) as measured by the abundance of herbaceous (forbs, ferns, and graminoids) as well as woody/semi-woody (shrubs, trees, and vine/shrubs) plant cover (Figure 2.5). At both sites, herbaceous species were capable

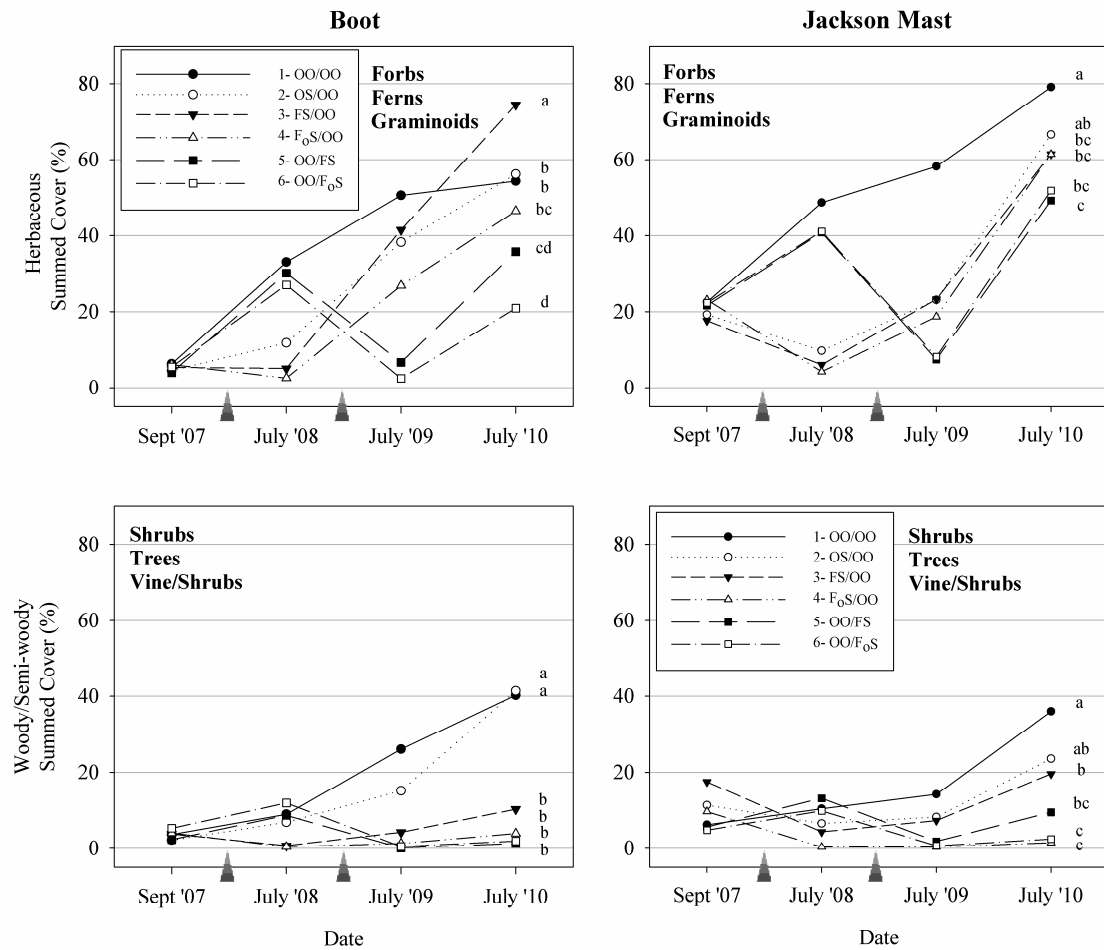


Figure 2.5: Herbaceous and woody/semi-woody development through the initial three years of plant community development. Herbaceous cover is the summed total of plants classified as forbs, ferns, and graminoids. Woody/semi-woody cover is the summed total of all plants classified as shrubs, trees, and vine/shrubs. Triangles represent approximate times of herbicidal application according to the treatment regimes. July 2010 data was analyzed as all treatments were at least one year beyond herbicide application. Treatment means with the same letter are not statistically different at  $\alpha=0.05$ .

of invading open conditions once the disturbances of harvest and/or chemical treatments were complete. Herbaceous plants at Boot had grown to between 21 and 74% cover as of July 2010. One year after herbicide use, treatments 5 and 6 had the lowest abundance of herbaceous plants at 36 and 21% (respectively) while those two years from herbicide use were over 47% (treatments 2, 3, and 4). Treatment 3 had the highest cover at 74%. This was primarily due to the colonization of a single species of fern, *Pteridium aquilinum* (data not shown). Orthogonal contrast three at Boot (Table 2.8) demonstrated that the inclusion of sulfometuron methyl in the fall site preparation resulted in a lower abundance of herbaceous plants in 2010. Delaying vegetation management according to treatments 5 and 6 resulted in less herbaceous cover in 2010 when compared to the companion treatments (3 and 4) applied one year earlier (Table 2.8).

At Jackson Mast, herbaceous plant cover was different among the treatment regimes (Table 2.6 and Figure 2.5). Herbaceous plants comprised 79% of the cover in the no-action control. Subtle differences existed among herbaceous cover present in treatments 2 through 6 spanning a range of 17 percentage points from 49 to 66%. The only significant orthogonal contrast on this site demonstrated a herbicide effect comparing the herbaceous cover of the no-action control to all other treatments (Table 2.8). As mentioned previously, the vegetation communities associated with treatments 2 through 6 at Jackson Mast rapidly increased from 2009 to 2010. Figure 2.5 illustrates the role herbaceous plants played in this response.

Woody/semi-woody vegetation was slower to respond to the harvest disturbance but was different among the treatment regimes at both sites in July 2010 (Table 2.6). In July 2010, four years had passed since the sites had been harvested and woody/semi-woody vegetation in the no-action control reached 40% and 36% cover at Boot and Jackson Mast, respectively (Figure 2.5). Woody/semi-woody vegetation growing in treatment 2 was not different from the no-action control at either site and was 41% cover at Boot and 24% at Jackson mast, respectively. At both study sites, the use of herbicides affected the abundance of woody/semi-woody plants (contrast 1,

Table 2.8: Orthogonal contrast results presented by site for the analysis of herbaceous and woody/semi-woody components of the vegetation community in July 2010. Bold font indicates a significant contrast at  $\alpha=0.05$ .

Boot	Contrast - Herbaceous 2010		DF	Contrast SS	F Value	Pr > F
	1. Trt 1 (OO/OO) vs All Trt's with herbicides	1	193.8021	1.56	0.2308	
	2. Trt 2 (OS/OO) vs. Trt's 3, 4, 5, and 6	1	445.3320	3.58	0.0778	
	3. Trt 3 and 5 (FS) vs. Trt 4 and 6 (F <sub>o</sub> S)	1	1832.9102	14.75	<b>0.0016</b>	
	4. Trt 3 vs Trt 5 (FS - one year apart)	1	2993.4453	24.10	<b>0.0002</b>	
	5. Trt 4 vs Trt 6 (F <sub>o</sub> S - one year apart)	1	1313.2813	10.57	<b>0.0054</b>	
	Contrast - Woody/Semi-woody 2010		DF	Contrast SS	F Value	Pr > F
	1. Trt 1 (OO/OO) vs All Trt's with herbicides	1	2712.2521	25.28	<b>0.0002</b>	
	2. Trt 2 (OS/OO) vs. Trt's 3, 4, 5, and 6	1	4395.6125	40.97	<b>&lt;0.0001</b>	
	3. Trt 3 and 5 (FS) vs. Trt 4 and 6 (F <sub>o</sub> S)	1	33.0625	0.31	0.5870	
	4. Trt 3 vs Trt 5 (FS - one year apart)	1	162.0000	1.51	0.2381	
	5. Trt 4 vs Trt 6 (F <sub>o</sub> S - one year apart)	1	8.0000	0.07	0.7885	

Jackson Mast	Contrast - Herbaceous 2010		DF	Contrast SS	F Value	Pr > F
	1. Trt 1 (OO/OO) vs All Trt's with herbicides	1	1468.2505	14.48	<b>0.0017</b>	
	2. Trt 2 (OS/OO) vs. Trt's 3, 4, 5, and 6	1	360.1883	3.55	0.0790	
	3. Trt 3 and 5 (FS) vs. Trt 4 and 6 (F <sub>o</sub> S)	1	6.5664	0.06	0.8026	
	4. Trt 3 vs Trt 5 (FS - one year apart)	1	300.1250	2.96	0.1060	
	5. Trt 4 vs Trt 6 (F <sub>o</sub> S - one year apart)	1	182.8828	1.80	0.1993	
	Contrast - Woody/Semi-woody 2010		DF	Contrast SS	F Value	Pr > F
	1. Trt 1 (OO/OO) vs All Trt's with herbicides	1	2035.6922	17.46	<b>0.0008</b>	
	2. Trt 2 (OS/OO) vs. Trt's 3, 4, 5, and 6	1	753.3781	6.46	<b>0.0225</b>	
	3. Trt 3 and 5 (FS) vs. Trt 4 and 6 (F <sub>o</sub> S)	1	631.2656	5.41	<b>0.0344</b>	
	4. Trt 3 vs Trt 5 (FS - one year apart)	1	205.0313	1.76	0.2046	
	5. Trt 4 vs Trt 6 (F <sub>o</sub> S - one year apart)	1	2.0000	0.02	0.8975	

Table 2.8). Orthogonal contrast results demonstrated that the woody/semi-woody cover at both sites was greater in the spring release only treatment (2) when compared to those treatments that also received a fall site preparation (3-6). At Boot, treatments 3, 4, 5, and 6 were not different from one another and ranged from 10% in treatment 3 to 1% in treatment 5. At Jackson Mast, woody/semi-woody plant response to treatments 3-6 had differences that spanned a range of 1 to 20%. Despite this low cover at Jackson Mast, treatments that included sulfometuron methyl in the fall site preparation (4 and 6) had a significantly lower abundance of woody/semi-woody plants when compared to those without the chemical (3 and 5) (Table 2.8).

#### 2.4.3 Environmental data

Boot is 209 km (130 miles) to the North/Northwest of Jackson Mast (azimuth 350°). Despite the relatively close circumstances west of the Cascades, mean total precipitation received at the two sites was different by over 700 mm with Boot at 1649 mm and Jackson Mast at 944 mm (Figure 2.6). From 2008 to 2010 the mean total precipitation during July, August, and September was 58.9 mm and 36.7 mm at Boot and Jackson Mast, respectively. This represented only 3.6% and 3.9% of the annual amount of precipitation. During these periods of drought, soil moisture can decline to low levels (Figure 2.7). Vegetation cover over 20% depleted soil moisture to very low levels decreasing to 5% at Jackson Mast, the driest of the two study sites. Reducing vegetation cover below 20% through the regimes tested increased soil moisture availability at 10 cm depth to greater than 0.30 m<sup>3</sup> H<sub>2</sub>O/m<sup>3</sup> soil at Boot and 0.14 m<sup>3</sup> H<sub>2</sub>O/m<sup>3</sup> soil at Jackson Mast.

A heat event occurred from 16 May to 18 May 2008 (Figure 2.8) that is believed to have affected the survivorship and growth of seedlings at Jackson Mast. The highest air temperature recorded during this three-day period at Jackson Mast was 39°C (102°F) at 1700 hrs local time on 16 May 2008. A relative humidity of 18% was also recorded at this time creating a vapor pressure deficit (VPD) of 5.73 Kpa. This

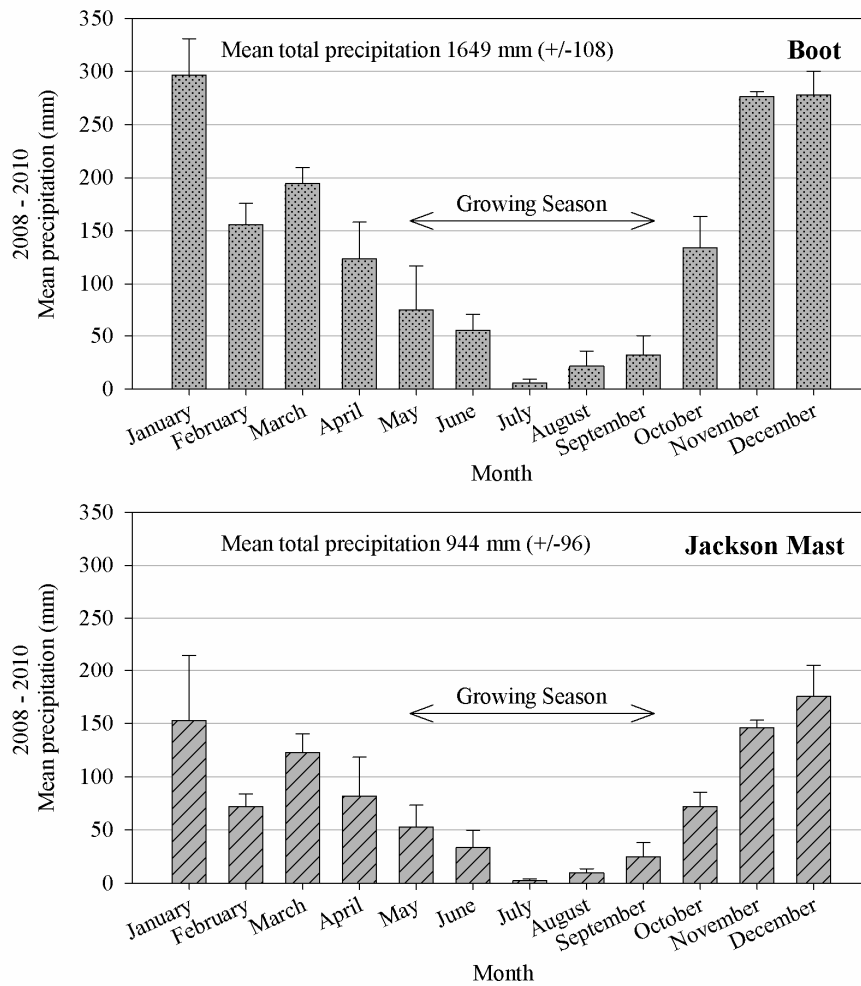


Figure 2.6: Mean monthly precipitation by site. Values calculated as the amount received each month across the three years included in the study (2008 to 2010). Approximate length of the growing season from May to September has been indicated by an arrow.

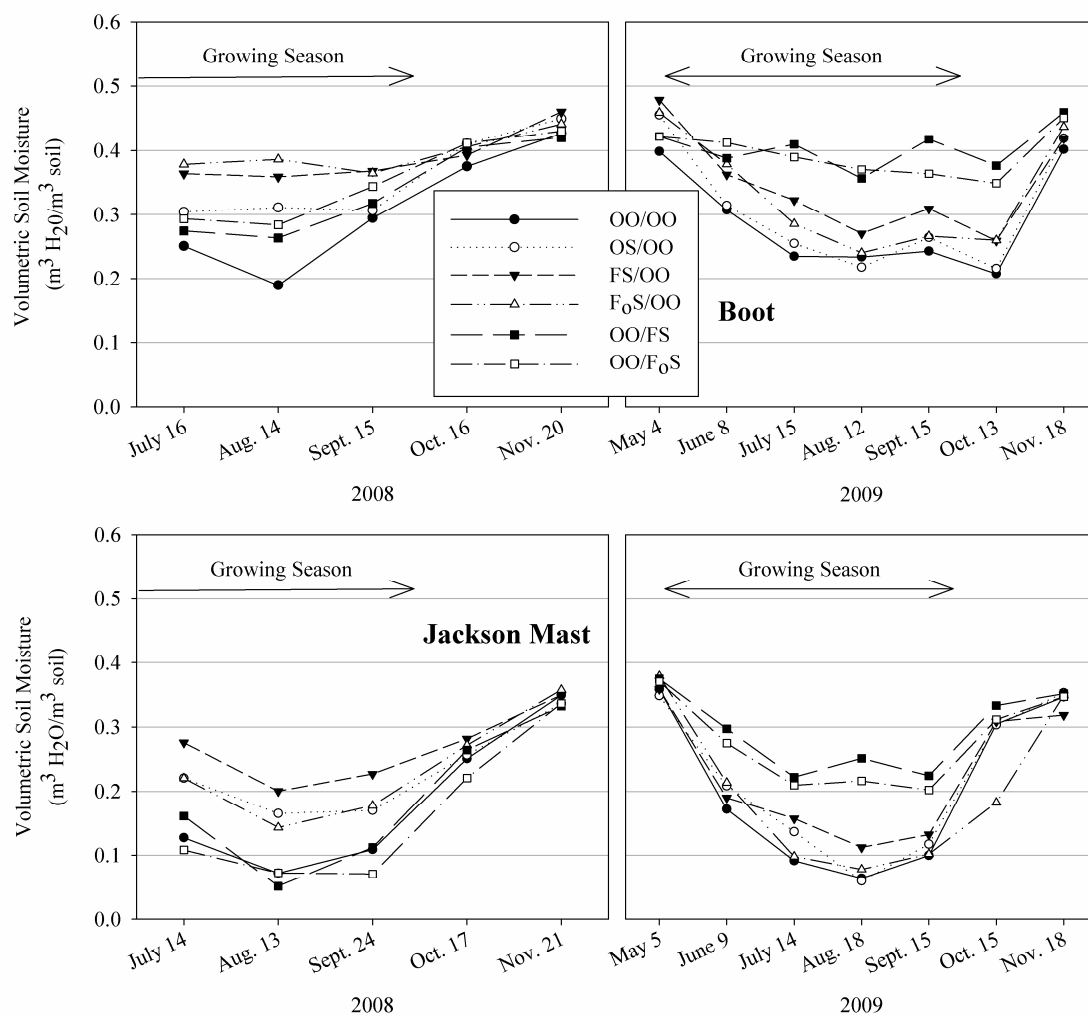


Figure 2.7: Soil moisture presented by year and site during 2008 and 2009. Approximate length of the growing season has been indicated by an arrow.

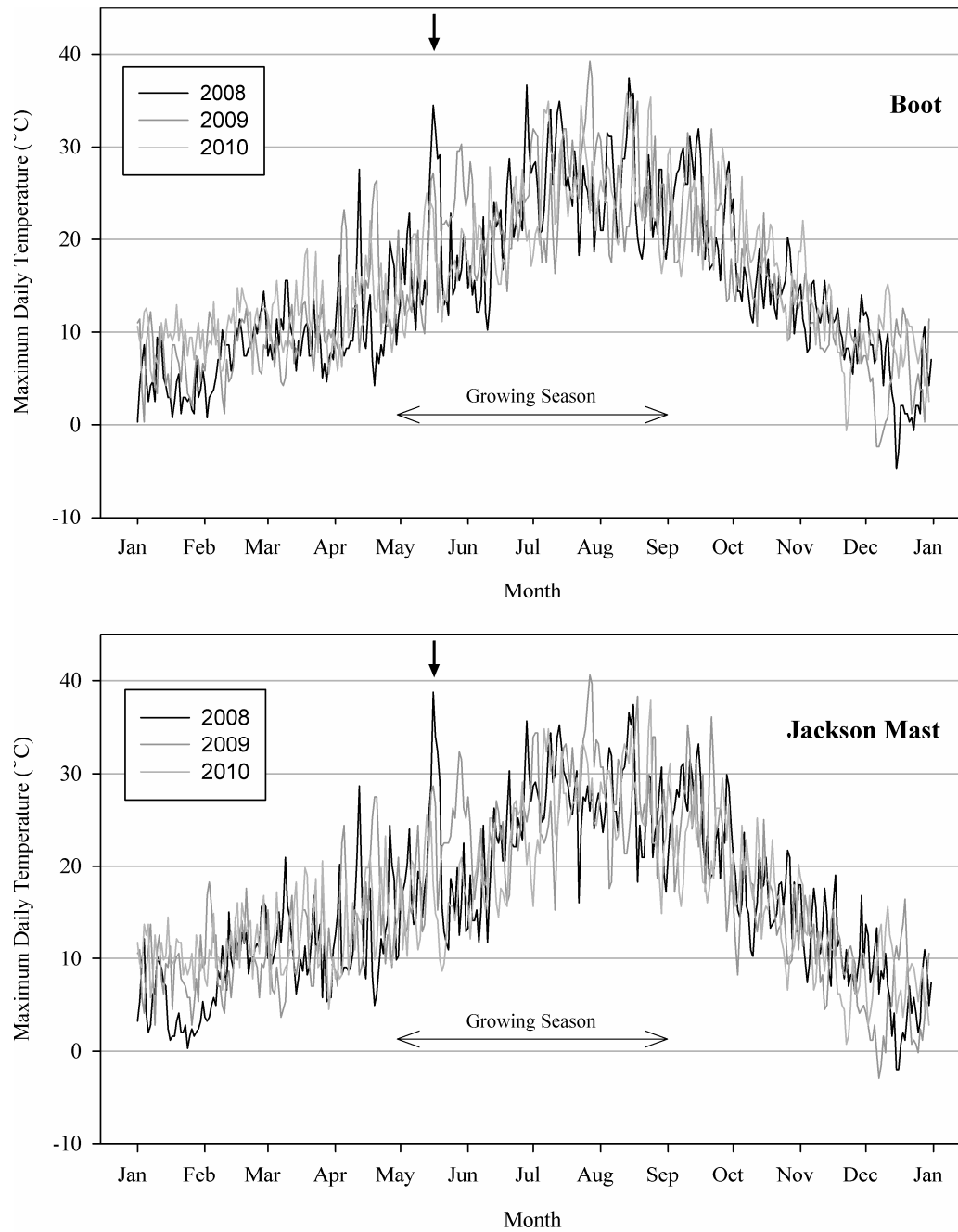


Figure 2.8: Daily maximum temperature presented by site and year. Bolded arrow on the top of each image indicates the peak of a three-day heat event observed on 16 May 2008. Approximate length of the growing season has been indicated by a double-ended arrow.

was the hottest temperature and one of the driest days recorded on that site during the entire 2008 growing season. The same day at Boot a high temperature of 34°C (93°F), relative humidity of 27%, and VPD of 3.88 Kpa was recorded. Similar May heat events were not recorded in 2009 and 2010. During these years, temperatures steadily increased through the spring until peaking in late-July or early August.

## **2.5 Discussion**

### **2.5.1 Delaying Forest Establishment**

This is the first study in the Pacific Northwest to evaluate the tradeoffs associated with whether or not delaying establishment and instituting a fallow year improved seedling growth through improved efficacy of vegetation management. Plantation establishment and competition control immediately following harvest (no fallow period) created seedlings that were physically larger than those planted after a delay. This result indicated that delaying silvicultural activities extended the time associated with forest establishment. During the initial season after planting, seedlings draw upon stored carbohydrate reserves as well as the photosynthetic output of current needles to establish intimate contact with soil and extend roots to acquire water and nutrient resources (Burdett 1990; van den Driessche 1987). Bud flush and future growth potential is directly tied to a seedling's ability to accomplish these tasks. Delaying the establishment of the next stand passed up this initial growth year.

Trees planted according to the delayed schedule did not respond to the level of vegetation control with growth that surpassed trees planted the prior year in treatments 2, 3, and 4. Agricultural crop production illustrated that minor losses in growth may occur with planting dates close together, but planting dates farther apart tend to have larger and more economically negative impacts on crop yield (Helms et al. 1990; Nielsen et al. 2002). The logistical considerations of applying the necessary seed bed preparation and appropriate weather conditions as well as the time required to cover large ownerships may prevent planting at the optimal time for the crop (Shaw 1996). Similar logistical considerations occur in forestry as variable weather patterns,

chemical effectiveness, delays in nursery production, and frost or heat events can influence the timing of establishment activities.

It is important to recognize that differences of approximately 30 to 50 cm in height, 0.5 to 1.0 cm in caliper, and 100 to 200 cm<sup>3</sup> in stem volume found during the initial three years between seedlings planted immediately and those delayed may not be biologically significant in the future. These are relatively short-term results and a temporal separation of one year may not be enough to slow timber production schedules such that rotation lengths are impacted. Conversely, planting immediately after harvest using the appropriate vegetation control regimes resulted in an automatic shortening of rotation length on that unit of land by one year. Budget constraints often dictate the length of time forest managers can control competing vegetation during the critical period (Nieto et al. 1968, Wagner et al. 1999, Rosner and Rose 2006) creating a high priority need to understand the most efficacious use of vegetation management regimes. Barring any large increases in chemical or application costs from year to year, treatments 3 and 5 as well as 4 and 6 should require similar funding. This would indicate that economically, the decision to establish a forest immediately or delay these activities will depend on the market on which mature trees will be sold. Projecting market conditions 40 or more years into the future is unpredictable.

### 2.5.2 Specific Seedling Responses

Initial seedling characteristics measured by Seedling Quality and Evaluation Services (Figure 2.1) demonstrated the potential for year to year variability in seedling size. This study ensured seedlings came from the same nursery using the same seed source and was not designed to manage nursery cultural practices. These practices as well as weather conditions work jointly to influence the morphology and physiology of seedlings destined for reforestation sites (Burdett 1990). Reactive measures like frost protection and active management of irrigation, fertilization, pest control, wrenching and/or root pruning are used to direct seedlings toward target characteristics (Rose et al. 1990; Burdett 1990). The year to year difference in root to

shoot volume data at Boot demonstrated how these physical characteristics can change in operational silviculture.

The mid-May heat event in 2008 (Figure 2.8), physical site characteristics, and the vegetation competition at Jackson Mast provided a plausible explanation for the slow plantation establishment reported. In 2008, actual bud break and leader extension occurred during the early part of May (Fuchigami et al. 1982; Burney and Jacobs 2009, personal observation) so bud scales were open and foliage exposed when the high temperature and VPD were recorded on 16 May. Jackson Mast is situated on a SW to W facing slope at angles that range between 10 to 25° so the surface of the soil is tilted toward incoming solar radiation at angles that improve the transfer of light energy (Maguire 1955; Holbo and Childs 1987). It is proposed that this heat event coupled with the physical attributes of Jackson Mast provided an initial dessicating shock that exceeded the ability of seedlings with limited root systems to supply water to growing tissues (Barnes et al. 1998; Hobbs et al. 1992; Larcher 2003).

Plant community competition contributed to the poor seedling growth and 44% survivorship in the no-action control by drawing soil water to low levels at Jackson Mast. Substantiating this argument was the fact that trees in treatments 2, 3, and 4 experienced the heat event, but had less than 16% vegetation cover and soil moisture over 14%  $\text{m}^3 \text{H}_2\text{O}/\text{m}^3$  soil. Survivorship was improved to between 63 and 69% (still an unacceptable number for forest managers). Trees planted in January 2009 were exposed to similar chemicals and low cover values, but did not experience hot dry conditions in mid-May. Seedling survivorship was over 88% and their growth response may put them on a trajectory to surpass seedlings planted the previous year (treatments 2, 3, and 4). Boot was a very different site but provides support for this discussion. It faces north at 0 to 5° so incident light on this site was at a more oblique angle and temperature and VPD were lower during the mid-May heat event in 2008. Seeding survivorship and growth at Boot was much improved.

Seedlings in the open setting of a regenerating unit must contend with high temperatures, various levels of competition for limiting site resources, and extend

roots and shoots in a manner that allows them to steadily increase dominance over the course of stand development (White and Newton 1989; Nyland 2002; Walstad and Kuch 1987). Douglas-fir and other true firs can be highly susceptible to heat damage in young conifer plantations when high surface soil temperatures occur on south and southwest facing slopes (Newton 2008; Livingston and Black 1987). If extreme weather events in the PNW become more common in future years, changes to nursery cultural practices and silvicultural regimes may be required. Nursery managers may need to target seedlings with lower shoot to root ratios increasing the volume of roots relative to the shoots, a measure linked to reforestation success on dry outplanting sites (Rose et al 1990; Rose et al. 1997; Hobbs et al. 1992). In a physical setting like Jackson Mast, harvesting patterns may need to change to a shelterwood or strip cut (oriented to cast shade in the afternoon) which may provide more ameliorated conditions for the initial years (Holbo and Childs 1987). If crop failures occur on a more regular (although unpredictable) basis, reforestation budgets will need to be adapted such that funds are available for replanting. Lastly, additional years of vegetation control may also be required to reforest difficult sites. There are recognized tradeoffs associated with each of these options which revolve around the efficiency of forest management as well as the ecophysiology requirements of the conifer crop species.

At the rates, timing of application, and soils on these sites, the presence of sulfometuron methyl in the fall site preparation tank-mix did not noticeably influence bareroot Douglas-fir seedling growth. This study offered the opportunity to investigate the effect of sulfometuron methyl at a rate and timing of application common to operational practices. It was not conducted to define the exact phytotoxic level of the chemical. A phytotoxicity study using carefully controlled conditions and a gradation of sulfometuron rates would need to be done in order to assess the exact level that impacts bareroot Douglas-fir 1+1 seedlings on a particular soil. This study is indirect confirmation that when used in a fall site preparation tank-mix on these soils and at the rates tested (always according to label instructions), sulfometuron

methyl did not damage Douglas-fir bareroot 1+1 seedlings in a manner that was statistically detectable.

Much controversy about the effects of sulfometuron methyl has existed among forest managers in the region. The chemical inhibits the activity of acetolactase synthase, an enzyme responsible for the production of proteins necessary for normal growth and cellular function (Ahrens et al. 1994; Colquhoun 2001; Kearney and Kaufman 1988). It is soil active and depending on site characteristics and environmental conditions, has a half-life of up to one month (Ahrens 1994; Trubey et al. 1998). The soil residual nature could increase the potential for seeding roots to come in contact with the herbicide and detrimentally impact initial growth. Substantiating this debate, Burney and Jacobs (2009) reported that the chemical had short-term impacts on containerized (415D or styro-10) Douglas-fir seedlings planted near Tillamook, Oregon. Results indicated that sulfometuron methyl (Oust<sup>®</sup>) applied as a fall site preparation at 0.16 kg ai ha<sup>-1</sup> reduced root growth more than five centimeters below the surface by 30 to 49% when compared to a no action control (Burney and Jacobs 2009). The chemical did not impact above-ground growth characteristics and the negative effect on root growth disappeared 15 months after the fall site preparation. Root growth was not measured in the current study and the bareroot seedlings used had no media surrounding root systems. However, consistent with findings presented by Burney and Jacobs (2009), there were no detectable differences among the above-ground morphologic seedling measurements.

### 2.5.3 Vegetation Community Response

Plant community development in the no-action control illustrated the rapid colonization of herbaceous plants and gradual increase in dominance of woody/semi-woody species according to successional trends. The disturbance of harvesting substantially modified the light environment, decreased demand for water, and due to the movement of logs and machinery, exposed mineral soil. These actions provided the appropriate germination and early growth conditions that favor wind-disseminated

seed as well as the stimulus required for species in the seed bank (Duke 1985). Many of these herbaceous species are known to have rapid photosynthetic rates, high growth rates, prolific root systems, and produce large amounts of seed (Antos and Halpern 1997; Dyrness 1973; Larcher 2003; Michaux 1989; West and Chilcote 1968). These characteristics enable them to dominate a site creating intense competition for resources in a relatively short period of time (Dinger and Rose 2009, Newton and Preest 1988). Woody/semi-woody plant species germinate from seed and can regenerate vegetatively from root stocks or stump sprouts (Antos and Halpern 1997). These species tend to have slower photosynthetic rates and hence slower growth rates, but as leaf area continues to develop, they can become strong competitors (Larcher 2003; White and Newton 1989). In this forestry context, these residual woody/semi-woody species can shift from what Grime (2002) described as “stress tolerators” in the previous stand to intense “competitor” species in this open environment. If left unchecked, they can become a longer-term hindrance to forest growth (Harrington and Tappeiner 1991; Reynolds and Roden 1995; White and Newton 1989).

This study has shown various methods with associated tradeoffs that can be used to limit plant community growth on managed lands with the potential for invasion from diverse mixes of herbaceous and woody/semi-woody plant communities (Balandier et al. 2006; Newton 2008; Wagner et al. 2006). From a forest management perspective, these herbaceous and woody/semi-woody plants do not hold the same economic value as the trees these sites are managed for and are thus, deemed to be weeds. Vegetation management regimes introduce additional short-term disturbances which interrupt floristic successional models (Connell and Slatyer 1977). These alternate pathways disrupt plant community development by introducing planned disturbances in an attempt to minimize competition for limited site resources during the initial establishment phase.

Treatment 2 (OS/OO) demonstrated how herbicides utilized in a spring release treatment have the ability to selectively control herbaceous species. Spring release treatments utilize chemicals and rates that can be broadcast applied over dormant tree

seedlings while controlling undesired plants that germinate in the winter and early spring. Of the chemicals applied in the spring release, hexazinone has the longest residual nature with a reported half-life of 90 days (Ahrens et al. 1994). This characteristic in conjunction with droughty summer conditions common in the PNW serves to limit plant community growth across a growing season improving efficacy and longevity of the treatment (Rose and Ketchum 2002). This treatment represents a potential cost savings and a vegetation management option that uses a smaller amount of herbicides.

Applying only a spring release (treatment 2) did not provide long-lasting control of woody/semi-woody species at either site. In fact, results indicated that the elimination of the herbaceous component in these plots served to release both the Douglas-fir seedlings and the woody/semi-woody plants. The chemicals at the rates applied were not necessarily designed to control established residual plants. Herbicidal chemicals with soil residual behavior typically bind to organic matter and clay colloids in the upper profile owing to the high cation exchange capacity (Ahrens et al. 1994; Brady and Weil 2002). Established plants in these forested settings have deep root systems (Antos and Halpern 1997) and the chemicals may not penetrate far enough to negatively impact growth (Michael et al. 1999; Koskinen et al 1996; Newton et al. 2008). Deep roots and limited development of foliage at the time of application may allow certain plants the mechanisms necessary to avoid lethal herbicidal doses. While hexazinone can control woody species at higher rates or if significant foliage exists, there is a real danger to crop trees (acknowledged on the herbicide information label). The treatment has resulted in seedling growth improvements, but it remains to be seen if early responses change in future years as woody/semi-woody vegetation continues to compete with planted trees.

It was the combined application of a fall site preparation and a spring release (treatments 3-6) that reduced both the herbaceous and woody/semi-woody components of the plant community. Fall site preparation treatments often use a mix of two or more chemicals designed to increase the potential for absorption through foliar as well

as root contact (Ahrens et al. 1994; Colquhoun 2001). These chemicals are applied at rates that result in the adequate control of problematic species (Newton 2008; Lauer and Quicke 2006). The phytotoxic effects of these chemicals degrade in the months that elapse from application to the next spring; a fact forest managers use to minimize damage to tree seedlings (Reynolds and Roden 1995; Newton 2008). A spring release treatment following this fall site preparation is applied to control plants that germinate in the presence of diminishing herbicidal influence and maintain low amounts of competition for the length of the initial growing season (Dinger and Rose 2009; Zutter et al. 1986; Sand and Nambiar 1984). The tandem approach of these two treatments resulted in the efficacious control of the vegetation community across the initial three years of establishment.

Including sulfometuron methyl with the combination of chemicals in the fall site preparation tank-mix slowed the regrowth of the vegetation community in the second season after application providing some amount of extended control. It is possible (yet untested by the current study) that these chemicals are behaving in an additive or synergistic manner when applied as a tank mix and represent a potential avenue for future forest vegetation management research. Agricultural literature has reported additive, synergistic, and even antagonistic interactions among chemicals applied for weed control (Damalas 2004; Zhang et al. 1995). The type of interaction is highly dependent on the weed species (e.g. monocot vs. dicot), mode of action, translocation, site of activity, timing of application, etc. Damalas (2004) reported that synergism can be more common with broadleaved weedy plant species and companion herbicides that belong to the same chemical group. It is possible that the chemicals included in the fall site preparation, specifically sulfometuron methyl and metsulfuron methyl (both sulfonylurea herbicides), could have interacted in a manner that slowed the reinvasion of treated plots. But, this remains only a question at this time. While vegetation regrowth was slowed with these regimes (i.e. 4 and 6), second season cover was above the threshold associated with successful vegetation management (Dinger

and Rose 2009; Harrington and Tappiener 1991). These subtle differences could be artifacts of the data and not necessarily significant on a biologic level.

Treatments delaying vegetation control (5 - OO/FS and 6- OO/F<sub>o</sub>S) may have an adverse affect on the speed with which the vegetation community reinvades open areas due to the potential for increased seed rain. The rapid increase in abundance of the vegetation community, specifically herbaceous plant species, associated with treatments 5 and 6 during the 2010 growing season at Jackson Mast may be partly due to the vegetation communities in and around the research site. The western edge of Jackson Mast is less than 500 meters from Interstate 5, open to the prevailing winds (predominant winds are from the S and W), and close to a small collection of farms and residential homes. Alternatively, Boot is located in a more sheltered location (low winds) with contiguous mature and maturing forest surrounding it. Similar rates of increase post-chemical disturbance were observed at Boot in 2009 and 2010 for the fall site preparation and spring release treatments (3 and 5 as well as 4 and 6). In locations where herbaceous wind-dispersed seed is expected to be a challenge, delaying plantation establishment may not be a wise decision unless additional years of vegetation control are used to restrain growth long enough to protect the critical period (Wagner et al. 1999).

Delaying plantation establishment through the introduction of a fallow period has not resulted in a lower abundance of woody/semi-woody plants at Boot and was only marginally different at Jackson Mast. This result indicated that the chemicals used in common operational vegetation management regimes were capable of reducing plant cover to low levels (less than 10%) regardless of the timing utilized in this study. The result also shows that one year of unhindered growth allowing the development of larger amounts of leaf area, did not necessarily create a more efficacious use of herbicidal chemicals. Given the tree results discussed previously, delaying vegetation control appeared to simply lengthen the time associated with this early-seral stage of forest regeneration except on a site and during a year experiencing an extreme weather event.

## 2.6 Conclusions and Management Implications

The primary objective of early silvicultural treatments is to ensure a fully stocked vigorously growing stand of trees that exceeds management expectations and both state and federal forest policies. Reforestation scenarios can challenge conventional wisdom and create opportunities to evaluate methods that can be used to support decision-making criteria necessary for effective land management. The foundation of the concept to delay establishment in order to improve chemical vegetation control is built upon the complexities of managing logistical considerations that integrate complex weather patterns as well as matching chemical choices, timing, and rates of application to the current and potential weed community. This study is unique in the forestry literature and is supported by agricultural research which illustrated that tradeoffs exist with various establishment strategies.

Delaying plantation establishment in a multi-year forest crop did not result in tree growth that surpassed seedlings planted one year earlier except on a site where physical characteristics accentuated an extreme weather event. Given the short time frame necessary to establish forests on production schedules and the length of the critical period for tree species, delaying establishment one year resulted in a stand of trees that is one year behind. These are short-term results and treatment differences could disappear with time so the long-term impact of introducing a fallow period remains to be seen. Sulfometuron methyl included in the fall site preparation at the rates tested did not appear to affect above-ground Douglas-fir seedling growth in a negative manner.

Results indicated that the chemical choices, rates, and timing of herbicide applications according to the treatments included in this study were sufficient to limit vegetation community growth (as measured by summed cover) during the year of application. Chemicals used in the treatment regimes altered the herbaceous and woody/semi-woody components of the vegetation communities. A spring release only treatment reduced herbaceous vegetation during the year of treatment, but did little to control woody/semi-woody plants. Even after one year of unchecked growth (the

fallow year in treatments 5 and 6), similar chemical regimes limited competing plant growth below 20%, a typical threshold for successful vegetation control. Fall site preparation treatments were necessary to provide longer-term control of woody/semi-woody plants. No single chemical regime was capable of providing long-term control of herbaceous plants. Sulfometuron methyl included as a component in the fall site preparation tank-mix did not result in reduced competitive cover during the year of application.

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## **CHAPTER 3.0**

### **COMBINING STEM AND VEGETATION MAPPING APPROACHES TO CHARACTERIZE ABUNDANCE AND DISTRIBUTION OF AN EARLY- SERAL FOREST PLANT COMMUNITY**

#### **3.1 Introduction**

Collecting and presenting data on forest plant communities in a designed experiment poses unique challenges. Various methodological approaches exist to quantify and characterize the abundance of plant species. The majority of these data collection techniques require a botanist to visually estimate cover percentages based on a specified number of subplots within larger experimental units. There are no known survey methods which can use remotely sensed images and computer modelling software to develop species specific cover values in these complex vegetation communities (Omasa et al. 2007; Hopkinson et al. 2004; Tarp-Johansen 2002). Presenting these results is often done in a tabular format of species lists and cover percentages (Halpern and Spies 1995), groupings by growth habit (Miller et al. 1995), a sentence describing the current or previous forest type (Peter and Harrington 2009), or graphical form in charts of various scatter plots, trend lines, or other figures. While these techniques all have merit, few opportunities exist to collect spatially explicit data on interspecific competition within experimental units and present that data in a graphical format.

Programs such as the Stand Visualization System (SVS) were designed to create images that demonstrate aspects of silviculture and forest development (McGaughey 1998; Stoltman et al. 2004). These programs have the capability of utilizing spatial information in a Cartesian coordinate system placing trees and other large objects of interest on a viewable plane. Plant growth habits physically smaller than shrubs and trees, however, are more difficult to present. Large numbers of individuals, close proximity, and rapid species turnover make it difficult to collect

spatial information on smaller plant lifeforms. Forest visualization software has thus disregarded herbaceous and woody/semi-woody components of the early-seral plant communities.

Mapping tree locations was initially presented by researchers in Canada during the late-1960's. Ek et al. (1969) developed stem mapping techniques that generated Cartesian coordinates (X and Y distances from a reference point) necessary to draw the location of trees. Payandeh (1974) carried on Ek's work by using this positional data to assess measures of uniformity or randomness among trees, shrubs, and other forest structures. Moeur et al. (1993) utilized a transit laser to stem map 0.3 to 0.9 ha plots from multiple positions within an established forest to assess the level of tree uniformity in older stands. Other techniques use triangulation (Boose et al. 1998) or measuring tapes and 90 degree prisms (Reed et al. 1989) to collect the necessary spatial data in mature forest stands. These techniques have the disadvantage of being slow and tedious in an established forest where line-of-sight is highly limited, an impediment to any ground-based data collection system. The use of ground-based survey lasers during the early stages of plantation establishment could, however, make it possible to map stand conditions when line-of-sight is not obstructed.

Plant communities are influenced by the silvicultural regimes used to establish forest stands on managed lands. Herbicidal vegetation control is one such prescription that has the potential to temporarily limit plant growth as well as create short-term shifts in the community composition based on the response to these regimes (Comeau and Harper 2009; Dinger and Rose 2010; Miller et al. 1999). Competition for limited site resources can be intense and has been shown to have a spatial component (Fischer and Miles 1973; Rose et al. 1999). As the weed-free area around a seedling increases, the probability of overlapping zones of competition decrease, enabling improvements in growth response (Casper and Jackson 1997; Fischer and Miles 1973; Rose et al. 1999; Rose and Rosner 2005). A technique has not been developed to characterize, even on a coarse-scale, the spatial changes that occur in plant communities during early succession as directed by silvicultural regimes.

Techniques that accurately and efficiently portray growth, development, and replacement of plant species are needed to increase our understanding of the complex phenomenon associated with plant community response to management activities. It is also a highly coveted situation within the sciences when a new technique can be used to challenge a well-established methodology in an attempt to test the validity of both. The objectives of the current study were to 1) present a methodology for relative mapping of tree locations and experimental unit features in a regeneration study, 2) present a new methodology for assessing, on a coarse-scale, whole-plot vegetation, and 3) compare the accuracy of this new whole-plot vegetation survey with the more common use of randomly located subplots to characterize plant community dynamics. Future applications and avenues for research will also be discussed.

### **3.2 Materials and Methods**

*Prologue: All distance measurements were intentionally presented in United States Customary Units throughout this paper. This is normally an unacceptable practice in scientific literature, but this decision was made in order to preserve the consistency of the data. Plot layout, survey instrumentation, visual measures of species abundance, as well as the applicability of the results to foresters in the region are all dominated by United States Customary Units (not metric) of measure.*

#### **3.2.1 Regeneration study site**

A single site, Boot, served as the basis for the current mapping study. It is located on a 0 to 5° north facing slope in the Oregon Coast Range (44° 46' 36.32" N, 123° 27' 57.63" W). Soils are classified as mesic typic Haplohumults and support a Douglas-fir site index of 135 feet at 50 years. Seedlings planted on this site in 2008/2009 represented the third generation of forest in recent ~100 year history. Several stumps over six feet in height with spring board notches suggest the forest was felled prior to the use of gas-powered chainsaws. Ring counts on the largest stumps harvested in the summer of 2007 ranged from 85 to 98 years indicating they were

established between 1909 and 1922. Dominant tree species on this site were *Pseudotsuga menziesii*, *Tsuga heterophylla*, *Alnus rubra*, and *Acer macrophyllum*. Common understory species were *Symphoricarpos albus*, *Polystichum munitum*, *Corylus cornuta*, *Sambucus* spp., and *Acer circinatum*.

A complete randomized block design was used to establish the regeneration study that served as the basis for the current mapping study. Six vegetation management treatment regimes (Table 3.1a) were randomly assigned and replicated four times on 60' x 60' measurement plots in the summer of 2007. Plot dimensions were based on slope distance measurements made with an Impulse Laser Rangefinder (Model 100; Laser Technology, Inc. Centennial, Colorado) and a double right angle prism (Sokkia Corporation; Olathe, Kansas). Plot corners were marked with five foot lengths of polyvinyl chloride (PVC) pipe. Using a measuring tape and colored pin flags, tree locations were marked on 10' x 10' grid prior to planting. The layout of the plots allowed 25 measurement trees to be located within each experimental unit. A single row of buffer trees was located between experimental units.

A professional crew was used to plant the Douglas-fir bareroot 1+1 seedlings in February 2008 (treatments 1-4) and January 2009 (treatments 5 and 6). Directions were given to place the seedlings in the best planting location that occurred as close as possible to each pin flag. Vexar<sup>®</sup> tubes were installed immediately after planting to protect seedlings from ungulate browse. Herbicide treatments were broadcast applied using a backpack sprayer according to the schedule outlined in Table 3.1b. Treatments were designed to test whether introducing a delay or fallow period into plantation establishment (i.e. treatments 5 and 6) improved vegetation control and in turn, early seedling growth (see Chapter 2). All sprouting *Acer macrophyllum* stumps were treated using a "hack and squirt" technique to apply an undiluted solution of imazapyr on the same date as the fall site preparation in 2007.

Each experimental unit was stratified into four equal quadrants and one permanent 1-meter radius vegetation survey subplot was randomly located between trees in each strata (n=96 on the site). During early to mid-July in the initial three

Table 3.1: Description of the six treatment regimes (A) and treatment regime details (B). Individual treatments were broadcast applied as a tank-mix using backpack pumps. Subscript “o” and the asterisk signify a fall site preparation with sulfometuron methyl included (treatments 4 and 6) in the tank-mix. Seedlings were planted in treatments 1-4 February 2008 while treatments 5 and 6 were planted January 2009.

<b>A</b>	Plantation establishment immediately following harvest		Delay plantation establishment one year	
Treatment	Fall Site Preparation	Spring Release	Fall Site Preparation	Spring Release
1. OO/OO	no	no	no	no
2. OS/OO	no	yes	no	no
3. FS/OO	yes	yes	no	no
4. F <sub>o</sub> S/OO*	yes	yes	no	no
5. OO/FS	no	no	yes	yes
6. OO/F <sub>o</sub> S*	no	no	yes	yes

<b>B</b>	Treatment Regime Details		
Regime	Application	Chemicals	Rates
Treatments 3 and 4	Fall Site Preparation 20 September 2007	Glyphosate (Accord)	3 qts/ac
		Metsulfuron methyl (Escort)	1 oz/ac
		Imazapyr (Chopper)	8 oz/ac
		Induce (adjuvant)	8 oz/ac
		Sulfometuron methyl (Oust) *	3 oz/ac
Treatments 2, 3, and 4	Spring Release 11 April 2008	Hexazinone (Velpar DF)	2.0 lbs/ac
		2, 4 D (Weedone LV6)	0.75 qts/ac
Treatments 5 and 6	Fall Site Prep 3 September 2008	Glyphosate (Forester)	3 qts/ac
		Metsulfuron methyl (Escort)	1 oz/ac
		Imazapyr (Chopper)	8 oz/ac
		Induce (adjuvant)	8 oz/ac
		Sulfometuron methyl (Oust) *	3 oz/ac
Treatments 5 and 6	Spring Release 15 April 2009	Hexazinone (Velpar DF)	2.0 lbs/ac
		2, 4 D (Weedone LV6)	0.75 qts/ac

Note: A “hack and squirt” treatment of the sprouting *Acer macrophyllum* stumps was conducted on 20 September 2007. Undiluted imazapyr was squirted into circumferential gashes made with a hatchet on all stumps at the site.

years of establishment, total cover of all vascular plants as well as cover of each species was recorded in these subplots. Total cover is defined as the percentage of a subplot covered by vascular plants. It was a number ranging from 0 (i.e. bare ground) to 100 (i.e. totally occupied by vascular plants). Hitchcock and Cronquist (1973) served as the basis for genus and species nomenclature. Information on species growth habit (forb, fern, graminoid, shrub, tree, vine/shrub) was incorporated into the dataset using the process outlined in Appendix 2. Vegetation survey information was divided into two principle groupings, herbaceous and woody/semi-woody. Forbs, ferns, and graminoids are referred to as herbaceous plants while woody/semi-woody plants are those classified as shrubs, trees, and vine/shrubs. The percentage totals for each species were summed based on these two components of the plant community to establish the amount of herbaceous or woody/semi-woody cover in a particular subplot. The four subplot values were used to calculate a mean and these means (n=24, 6 treatments x 4 replicates) were analyzed.

### 3.2.2 Stem Mapping

A permanent marker was established at least 3 meters from the SW corner of each experimental unit. This position tended to be physically uphill of each 60' x 60' plots. A Criterion<sup>®</sup> Survey Laser Series (Model 100; Laser Technology Inc., Centennial, Colorado) mounted on a monopod was used to ascertain the horizontal distance and magnetic bearing (azimuth) from this permanent marker. The laser location served as the origin (0, 0) and each point of interest within a plot was relative to this position. A slope correction was not used due to the low angles associated with the land on this site.

Three people collected these data. One person operated the laser while a second walked between points providing the "target." Positioned adjacent to the seedling, a two meter pole with a ping pong-style paddle attached as well as this person's body served as the "target" for each item of interest. The third person entered the data into a Tripod Data Systems (TDS) data logger (Model - Ranger;

Trimble Company, Corvallis, Oregon) and ensured accurate call back of information. While the order of measurement had no influence on the process, a strict order was adhered to so that all locations were measured. In this case, the four corner posts for the experimental unit were surveyed then border trees, followed by measurement trees, and finally vegetation subplot locations. This technique also allowed certain kinds of shrubs, trees, or sprouting stumps to be mapped.

### 3.2.3 Vegetation Mapping

Utilizing a feature of the experimental units, a coarse-scale vegetation survey was conducted that included 100% of the area within each 60' x 60' plot. Recall that all of the trees on the study site were planted on a roughly 10' x 10' grid inside each plot and Vexar<sup>®</sup> tubes were installed. These locations formed a grid that delineated 36 100 ft<sup>2</sup> polygons (Figure 3.1) marked by the location of the trees and/or the Vexar<sup>®</sup> tubes (if the tree was dead at the time of survey). Data collected within each of these 36 unique polygons could then be used to create a pixilated map of the vegetation community. For the purpose of simplification, these 36 - 100 ft<sup>2</sup> polygons within each experimental unit will henceforward be referred to as “vixels” or vegetation pixels.

A complete survey of all vixels on the site (n=864) was done in late-July or early-August each year (2008, 2009, 2010) following the detailed vegetation survey that occurred in the four randomly located subplots. Total vascular plant cover and square foot coverage of the top three most abundant species was visually estimated for each vixel. Generally, the key species in a vegetation community were a small number of individual species (Miller et al. 1995) and this determination required a subjective judgment. Similar to the subplot vegetation surveys conducted in July, Hitchcock and Cronquist (1973) served as the basis for nomenclature and information on species growth habit (forb, fern, graminoid, shrub, tree, vine/shrub) as well as separating these habits into herbaceous (forb, fern, and graminoid) and woody/semi-woody (shrub, tree, and vine/shrub) components. These data were added using the methods outlined in Appendix 2.

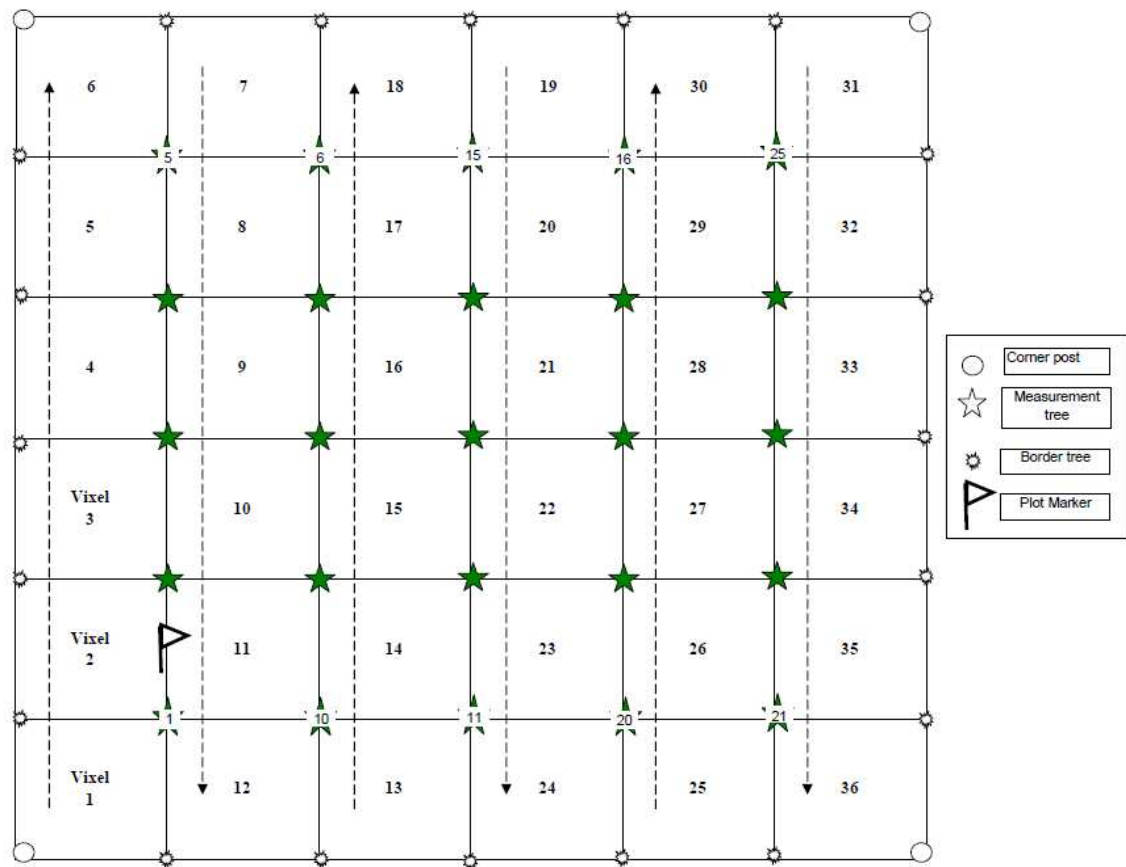


Figure 3.1: Conceptual map defining the vegetation pixel or "vixel." PVC corner posts are designated by the small circles. Border trees occur between PVC corner posts (small explosion symbols). Stars represent measurement trees and the flag symbol shows the location of a permanent plot marker between trees #1 and #2. Dashed arrows indicate the systematic order followed during vixel vegetation surveys. The beginning and ending rows of trees are numbered showing the approximate location of each measurement tree.

### 3.3 Data Management and Statistical Analysis

#### 3.3.1 Data Processing

Laser survey data were downloaded from the TDS data logger into Excel (Microsoft Corp.) where the horizontal distance and azimuth information was used to calculate X and Y distances from the laser location. This process is presented in steps one and two of Figure 3.2 and is similar to procedures outlined in Moeur et al. (1993). The survey point for each plot tended to be outside the SW corner. This meant that the majority of the points were located along azimuths that fell from North to East or 0 to 90 degrees, ascertained by the magnetic compass housed in the Criterion<sup>®</sup> laser. However, this was not always the case and azimuths toward the northwest or southeast created X Y coordinates with negative numbers. SVS does not permit negative numbers and in order to position each point of interest in a space recognizable by the program, 50 feet was universally added to both coordinates making all numbers positive and eliminating the issue.

These data were incorporated into SVS to produce individual plot maps similar to Figure 3.2 and visually inspected for erroneous data (Figure 3.3a). Out of the 1272 data points on the Boot study site, only 5.5% required any kind of correction. The majority of these mistakes were typographical errors and easily spotted using SVS as either the distance and/or azimuth were incorrect placing the element of interest in an impossible location. An adjustment was made to place the item in the appropriate location, but slight variations were not changed (Figure 3.3b). In this context, the “appropriate” location of trees and other markers was definable. Plots were established methodically and all tree locations marked on a strict grid pattern such that specific trees were in locatable positions (e.g. tree #6 should always be next to #5). It is recognized this technique would be an inappropriate practice if extreme accuracy and/or unbiasedness was an absolute requirement. Under these circumstances, a secondary data collection would be necessary to correct these points. For the purposes of this study, the small number of corrections was not deemed to be improper or result in bias which would compromise study results.

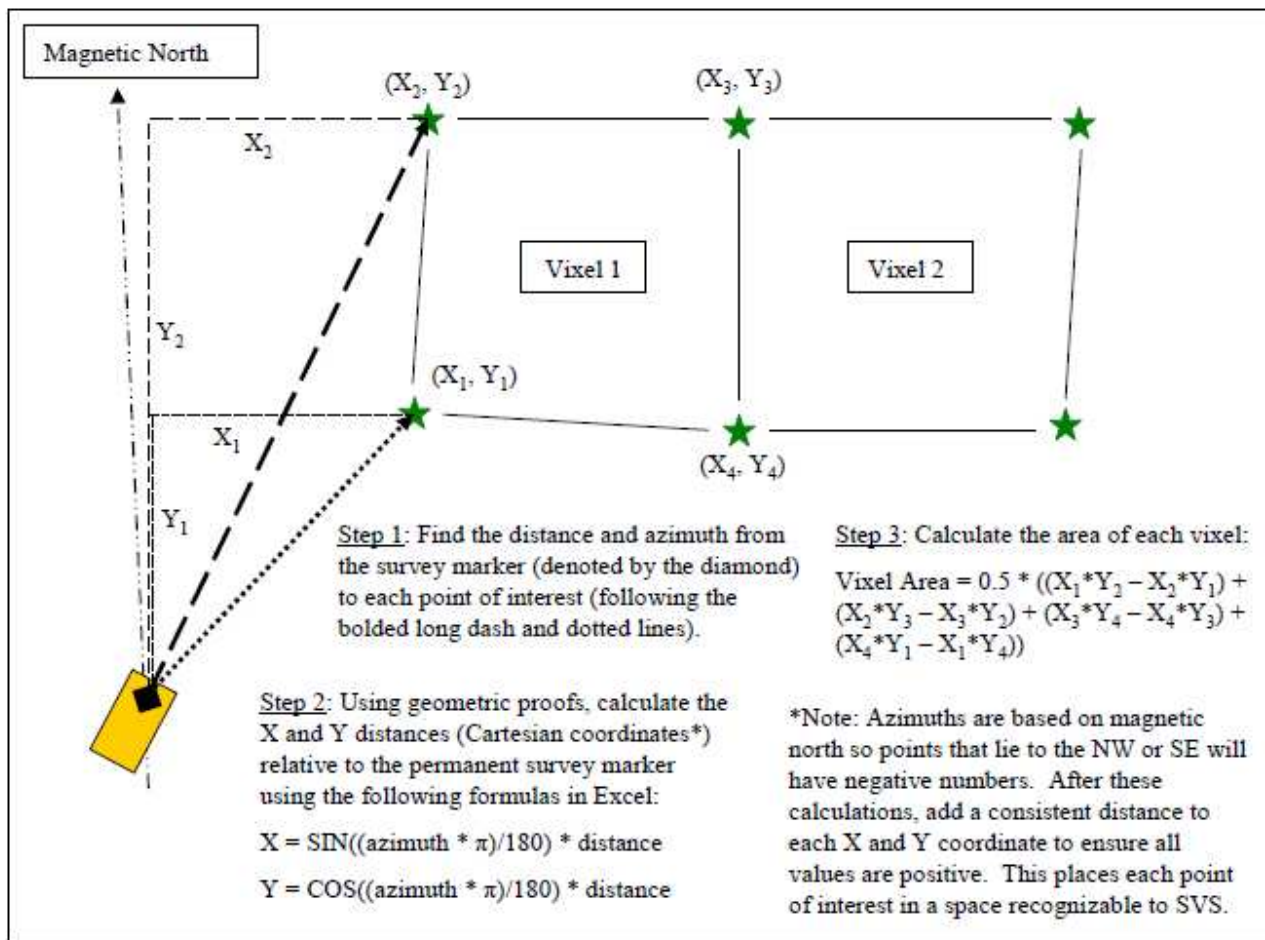


Figure 3.2: Explanation of the steps in the data collection process (step 1), calculating the X Y coordinates (step 2), and using these coordinates to calculate the area of each vixel (step 3). Seedlings are represented by filled stars and vixel borders are delineated by the solid lines. The diamond is the survey point and the rectangle over the diamond is the laser.

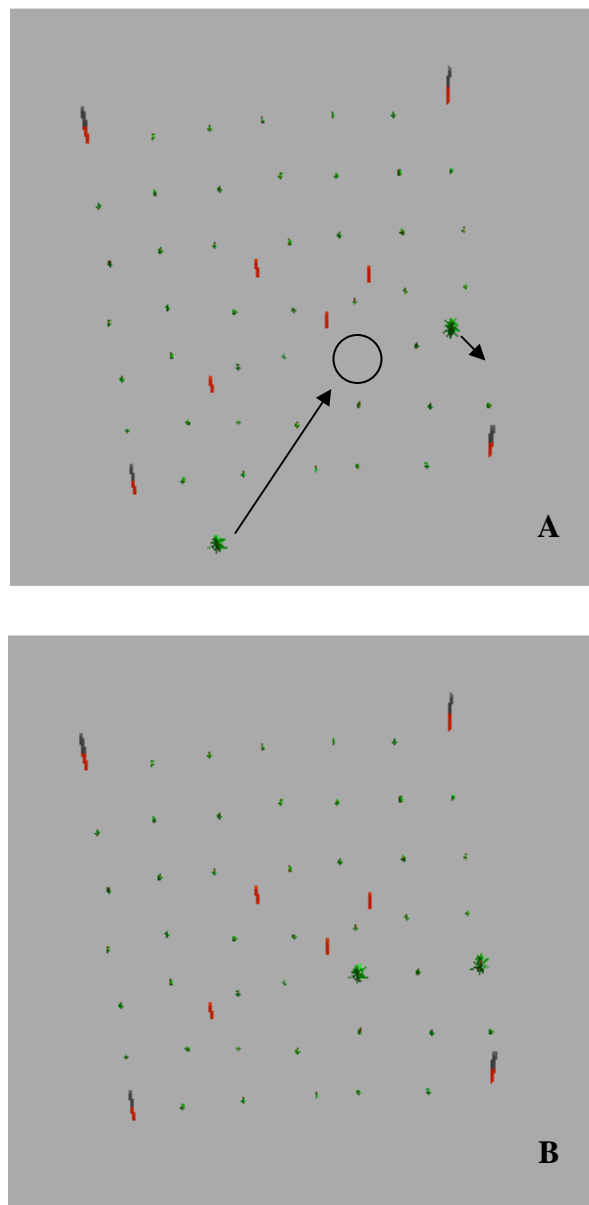


Figure 3.3: Stand Visualization System (SVS) images of raw data in Block 1 Plot 4 at Boot with two seedlings in unlikely positions (A) and the same plot with the two trees edited to be in the “correct” position (B). The two suspect trees were intentionally made larger than the rest of the seedlings in order to clearly illustrate how errors in data collection were discovered and corrected.

At the time of the vixel vegetation survey, the tree locations were used as a frame of reference to aid in determining species cover. The assumption was that at the time of survey, the space between the four trees was 100 ft<sup>2</sup> but terrain features, harvest residue, and planter decisions all resulted in tree locations that are not exactly 10 feet apart. This meant that not all vixels encompassed exactly 100 ft<sup>2</sup> and a correction was required to accurately portray the square foot coverage of individual species. The Cartesian system of X Y coordinates allowed the direct calculation of the area contained by each vixel using a geometric formula outlined in Step 3 of Figure 3.2. This area was then divided by 100 (what it was assumed to be at the time of visual assessment of the vegetation) to develop a correction factor. This correction factor was then used to adjust plant species cover values based on the size of each vixel (i.e. multiplying species cover by the correction factor). All plant species cover values presented in this paper have been adjusted in this manner.

### 3.3.2 Statistical Analysis

Statistical Analysis Software version 9.1 was used (SAS Institute, Inc., Cary, NC) for analysis. Regression analysis was conducted using the PROC REG function in SAS. ANOVA analyses were conducted using generalized linear models (PROC GLM). Assumptions of normality, linearity, and constant variance were examined on the residuals for each response variable tested. No transformations were required to meet model assumptions. Unless otherwise stated, an alpha level of 0.05 was used to determine significant differences.

Mean total cover percentages were individually calculated for each experimental unit by the described techniques (subplot and vixel vegetation surveys) across the three data collection years. These means were then compared using simple linear regression analysis to understand the relationship between these two methods of measuring a vegetation community. The dependent variable was the total cover percentages found through the four randomly located subplots while cover percentages found through the vixel assessment served as the independent variable. All three

initial years worth of data (2008, 2009, and 2010) were included in this analysis. This was a necessary step as the vegetation community in any one year did not provide the full range of observable cover values.

Treatment effects on 2010 total cover values provided by the subplot and vixel vegetation surveys were analyzed separately by data collection technique and year using ANOVA procedures. Fisher's protected least significant difference t-tests were used to compare treatment means at an alpha level of 0.05. Herbaceous (forbs, ferns, and gramioid) and woody/semi-woody (shrubs, trees, vine/shrubs) components of the 2010 vegetation community were analyzed separately by data collection technique using ANOVA procedures.

Common and/or abundant plant species are presented in mathematical and graphical format only. Due to the manner in which the data was collected, ANOVA procedures could lead to erroneous results. Only information on the top three species were collected at the time of the vixel vegetation survey which meant that the actual area and presence of a specific species created zeros in the dataset that could be a result of the sampling method not necessarily the treatment regimes being tested by ANOVA procedures. When means of a particular species are presented, these values were calculated by treatment over replication. Vixel images of individual plots are also graphically presented.

### 3.4 Results

*Summary: While stem mapping concepts are not new, this was the first known attempt to apply these techniques to a regenerating forest and vegetation community response to silvicultural regimes. Results indicated that visual representations of research plot conditions as well as the vegetation community dynamics can be presented, but not completely married into one image at the current time. A comparison of total plant cover evaluated through the four randomly selected subplots and that developed through vixel cover resulted in a linear regression equation with an adjusted  $R^2$  of 0.90. The two vegetation survey methods had less than a 12*

*percentage point difference in total cover when compared by treatment and year. Analysis of herbaceous and woody/semi-woody components of the vegetation community revealed similar response trends despite the differences between the two techniques.*

### 3.4.1 The Process of Mapping Stems and the Vegetation Community

Data collection in this regenerating forest took less than 20 minutes to map the points of interest located within each plot. Three people were required for the mapping process so data collection could proceed at a reasonable pace. The resulting datasets were then easily managed using standard computer software that included the free SVS program made available by the USDA Forest Service (McGaughey, 1998). Plots were carefully laid out to encompass 3600 ft<sup>2</sup> inside each 60' x 60' experimental units. Trees were planted on a 10' x 10' grid and vixels should encompass, on average, 100 square feet. Using the data produced from the mapping exercise, the mean plot area (n=24) with a 95% confidence interval was 3657 ft<sup>2</sup> (3614.8, 3699.3). The mean vixel area (n=864) with a 95% confidence interval was 101 ft<sup>2</sup> (99.73, 102.8).

Collecting vixel data was an intense dedicated effort. It took two to three days for one person to survey the entire plant community on the study site. Focusing only on total cover and the abundance of the top three species enabled data collection to progress faster than a vegetation survey recording every species encountered. Figure 3.4 presents an example of how vixel images portray the dynamic changes that occurred on the site. This experimental unit served as a no-action control and the corner posts (cylinders in the four corners), vegetation survey locations (smaller cylinders), all trees (small green dots), and one bigleaf maple (*Acer macrophyllum*) stump that escaped treatment in the fall of 2007 are clearly presented. Tree measurements (height and diameter) as well as measures of the maple stump (canopy radius and height) were included in 2008 (left) and updated using the same measures in 2010 (right). X and Y grid lines were included in this vixel image to show the

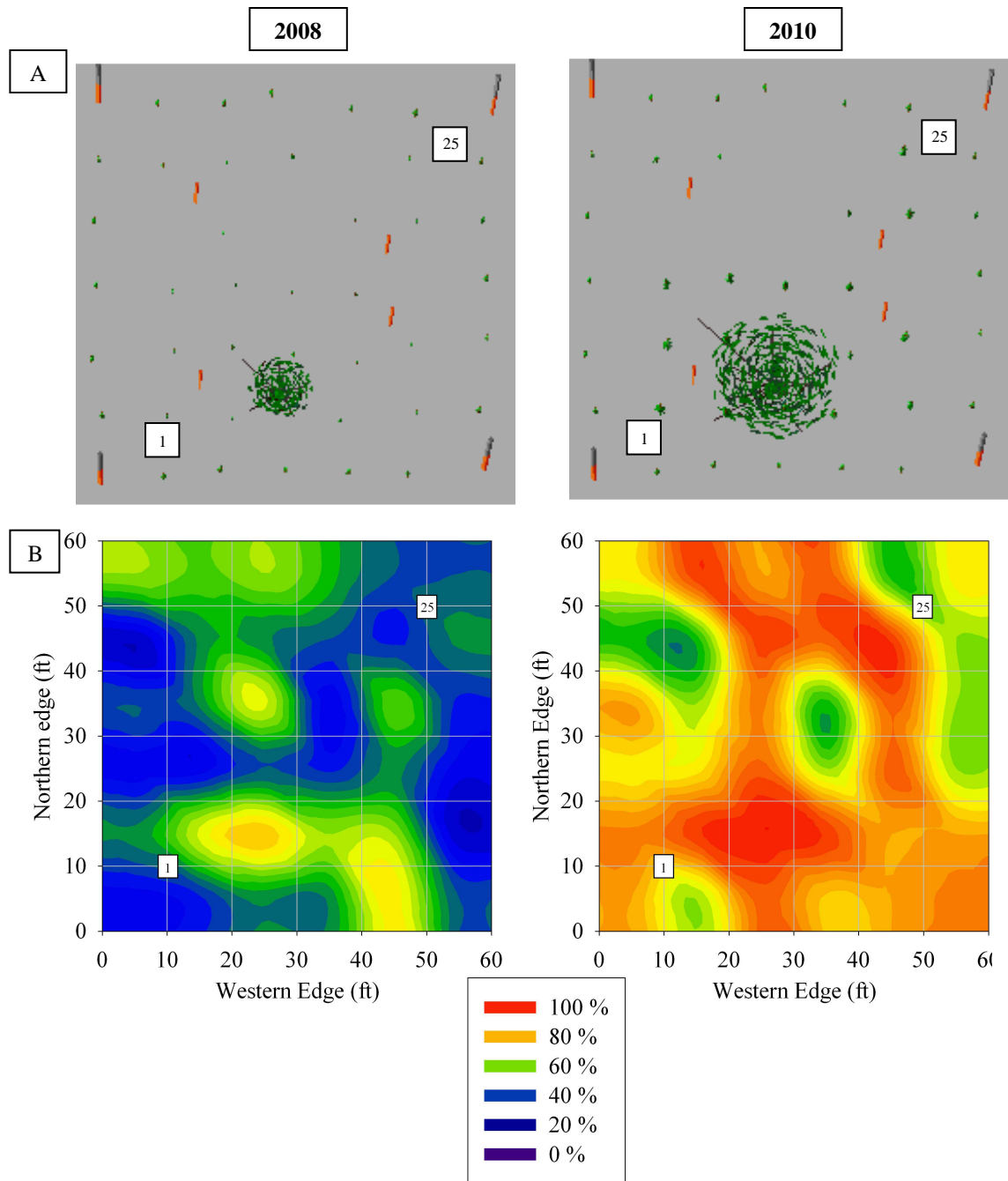


Figure 3.4: SVS images of a no-action control in 2008 (left) and 2010 (right) (panel A) as well as the total cover (%) of the vegetation community (panel B). Mapped tree locations are presented in panel A with corner posts represented by double high cylinders and vegetation survey markers as single high cylinders. Sigmaplot<sup>®</sup> does not allow specified positions to match the voxel concept so the straight grid lines were utilized in panel B to show approximate tree locations. The approximate location of tree 1 and 25 were inserted with small labeled boxes.

approximate location of all trees in the plot. Vixel survey results are presented in panel B (lower). The technique detected the competitive epicenter shown by the 100% cover in the bottom central portion of the vixel image.

### 3.4.2 Comparing the Accuracy of Subplots and Vixels

Regression analysis between total cover assessed through the vixel survey (x-axis) and that resulting from the subplot survey (y-axis) is presented in Figure 3.5. The linear regression explained over 90% of the variation among the two techniques of measuring total plant cover (adjusted  $R^2$  of 0.9082).

Analyzing total cover assessed through either technique as well as the amount of herbaceous and woody/semi-woody vegetation in 2010 revealed significant treatment responses in the first three years of establishment (Table 3.2). Treatment means resulting from both techniques are presented in Table 3.3. By either the vixel or subplot method, herbicide application resulted in 2008 total cover values below 20%. Treatment 2, which received only a spring release, restrained cover to 14% and 17% during 2008 found through the subplot and vixel techniques, respectively. Plots receiving a fall site preparation in addition to this spring release (treatments 3 and 4) were limited to less than 6% cover. The no-action control and delayed regimes (treatments 5 and 6) had cover values between 30 and 39% in 2008. The application of treatments 5 and 6 in 2009 reduced total cover below 9%. Plants in the no-action control increased to over 73% cover by the time of the 2010 surveys. Treatments 2 and 3 were not different compared to the no-action control in 2010 by either vegetation survey method ranging from 68 to 73% cover. Total cover abundance increased in treatments 4, 5, and 6 from 2009 to 2010 but remained statistically lower than treatments 1, 2, and 3.

There were, however, differences that existed between the values presented by the subplot or vixel techniques (Table 3.3). The vixel method suggested that treatments 4, 5, and 6 were not different from one another in the 2010 survey and were separated by less than 10 percentage points. The subplot analysis showed a difference

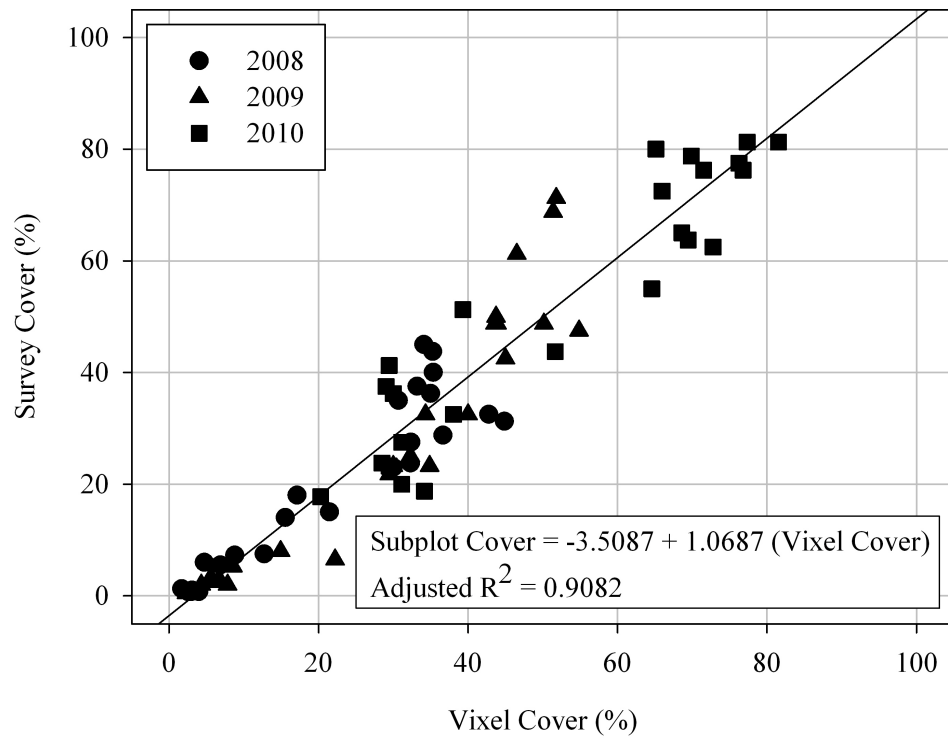


Figure 3.5: Regression analysis of the relationship between total cover measured using the vixel assessment (x-axis) and total cover assessed using four random permanent subplots (y-axis). Note that three years worth of data were included in order to provide a more complete range of data for the regression analysis.

Table 3.2: ANOVA tables of total cover response to treatment regimes by year as well as the analysis of herbaceous and woody/semi-woody vegetation community components in July 2010. The vixel survey is in the upper panel while the subplot is in the lower. Asterisk indicates significant treatment effects at  $\alpha=0.05$ .

Vixel	Parameter	Source	DF	Type III SS	Mean Square	F value	Pr>F	
	2008 Total Cover	Block	3	70.9355	23.6452	3.32	0.0485	
		Treatment	5	4788.8736	957.7747	134.67	<0.0001	*
	2009 Total Cover	Block	3	87.1840	29.0613	1.13	0.3694	
		Treatment	5	7078.4110	1415.6822	54.93	<0.0001	*
	2010 Total Cover	Block	3	69.8265	23.2755	0.49	0.6913	
		Treatment	5	9374.9841	1874.9968	39.86	<0.0001	*
	Parameter	Source	DF	Type III SS	Mean Square	F value	Pr>F	
	2010 Herbaceous	Block	3	540515.3610	180171.7870	1.27	0.3209	
		Treatment	5	3663684.5160	732736.9030	5.16	0.0060	*
	2010 Woody/ Semi-woody	Block	3	646999.4750	215666.4920	1.80	0.1899	
		Treatment	5	9648213.3600	1929642.6720	16.13	<0.0001	*

Subplot	Parameter	Source	DF	Type III SS	Mean Square	F value	Pr>F	
	2008 Total Cover	Block	3	165.3620	55.1207	2.50	0.0994	
		Treatment	5	4579.1068	915.8214	41.48	<0.0001	*
	2009 Total Cover	Block	3	462.3412	154.1137	1.72	0.2058	
		Treatment	5	10703.6172	2140.7234	23.88	<0.0001	*
	2010 Total Cover	Block	3	237.1979	79.0660	1.71	0.2081	
		Treatment	5	11501.4063	2300.2813	49.69	<0.0001	*
	Parameter	Source	DF	Type III SS	Mean Square	F value	Pr>F	
	2010 Herbaceous	Block	3	736.2188	245.4063	1.98	0.1610	
		Treatment	5	6778.7708	1355.7542	10.91	0.0001	*
	2010 Woody/ Semi-woody	Block	3	468.7813	156.2604	1.46	0.2662	
		Treatment	5	7310.9271	1462.1854	13.63	<0.0001	*

Table 3.3: Treatment means of total plant cover (%) presented by year and method. Values within a column sharing the same letter are not statistically different at  $\alpha=0.05$ .

	2008		2009		2010	
Treatment	Subplot	Vixel	Subplot	Vixel	Subplot	Vixel
1. OO/OO	36 a	39 a	61 a	49 a	75 a	73 a
2. OS/OO	14 b	17 c	44 b	47 a	75 a	73 a
3. FS/OO	4 c	6 d	36 bc	38 b	68 a	69 a
4. F <sub>o</sub> S/OO	2 c	3 d	22 c	30 c	43 b	38 b
5. OO/FS	33 a	34 b	5 d	9 d	30 c	32 b
6. OO/F <sub>o</sub> S	30 a	32 b	2 d	5 d	20 d	29 b

which spanned 23 percentage points. These differences altered the results of the multiple comparisons causing some treatments to be statistically different in the subplot technique but not in the vixel survey. The single largest of these differences occurred during 2009 in the no-action control where a 12 percentage point difference was found. Other differences between the techniques tended to be more subtle and were less than 9 percentage points.

Treatment regimes influenced herbaceous and woody/semi-woody components of the vegetation community (Table 3.2). Abundance of herbaceous vegetation assessed through the subplot method (upper panel) and vixel assessment (lower panel) are presented in Figure 3.6. Treatment regimes that used herbicides in 2008 or 2009 reduced the abundance of herbaceous vegetation below 12% (subplot) or less than 330 ft<sup>2</sup> (vixel). Once treatment regimes ceased in 2009, herbaceous plants in all plots increased in abundance. Treatment 3 received a fall site preparation (no sulfometuron methyl) and a spring release and had the greatest amount of herbaceous vegetation with 74% or 2050 ft<sup>2</sup> coverage by the subplot and vixel techniques, respectively. The subplot method detected differences among treatments 2, 4, 5, and 6 that spanned a range of 21 to 56% herbaceous cover in July 2010. Spanning a range of 922 to 1380 ft<sup>2</sup> of coverage, these same treatments were not statistically different from one another in August 2010 as measured by the vixel method.

Despite the difference in techniques, assessments of the woody/semi-woody cover resulted in identical treatment responses (Figure 3.6). The no-action control and spring release only treatments were not different from one another in 2010. Surveying the subplots revealed both treatments had nearly 40% cover. Using the vixel method, these same treatments had 1661 ft<sup>2</sup> and 1384 ft<sup>2</sup> in the no-action control and treatment 2, respectively. The remaining treatment regimes were grouped between 1 and 10% using subplots or 95 and 587 ft<sup>2</sup> with the vixel survey.

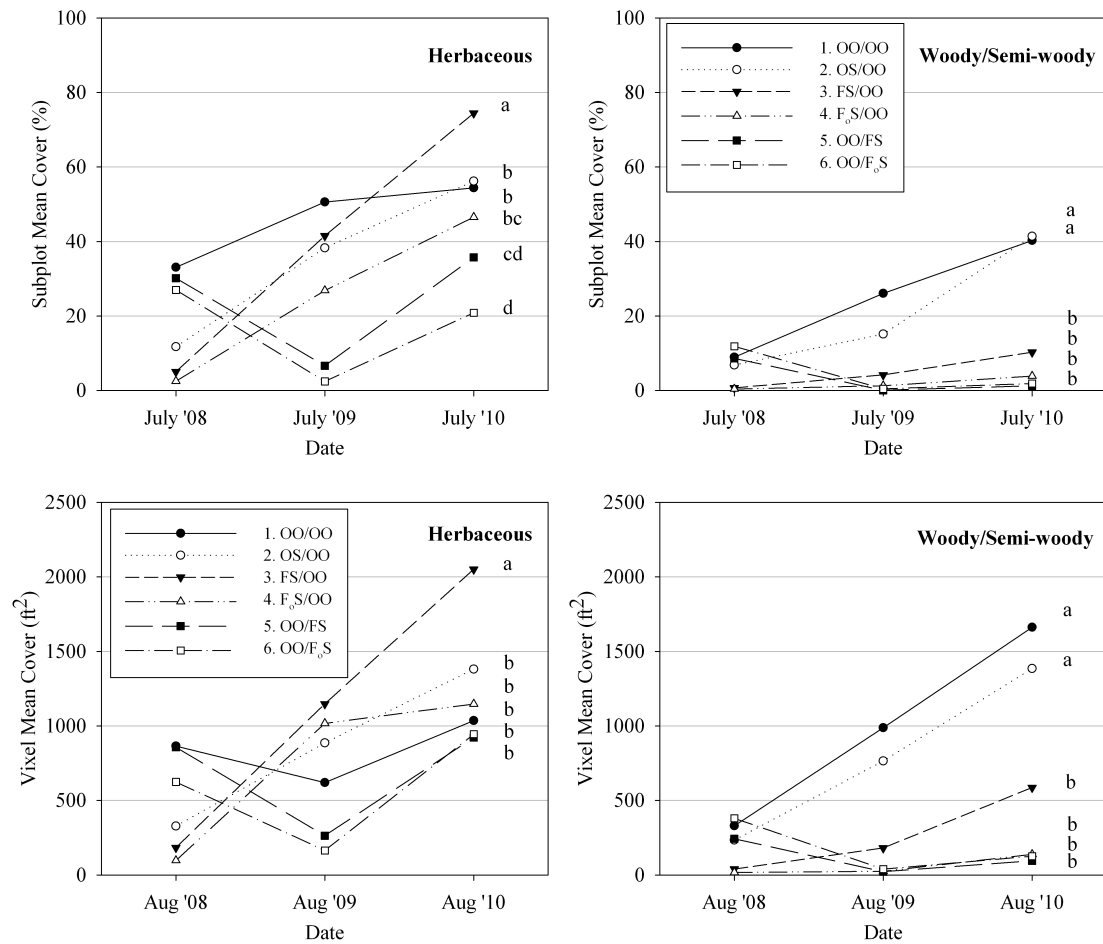


Figure 3.6: Herbaceous and woody/semi-woody cover assessed by the subplots (upper panel) and vixels (lower panel). Herbaceous plants are those classified as forbs, ferns, and graminoids while woody/semi-woody plants are trees, shrubs, and vine/shrubs. Please note the different scales and units of measure on the y-axis as well as the different dates of survey on the x-axis. Treatments within a figure that have the same letter are not different from one another in 2010 at  $\alpha=0.05$ .

### 3.4.3 Species Specific Responses to Treatment Regimes

The plot presented in Figure 3.7 received a fall site preparation and spring release in 2007/2008. It was physically adjacent to the no-action control presented in Figure 3.4 and had less than 6% competitive cover in the first growing season (2008). This low amount of competition did not persist into future years. Recall that this treatment had had a dramatic increase in the abundance of herbaceous plants found through both the vixel and subplot assessments of total cover (previously shown in Figure 3.6). The red and orange areas within the central portion of the plot were dominated by a single herbaceous species, *Pteridium aquilinum* (western brackenfern) that quickly colonized all replicates of treatment 3. A treatment mean of 1282 ft<sup>2</sup> cover by this species was found in 2010 (Table 3.4). This amount was nearly three times the 436 ft<sup>2</sup> observed in the no-action control.

The purpose of a fall site preparation was to reduce the abundance of perennial species capable of competing long-term with desired trees. Despite the intensity of the regimes tested, *Rubus ursinus* (trailing blackberry) was not eliminated from being among the top three most abundant plant species in any treatment (Table 3.4). Treatment 2 (OS/OO) did little to control *Rubus ursinus* which increased from 122 ft<sup>2</sup> in 2008 to 830 ft<sup>2</sup> in 2010. Treatments 3-6, which all had two herbicide applications, adequately controlled this species limiting coverage to less than 20 ft<sup>2</sup> in the year following application. Whether it was through seed dispersal or partial control of established plants, the species progressively increased in cover post-herbicidal control.

*Senecio sylvaticus* increased dramatically in certain treatment plots then nearly disappeared from these areas (Table 3.4). The species was present in all treatment plots on the site at some point during the development of the vegetation community from 2008 to 2010. However, the greatest abundances of *Senecio sylvaticus* were found in treatments 4 and 6 in the year following the application of these treatment regimes which included sulfometuron methyl in the fall site preparation. In 2009, treatment 4 had over twice the amount of *Senecio sylvaticus* at 491 ft<sup>2</sup> when compared to treatment 3 at 181 ft<sup>2</sup>. A similar response was observed in treatments 5 and 6 in

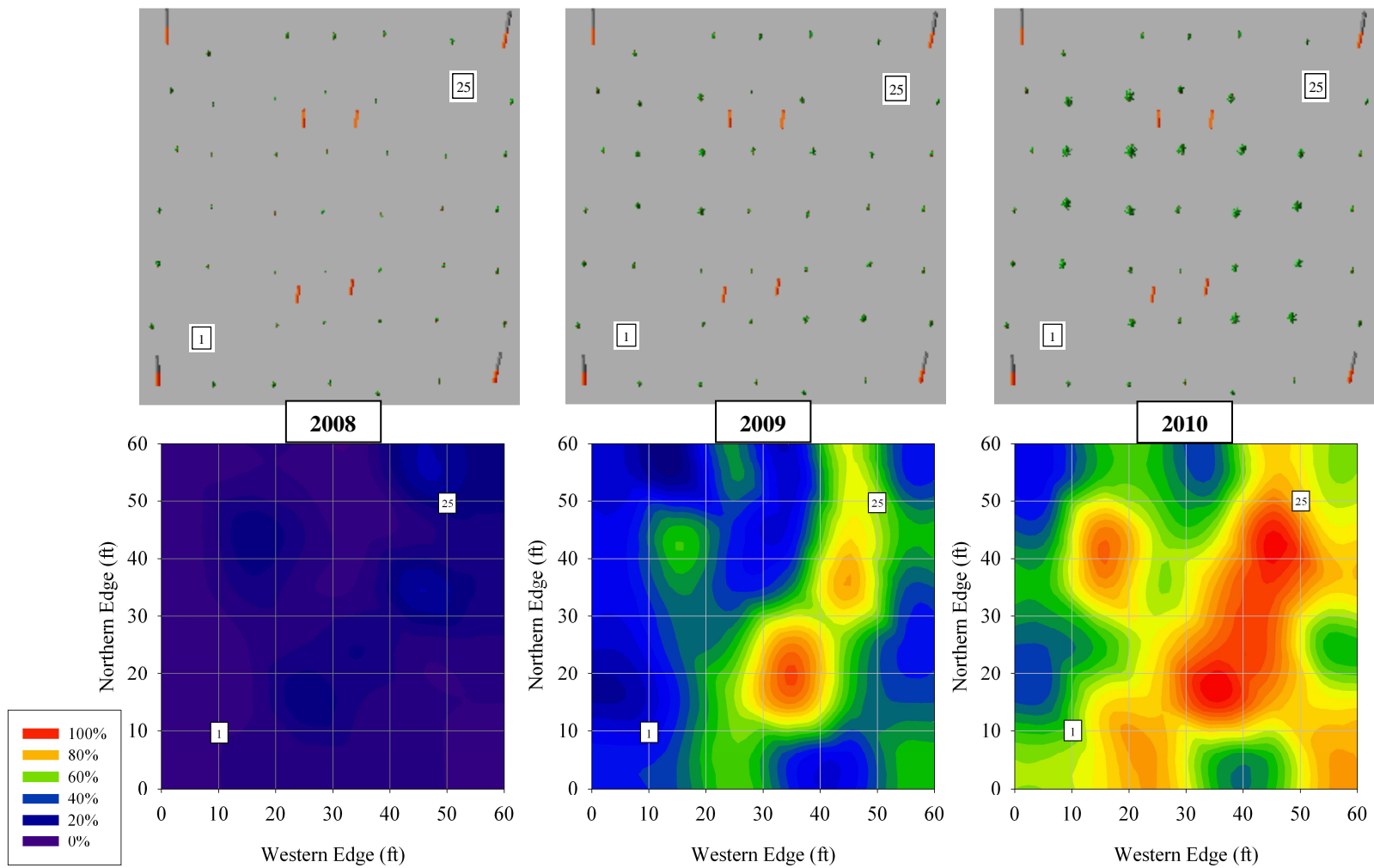


Figure 3.7: Images of the seedling locations within a treatment plot (upper panel; FS/OO, block 2) and total plant cover (lower panel) from 2008 to 2010. For reference, 2008 was a treatment year. Tree 1 died in 2008 and tree 25 died in 2009.

Table 3.4: Summed cover development for three abundant species; *Pteridium aquilinum*, *Rubus ursinus*, and *Senecio sylvaticus* presented by treatment regime. Means and standard errors were calculated by species over replications. Grey boxes indicate years when herbicidal control was applied according to the regimes tested.

*Pteridium aquilinum* Cover (ft<sup>2</sup>)

Treatment	2008	SE	2009	SE	2010	SE
1. OO/OO	73	37	228	110	436	160
2. OS/OO	80	43	455	258	822	445
3. FS/OO	95	37	609	222	1282	474
4. F <sub>o</sub> S/OO	17	9	152	71	484	183
5. OO/FS	40	25	30	16	143	59
6. OO/F <sub>o</sub> S	70	40	12	8	102	54

*Rubus ursinus* Cover (ft<sup>2</sup>)

Treatment	2008	SE	2009	SE	2010	SE
1. OO/OO	107	30	573	67	988	128
2. OS/OO	122	36	487	85	830	119
3. FS/OO	3	1	72	31	270	86
4. F <sub>o</sub> S/OO	3	2	3	2	45	11
5. OO/FS	91	32	9	6	66	34
6. OO/F <sub>o</sub> S	127	36	17	6	65	22

*Senecio sylvaticus* Cover (ft<sup>2</sup>)

Treatment	2008	SE	2009	SE	2010	SE
1. OO/OO	65	21	35	22	5	5
2. OS/OO	13	11	151	31.7	6	4
3. FS/OO	12	5	181	62.7	3	3
4. F <sub>o</sub> S/OO	15	8	491	93.4	62	24
5. OO/FS	60	21	80	33	72	30
6. OO/F <sub>o</sub> S	54	45	79	19	372	43

2010 which had the same chemical regimes after a one year delay. Treatment 6 (OO/F<sub>0</sub>S) had 372 ft<sup>2</sup> of *Senecio sylvaticus* while treatment 5 (OO/FS) had only 72 ft<sup>2</sup>. Figure 3.8 illustrates these colonization patterns in response to similar treatment regimes within a single block (replicate). The upper panel is treatment 4 and lower is treatment 6. *Senecio sylvaticus* was quick to colonize open conditions in 2009 after the application of treatment 4. In the delayed treatment, the species was present during 2008, absent in 2009 due to the chemical control measures, then came back to this plot in 2010.

### 3.5 Discussion

#### 3.5.1 Collecting Spatially Explicit Data During Early Forest Establishment

The mapping techniques presented in this paper show how, from a single position, data may be collected on locations of interest within experimental units. Peet et al. (1997) compared a similar laser-based survey instrument made by Criterion<sup>®</sup> with a standard surveyor's total station proving that these laser-based instruments were accurate within certain technical limitations. Results indicated that given the magnetic compass' possible error rate of +/- 0.3 degrees, the theoretical working distance that would allow stem mapping to achieve +/- 10 inch (~25 cm) accuracy is 156.5 ft (47.7 meters) (Peet et al. 1997). If the laser position forms the origin (0, 0) and a distance of 156.5 ft (47.7 meters) on a 45 degree angle is used, this system could map experimental units 0.25 acres (0.10 ha) from a single location with an accuracy of +/- 10 inches (~25 cm). Larger experimental plots (or other sites of interest) could be mapped from multiple survey positions that are located up to 47.7 m (156.6 ft) away.

Accuracy of stem mapping could be improved in several ways minimizing the need to estimate tree location corrections. First, a tripod could be used to hold the laser minimizing any sway that can occur with the use of a monopod. Second, a stadia rod with two levels 90° apart could be used by the person walking between the points. If a target such as a piece of plywood is fixed at particular height (e.g. 4.5 feet from the ground), this would ensure a more consistent backdrop for the laser and could

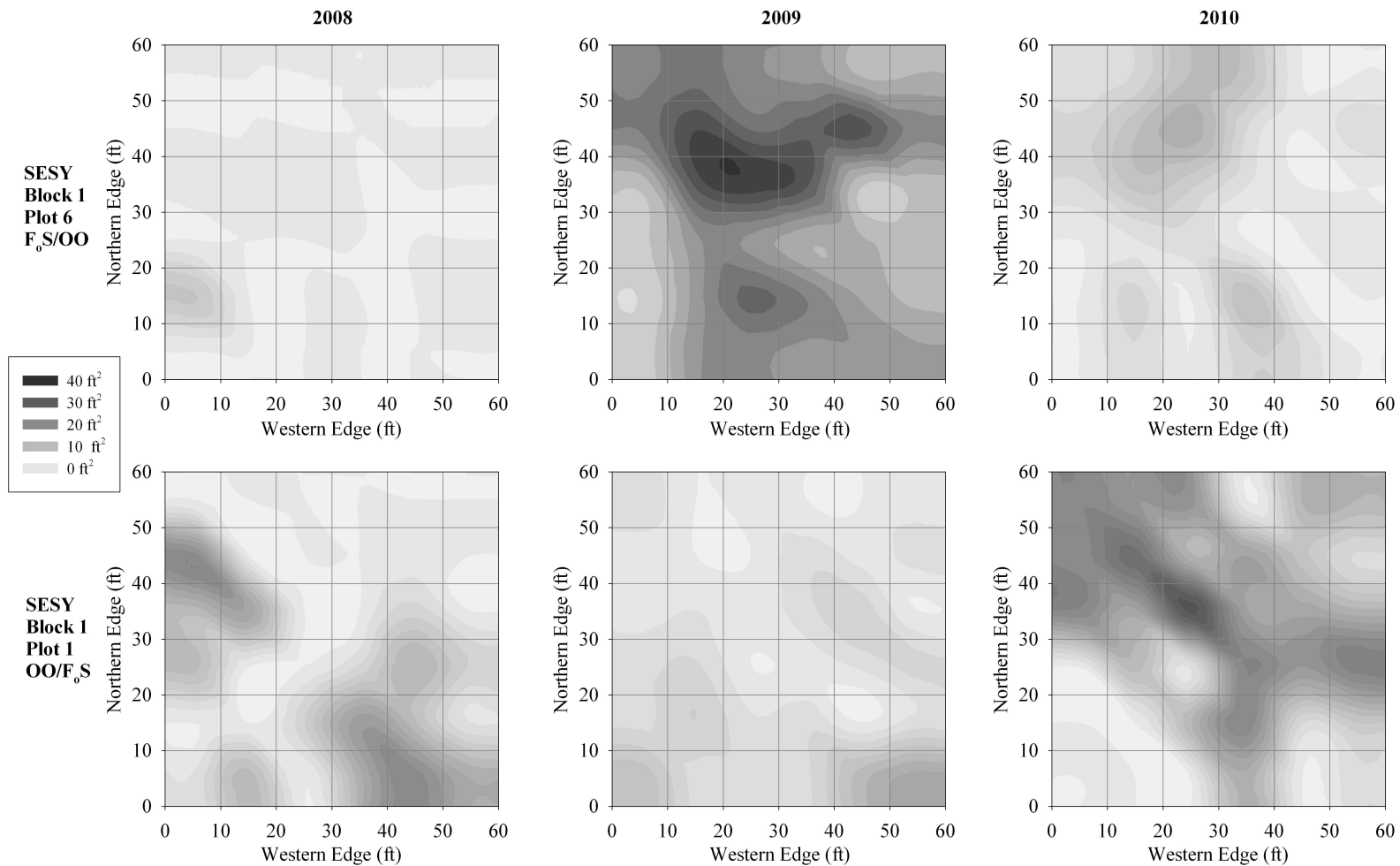


Figure 3.8: Yearly distribution (ft<sup>2</sup>) of the annual *Senecio sylvaticus* across three years in two plots that exist in one block (replicate) on the Boot study site. Upper panel is a plot that received treatment 4 (F<sub>0</sub>S/OO) making 2008 a treatment year. The plot in the lower panel received the same chemical treatment but delayed a single year so 2009 was a treatment year (OO/F<sub>0</sub>S).

simplify calculations in the data management process. Third, if extreme accuracy was vital to project success, using a surveyor's total station would be more appropriate.

Various mapping techniques utilize distance and azimuth readings to report forest stand characteristics (Parish et al 1999; Toney et al. 2009; Moeur 1993; Wells and Getis 1999), but this study was the first known attempt to collect data during establishment when line-of-sight was not a hindrance. In this initial phase of forest growth, data collection was rapid and took three people one eight hour day to map the entire study site (a total of 24 man-hours). Collecting this spatial information was an investment, but it is good for the life of the stand and tree growth data only need be updated when measurements are taken.

Utilizing a physical feature of an experimental unit to define polygons whose area can be calculated provides a new method for collecting spatially explicit data on plant community abundance and distribution. Collecting vixel data required two or three days for a single person to survey the entire plant community on this site (16 to 24 man-hours). Depending on the needs of the project and botanical experience of those assessing the vegetation community, the process could be increased or decreased in complexity and spatial scale. Vegetation surveys could include all species found in each vixel allowing more rigorous statistical testing of treatment effects in designed experiments. Vixel resolution could be changed to enable the foundations for spatial analysis at different scales including measures of interspecific competition proximity (e.g. if the area within a vixel, as defined in this study, is subdivided) or larger landscape level changes to plant communities (e.g. if vixels are coalesced into larger units). Vixel surveys could be made simpler and include only total cover measures or cover by growth habit (grasses, forbs, ferns, shrubs, trees, etc).

If the capability to integrate SVS and vixel maps into a single image were developed, a stand as well as the corresponding plant community could be quantified and visually presented from the initial years of establishment through all phases of forest development. Forest visualization programs were developed to serve as communication tools (McGaughey 1998; Stoltman et al. 2004) and have been shown

to provide an effective educational foundation for audiences unfamiliar with silvicultural practices (Roth and Finley 2007). The techniques outlined in this paper add a means of illustrating the effects of vegetation management, an aspect of forestry that has been difficult to present. Vegetation management carries with it the potential to ensure vigorously growing trees, but has associated social stigmas (Wagner 1993). From a manager's perspective, having the ability to map and present the response and development of forest vegetation under various management regimes could greatly improve the communication of these silvicultural practices to the public, adjacent land owners, as well as other interested groups.

### 3.5.2 Comparison of Survey Techniques to Present Vegetation Communities

The combination of mapping and analysis of the vixel vegetation survey has provided an alternate validity check that challenges results provided through commonly used vegetation surveys of randomly distributed subplots. Regardless of the vegetation survey method, treatment effects were apparent and began to disappear as herbicidal influences diminished allowing plant species to colonize these areas. Results of the linear regression analysis illustrated the strong relationship between the two measures of total plant cover. The nearly 1:1 slope of the presented regression line indicated that indeed randomly selected subplots were a valid method for assessing larger vegetation community response to treatment regimes.

Subtle differences that exist between the two techniques may be due to the increased precision that could result from a larger sample size as produced through the vixel survey. A second valid explanation for these differences revolves around the timing of the surveys. While the same vegetation community was measured, the four random subplots were assessed in early to mid-July which is generally the height of the growing season in these non-irrigated situations (Rose and Ketchum 2002). The vixels were assessed in late-July 2008 and mid-August in 2009 and 2010 creating a difference of three to five weeks between the two surveys during the hottest and driest part of the PNW Mediterranean summer (UW 2007). The ability to visually detect the

amount of leaf area and subsequently the amount of cover for a particular vegetation community was influenced by the life stage, development, and senescence patterns unique to a species and year. The comparison of woody/semi-woody analysis supported this explanation of timing influencing survey results. Woody/semi-woody plants have leaf area that does not necessarily senesce in the presence of typical PNW summer drought. By either the vixel or subplot method, results showed similar treatment responses as well as significant differences through the multiple comparison procedures cross-validating these measures of the plant community.

Forest mapping literature has largely ignored this aspect of early-seral competition that can affect the distribution of forest trees observed at some future time. A review of the relevant literature was conducted and the studies found focused on the distributions of older stands using observational study designs. The majority of this research was done to ascertain the degree of clustering in mature forest stands (Cromack 1979; Chen and Bradshaw 1999; Getis and Franklin 1987; Moeur 1993), define the amount of intraspecific tree competition (Shi and Zhang 2003), or utilize dendrochronology studies to understand previous disturbance history (Donnegan and Rebertus 1999; Parish et al. 1999; Wells and Getis 1999). Among these studies, competition acting over a period of time was often cited as the single most important factor determining the spatial distribution and composition of the canopy layer for a particular forest.

The current study adds to this body of literature by providing the ability to collect and present spatially explicit data on the response of these early-seral communities to vegetation management regimes that set the course for future stand characteristics. The level and duration of interspecific competition during the initial years of establishment can lead to a delay, reduction, or even failure of a stand to develop into the dominant vegetation type on a site (Tappeiner and Wagner 1987). The ability for a perennial shade-intolerant woody species, such as Douglas-fir, to develop into this dominant lifeform is linked to the ability to capture site resources necessary for survival and growth (Larcher 2003; Maguire and Forman 1983; Smith et

al. 1997; Petersen et al. 1988). This process begins at establishment. Seedlings incapable of acquiring these resources in adequate amounts have reduced physiological potential (Brix 1979; Larcher 2003; Sands and Nambiar 1984; van den Driessche 1987) that negatively impacts growth response (Cole and Newton 1987; Harrington and Tappeiner 1991; Hughes et al. 1990; Stransky 1961). Depending on the amount, composition, and duration of competition, seedlings experiencing low amounts of necessary resource(s) during these initial years have the potential to become trees of lower crown position or succumb and die as these stands mature (Cole and Newton 1987; Smith et al. 1997; Zedaker et al 1987).

### 3.5.3 Future Avenues of Research and Development

With the right development, forest growth models could use these kinds of spatial data to project stand growth beginning in the early stages of establishment. This effort would require the layout and mapping of strategic plots in a replicated manner across management units. These mapped plots would create an information network established to provide long-term information on vegetation communities, forest growth and yield, as well as site productivity. The network could also be used to aid prediction accuracy of forest models through the ability to ground-truth outputs on known research plots. If the survey positions (the point where the laser was placed for the mapping) were accurately located using Global Positioning Systems, it may be possible to incorporate all of these data into a Geographic Information Systems layer, opening a new avenue of forest planning and productivity research.

A secondary level of modeling at a regional level could also benefit from this proposed network of vixel data. Carbon sequestration research could utilize the cover estimates of various shrubs or other non-commercial tree species. These additional measures of terrestrial carbon stores could continue to improve the accuracy of these models by characterizing this component of forested plant communities and the effect management activities have on these lands. This could increase the understanding of

how plant communities respond to disturbance and, if collected over a period of time, the response to proposed climate shifts.

In support of these modelling efforts, additional work will be needed to link abundance data such as these with the ecophysiology of site resource use, specifically the use of soil water. While above-ground levels of vegetative cover have been correlated with the degree of competition (Rose et al. 1999; Cole and Newton 1986), few studies have been published that define the photosynthetic and transpiration rates of plant species common to regenerating sites in the PNW. If it is possible to quantify how individual plant species work on a physiologic level, it may be possible to define clear above-ground competitive thresholds (Wagner et al. 1989; Cousens 1988) when control measures are needed. This may become a reality if spatial analytic procedures (assuming the appropriate scale) are capable of aiding in the analysis of these proximity relationships. Ultimately, the goal would be to clearly define practical thresholds allowing the targeted control of problematic species that remain as barriers to productive forest growth.

Ecologic research could utilize these techniques to better understand the dynamic principles involved in plant community development. Measures of species abundance, diversity, as well as distribution across a landscape could be analyzed at a variety of spatial and temporal scales providing a basis for a number of plant related fields. These mapping techniques could aid in understanding the invasion patterns of alien plant species. Forest health scientists could map the spread of insects and pathogens. Holland et al. (2007) used a larger grid pattern concept to map and explore the distribution patterns of beneficial beetles that were known to occur in barley and wheat fields in the United Kingdom. Their results showed that current year's emergence patterns of the beetle species were related to previous populations in specific soil moisture patterns. Wildlife biologists could quantify the abundance and distribution of plant communities (Harrington and Nicholas 2007) for the analysis of forage and habitat suitability as well as the impact of spatial distribution on faunal species of interest. A plant ecologist could study seed dispersal, colonization, and

response to disturbances similar to results presented by Kooijman (1977) who studied barnacle colonization patterns using a spatial grid on the side of a ship hull in the Netherlands.

One example of how these techniques could be useful to the plant ecologist is presented in Figure 3.9. Two species of thistle *Cirsium arvense* and *Cirsium vulgare* are common to regenerating sites in the PNW and the image presents two research plots that had some of the highest concentrations of these plants. *Cirsium arvense* (upper panel) is an introduced perennial species that can colonize through wind disseminated seed as well as vegetatively through spreading root systems (Donald 1990; Tiley 2010). *Cirsium vulgare* (lower panel) is an introduced biennial species and is only able to colonize through wind disseminated seed (Michaux 1989; Mitich 1998). The first season (2008) both plots were treated with a spring release application which maintained low abundances of all plants. Neither species was found to be among the top three most common of the few that remained. In 2009, *C. arvense* colonized one location in the north central portion of this plot and continued to expand its influence into the 2010 growing season. *C. vulgare* was not among the top three most common while in the rosette stage during 2008, but owing to its biennial habit, bolted and flowered in the 2009 growing season. The plants then died and the species did not appear among the top three species during 2010.

Figure 3.9 visually illustrates why knowing the autecologic strategies of plants is important to decisions regarding effective management. *C. arvense* has been a researched problematic plant for over 100 years (Prentiss 1889). Prentiss (1889) found that a root fragment larger than 1/8 of an inch in diameter and 1/2 inch long possessed enough vegetative material to enable the plant to regenerate. This root sprouting behavior makes manual control difficult and may result in simply propagating the species where as chemical control is much more effective (Donald 1990). While *C. vulgare* is also controlled with herbicides, mechanical measures can be effective on this species due to the lack of vegetative reproduction.

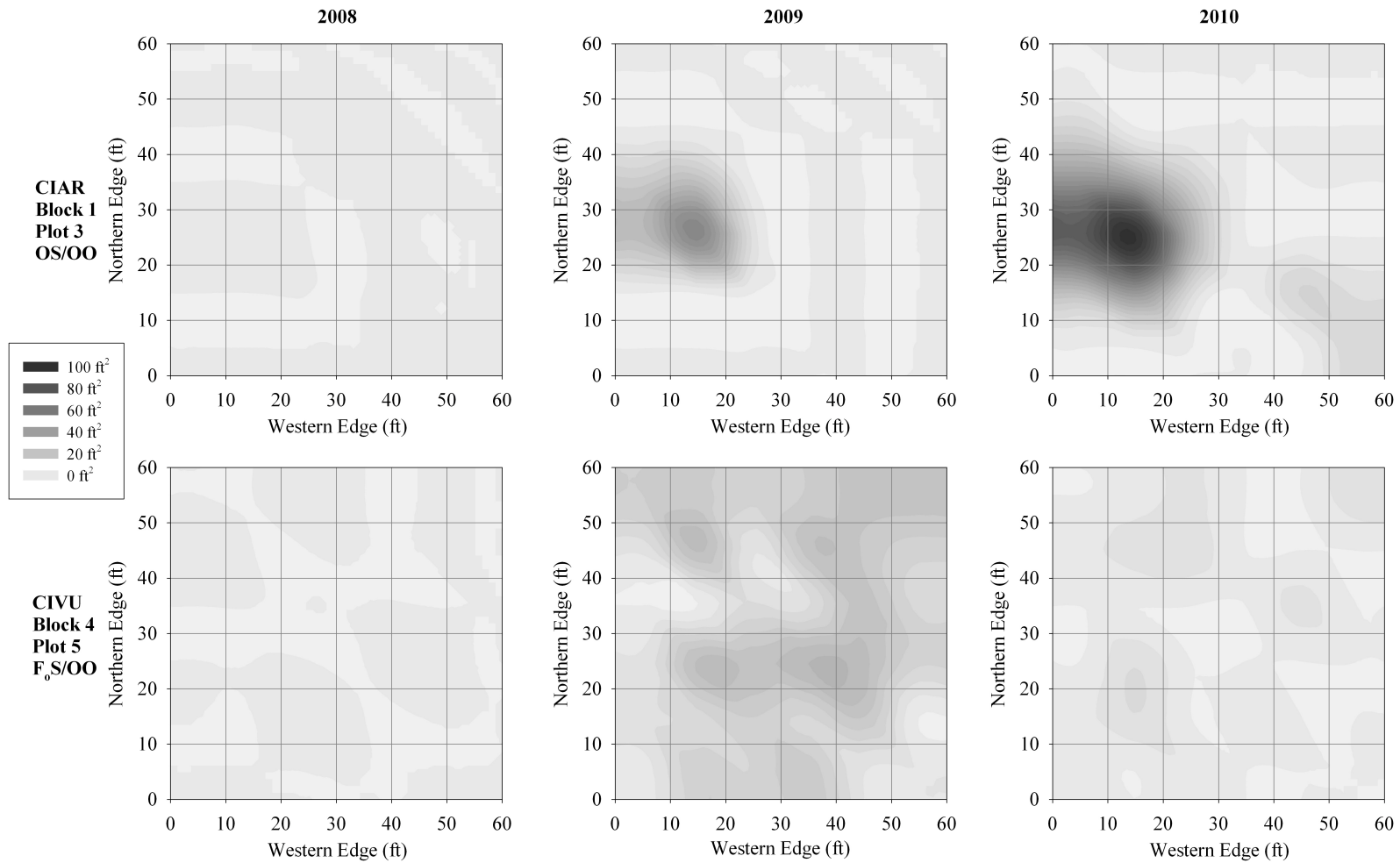


Figure 3.9: Perennial nature of *Cirsium arvense* (CIAR, upper) and ephemeral nature of *Cirsium vulgare* (CIVU, lower) from 2008 to 2010. Vegetation control was applied in 2008 as a single spring release (upper, OS/OO) or a more intense treatment (lower, F<sub>0</sub>S/OO). These two plots are physically separated by three plots, a distance of 180 feet (see site map, Appendix 1).

### 3.6 Conclusions

Stem and vegetation mapping methods outlined in this paper enable the presentation of forest conditions unique to the establishment period. Vixel surveys encompassing 100% of the area within experimental units have challenged standard survey methods and results indicated that both techniques depicted similar changes during this phase of forest succession. The vixel data provided an additional aspect to plant community data, the ability to present a spatially explicit image. Land managers could use these methods to educate audiences unfamiliar with forest practices as well as meet internal and external goals to steward forests in the most appropriate manner. These data could help direct the application of silvicultural investments (Dubois et al. 2001) in such a way that would maximize economic efficiencies and minimize environmental costs. The techniques also open a new research direction within the field of forest mapping that may assist with modelling forest competition, plant species abundance and diversity, plant ecophysiology, autecology, and potentially wildlife habitat utilization. Decision support tools such as these may allow broader management objectives to be tested, assist with compliance to sustainable forestry practice policies, and provide what McGaughey (1998) deemed to be “data-driven solutions.”

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## **CHAPTER 4.0**

### **SEASON-LONG GAS EXCHANGE AND BIOMASS PARTITIONING OF SELECTED WEEDY PLANT SPECIES ASSOCIATED WITH A REGENERATING PACIFIC NORTHWEST FOREST**

#### **4.1 Introduction**

Plant communities in regenerating forests are complex assemblages of species that can number well over 100 on sites in the Oregon Coast Range (Chen 2004). Vegetation management prescriptions are often used to limit the growth of this community and improve site resource availability for planted tree seedlings (Dinger and Rose 2009; Newton and Preest 1988; Sands and Nambiar 1984; Zutter et al. 1986). The goal of these activities is to interrupt successional trends (Connell and Slayter 1977) in an effort to favor the establishment of crop trees. These prescriptions are designed to temporarily minimize (Boyd et al. 1995; Comeau and Harper 2009; Lindgren and Sullivan 2001; Miller et al. 1995) the abundance of a significant portion of the plants on a site without damaging tree seedlings (Newton 2008; Walstad and Kuch 1987). Large seedling growth gains have been reported (Creighton et al. 1987; Harrington and Tappeiner 1991; Maguire et al. 2009; Yildiz et al. 2011). However, minimizing the abundance of an entire plant community would suggest the competitive importance of these species is equally weighted. With the appropriate information, it may be possible to selectively target certain species that are more competitive leaving other portions of the vegetation community unhindered. Information on a physiological level could supplement the commonly used abundance data providing a more detailed understanding of the competitive mechanisms at work during forest regeneration.

Forestry studies have compared specific types of vegetation common to regenerating units in an attempt to understand which species (or group of species) may be more competitive and thus more important to control. Studies have measured the biomass response of crop trees to either a standardized density or additive mixtures

showing that indeed, not all species were equally competitive for site resources (Willoughby et al. 2006; Perry et al. 1993; Davis et al. 1998; Britt et al. 1990; Bell et al. 2000; Morris et al. 1993). Other studies have demonstrated the negative physiologic responses of seedlings to specific vegetation communities, again illustrating that certain species can have a more pronounced impact on crop trees (Randal and Rejmànek 1993; Gordon et al. 1989; Parker et al. 2010; Morris et al. 1993).

A smaller cohort of forestry studies has assessed the weedy plant species themselves in an attempt to understand how they compete on a physiological level (sometimes in conjunction with the tree species). Conard et al. (1997) compared the leaf area, xylem water potential, and stomatal conductance of three shrub species in Southwest Oregon and reported that xylem water potential and stomatal conductance were different among the species and across the season. Differences among the species were attributed to rooting depth as well as species specific control of stomata. Hangs et al. (2003) explored the nitrogen uptake capabilities of Jack pine and three competitor species that spanned a range of life forms. Statistical differences were found among the species with the grass, *Calamagrostis canadensis*, being highly competitive for nitrogen. Lastly, Bell et al. (2000) reported that as competition density increased, Jack pine as well as four common weedy species, decreased photosynthetic and transpiration rates as well as nitrogen use efficiency. Results indicated that herbaceous species had the greatest negative impact on Jack pine early in the experiment, but diminished in time as woody vegetation steadily became more competitive (Bell et al. 2000).

A significant amount of research has been done to define various physiologic aspects of competition for species occurring in settings other than forests. Comparative approaches have been used to evaluate co-occurring plants or congener species to identify traits that confer advantages toward invading new habitats (Daehler 2003; Vilà and Weiner 2004). McAlpine et al. (2008) studied five shrubs to quantify the invasive strategies of *Berberis darwinnii* in conjunction with four native species.

Results showed that *B. darwinnii* had greater rates of seed germination and survival as well as a maximum light-saturated photosynthetic rate over  $15 \mu\text{mol m}^{-2} \text{s}^{-1}$ , a rate that was nearly twice that of the four natives. These traits were concluded to provide a significant physiologic advantage for the species in full-light conditions. Blickler et al. (2003) compared the water use capabilities of *Centaurea maculosa*, an invasive forb, with three native grasses in a semi-arid grassland. Findings indicated that the species did not use more water than native grasses and possessed an intermediate level of water use efficiency (approximately 2 g dry mass/kg H<sub>2</sub>O). *C. maculosa* did, however, use water later into the season. The authors proposed that this luxuriant use of soil water depleted resources below levels required by native species, aiding in its competitive success. Brock and Galen (2005) demonstrated that two congener *Taraxacum* (dandelion) species had no differences between photosynthetic and transpiration rates. The higher water use efficiency as well as low specific leaf area of the native (*T. ceratophorum*) was proposed to make it more tolerant to drought and hence more competitive in high alpine environments. Other studies have evaluated various competitive mechanisms enabling certain species to adapt to changes in resource availability (Houssard et al. 1992; Touchette et al. 2007), increase growth rate and biomass in different environmental conditions (Wang et al. 2006; Feng et al. 2008), phenology and seasonal physiology (Xu et al. 2007), or explore physiologic aspects unique to a species (Bossard and Rejmanek 1992; Nilsen et al. 1993).

A study has not been found which simultaneously compares gas exchange and morphologic attributes of plant species capable of rapidly colonizing disturbed forest landscapes in the Pacific Northwest (PNW). Published research results (Halpern 1989; Dyrness 1973; Schoonmaker and McKee 1988; West and Chilcote 1968; Rose and Ketchum 2002) as well as those presented earlier in this dissertation have illustrated how *Cirsium arvense* (CIAR), *Cirsium vulgare* (CIVU), *Rubus ursinus* (RUUR), and *Senecio sylvaticus* (SESY) are adept at invading disturbed forest environments. From a management standpoint, these species also represent a range of prescriptions that can be used to control their abundance. The two *Cirsium* species are

of known worldwide importance (Skinner et al. 2000) and work has been done to define their autecology (Tilley 2010; Kay 1985; Michaux 1989; Mitich 1998; Downs and Cavers 2002; Klinkhamer and de Jong 1993) as well as physiologic aspects of invasion and competition (Powell 2011; Lalonde and Roitberg 1994; Ziska 2002). The majority of this work has been done in agronomic systems.

McDowell (2002) and McDowell and Turner (2002) presented the photosynthetic and reproductive capabilities of four *Rubus* species, one of which was *R. ursinus*, a PNW native. Gas exchange rates, as defined by CO<sub>2</sub> response curves, were higher and reproductive effort greater for the two introduced *Rubus* species when compared to the two natives. These results provided a basis for comparison, but the observational study design and other methodological differences make it difficult to extend these results to a regenerating forest.

The underlying mechanisms of the ephemeral invasion characteristics of *Senecio sylvaticus* was first studied by West and Chilcote (1968). Other studies have sought to describe this species based on genetic information (Koniuszek and Verkleij 1982; Kumler 1969), soil nutrient usage and allocation (van Andel and Vera 1977; van Andel and Jager 1981), as well as interspecific competition (Halpern et al 1997). While photosynthetic measurements have been presented within the genus *Senecio* (Fioretto and Alfani 1988), gas exchange rates and the seasonal analysis of growth in the PNW for this species have not been found in the scientific literature.

Higher rates of gas exchange, greater use of water, increased water use efficiency, larger amounts of viable seed, fast growth, as well as greater root mass and nutrient acquisition may govern the competitiveness of species, but little of this information is available for these PNW species of interest. Differences in species, study objectives, habitat, and regions limit the ability to extrapolate results from previous studies to CIAR, CIVU, RUUR, and SESY growing in a regenerating forest in the Oregon Coast Range. The PNW has a pronounced summer drought period and it is unknown how the seasonal physiology and developmental patterns of these species are affected by declining soil water availability. Of particular interest to forest

ecologists as well as managers, is the link between the water utilized by these species and the competitive effects on forest growth. Detailed physiology data could lead to a greater understanding of the ecological significance of these species as well as more precise decision-making criteria for control measures. It is for these reasons that this study was designed to 1) compare instantaneous measures of gas exchange (photosynthesis, transpiration, and water use efficiency) across a growing season for CIAR, CIVU, RUUR, SESY, species commonly found in association with regenerating forests in the PNW, 2) measure the affect soil water availability has on gas exchange, 3) quantify biomass development and partitioning, and 4) quantify detectable soil moisture utilization among the four species.

## 4.2 Materials and Methods

### 4.2.1 Brief Description of Selected Species

*Cirsium arvense* (Canada thistle) is an introduced forb species in North America. This dioecious species can behave as an annual, biennial, or perennial and spreads through wind disseminated seed as well as vegetatively through networks of underground root systems (Lalonde and Roitberg 1994). It requires high light and bare mineral soil for successful germination and early growth (Tiley 2010) so from a forestry perspective, it is often confined to disturbed habitats with canopy gaps large enough to provide these conditions (Schoonmaker and McKee 1988).

*Cirsium vulgare* (bull thistle) is an introduced biennial forb. The plant spends the first year developing a basal rosette of leaves and a large taproot (Mitich 1998). In the second year, the species bolts, flowers, and can produce over 18,000 wind dispersed seed from a single plant (Michaux 1989). These seeds have large papas enabling extended flight, but the majority fall within a few meters of the maternal plant (Michaux 1989). In keeping with the biennial growth habit, the individual plant dies upon the completion of flowering and seed dispersal. Within regenerating forests, the species tends to invade rapidly during the early years of establishment (Halpern 1989) then declines in abundance as time progresses (Schoonmaker and McKee 1988).

*Rubus ursinus* (trailing blackberry) is a native vine/shrub species capable of existing in the lower light environment of a mature forest stand. It can, however, take rapid advantage of openings in the overstory through extensive growth (Dyrness 1973; McDowell 2002). The plant reproduces vegetatively through the rooting of canes at nodes along its length, but also produces edible fruit dispersed by various faunal species (McDowell and Turner 2002).

*Senecio sylvaticus* (woodland groundsel) is an introduced annual forb. In the PNW, this species is known for its ability to rapidly colonize reforestation sites (West and Chilcote 1968; Dyrness 1973; Halpern et al. 1997; personal observation). This often occurs in the year following disturbance (e.g. harvesting, burning, or chemical control) and is accomplished by the large amount of wind dispersed seed enabling long-distance travel. *S. sylvaticus* requires high available nutrients (van Andel and Vera 1977; West and Chilcote 1968) and light during the various phases of germination and growth which begin in the late-winter (Halpern et al. 1997). The species is considered a ruderal with little capacity for extended competition beyond its single year of growth.

#### 4.2.2 Study Sites and Plant Selection

Boot, a site managed by Forest Capital Partners LLC, was harvested in the late-spring of 2007. Mechanized equipment was used to load logs onto trucks near the road. Harvest residues in these localized areas were piled and burned in the winter of 2007/08. Vegetation management and seedling planting were delayed in the area immediately surrounding the study site, giving this land a fallow year where no establishment activities occurred until September 2008. At this time, the same site preparation chemicals applied according to treatment 6 of the Delayed Planting study (Chapter 2) were applied by backpack to the surrounding area on 3 September 2008. A tank-mix of glyphosate at 3.77 L a.i./ha, metsulfuron methyl at 42 g a.i./ha, imazapyr at 0.16 L a.i./ha, sulfometuron methyl at 158 g a.i./ha, and the adjuvant Induce<sup>®</sup> at 0.58 L/ha (8 oz/ac) was broadcast applied using a backpack sprayer (47

L/ha or 5 gal/ac spray volume). Douglas-fir bareroot 1+1 seedlings were planted on 28 January 2009. A spring release comprised of hexazinone at 1.68 kg a.i./ha and 2,4-D at 1.52 L a.i./ha was applied as a tank-mix on 15 April 2009. Little vegetation was observed in these areas through the 2009 season until precipitation returned in the fall of 2009, at which time, seeds began germinating (personal observation).

Hexazinone and 2,4 -D have half-lives of 90 and 10 days, respectively (Ahrens et al. 1994). More than eleven months had elapsed between the spring release application and plant selection in late-March 2010. This period of time encompassed nearly four half-lives (based on the longer-lived chemical, hexazinone) that would allow herbicidal influences to diminish (Ahrens et al 1994). In order to represent how these four species grow in a regenerating forest, plants were selected from this area north of the study site and above the road (Appendix 3). For each species of interest, four general areas were found that had many individual plants. The developmental stages and selection criteria for the plant species were as follows: SESY germinated but not bolting, CIVU as a basal rosette and not bolting, CIAR plants that were not bolting, and RUUR with independent canes (i.e. not rooted at both ends) less than 1 meter in length. Burned areas associated with slash piles were avoided.

Twenty plants in each general area were marked with pin flags; eight were randomly chosen and transplanted (n=128, 32 plants of each species x 4 species) while the remaining twelve were left undisturbed in the field. Transplanting procedures involved digging up plants leaving as much intact soil around the root systems as possible. Soil from the upper profile was used as the growing medium in 14 liter (3.7 gallon) plastic pots (Model #5, Nursery Supplies, Inc., McMinnville, OR). All study plants of one species were transplanted in a single day and taken to Oregon State University in a covered truck. SESY, CIAR, RUUR, and CIVU were collected on 2, 3, 5 and 12 April 2010, respectively. The potted plants were placed in a fenced outdoor area to prevent ungulate browse, watered as needed and monitored until the beginning of the greenhouse portion of the study. Transplant shock was minimal and consisted of temporary leaf discoloration and/or loss of turgor. Survival of these

plants was 100% and they were vigorously growing when moved into the greenhouse in late-May. All potted plants were hand-weeded to ensure competition free conditions. Extending canes of RUUR were not permitted to root at either site. Throughout the course of the experiment, notes were taken on the phenologic development of the study plants at both the greenhouse and field sites.

#### 4.2.3 Greenhouse Setup and Conditions

A Cravo<sup>®</sup> greenhouse (Cravo Equipment Ltd., Brantford, Ontario) with retractable walls and roof was used for this experiment. The greenhouse is situated on an East/West line and was programmed to close at temperatures below 12.8°C (55°F) and/or if it began to rain. The walls never closed during the experiment and the roof only closed a few times during brief summer rain showers. Special benches were constructed (see Appendix 4a for measured drawings) to allow four pots of each species in an experimental unit, the application of two irrigation treatment regimes, and four replicates or blocks (4 species x 4 pots x 2 irrigation x 4 replicates = 128). This arrangement constituted a factorial complete randomized block design. Each experimental unit was divided in half allowing two positions (A and B) to be used for plants that would be measured across the season while the other two positions (C and D) were available for plants that would be randomly selected for monthly multi-leaf gas exchange measurements and harvest (Appendix 4a).

Irrigation was supplied through a simple manifold system (Appendix 4a) using 3.8 L (1 GPH) pressure compensating drip emitters set to water for either 5 minutes or 15 minutes for the droughty and well-watered treatment regimes, respectively. A test was run to assess the published flow rates of the drip emitters. Three emitters per block were randomly chosen in both the well-watered and droughty treatment regimes (12 for each irrigation treatment regime). A graduated beaker was placed under each emitter to collect the water and a timer was set for either 5 or 15 minutes (Model 9001D, Dig Corporation, Vista, CA). Three trial runs were conducted for each irrigation regime to measure if the emitters provided 315 ml of water at 5 minutes and

945 ml of water at 15 minutes, the amount that should have been released according to the published flow rate. The mean and standard error of the droughty emitters set to water for 5 minutes was 364 ml (19 ml) and the well-watered emitters set to water for 15 minutes was 1033 ml (52 ml).

Plants were randomly assigned to the treatments and moved into the greenhouse on 31 May 2010. Plants were equally watered until 7 June, at which time irrigation treatment regimes began. Determining when and how much irrigation water to apply was done using a Hydrosense TDR probe with 20 cm prongs (Model # CS-620 Spectrum Technologies, Plainsfield, IL) to measure soil moisture one to three times per week. This sensor was used to assess the volumetric soil water in each pot and watering was applied as needed using this sensor data. The droughty treatment required a period of no additional water to dry down the soil after the first gas exchange survey then occasional additions of water in 5 minute increments. The objective of this treatment was to stress plants without killing them. Well-watered conditions were achieved through the addition of water in 15 minute increments in an attempt to maintain high levels of soil water availability. Irrigation water was not added within the 16 hour period before a gas exchange survey date.

A weather station less than 30 meters from the greenhouse in an open location provided on-site environmental data. A Hobo Microstation (Model# H21-002, Onset Computer Corporation, Bourne, MA) was connected to a relative humidity/air temperature gauge (Model # S-THA-M002), a tipping bucket rain gauge (Model # S-RGA-M002), and a photosynthetically active radiation sensor (Model # S-WSA-M003). The microstation was programmed to take hourly measurements.

#### 4.2.4 Field Conditions

The twelve plants remaining on the Boot site comprised the field portion of the study in a completely randomized design. Fences were constructed around two of the plants that occurred within a 2 m x 2 m square (see Appendix 3). These fences were built to prevent accidental crushing of plants that would be studied for the length of

the growing season. Four 152 cm (5 foot) pieces of PVC (polyvinyl chloride) pipe were used as the corner posts and bamboo was lashed to these pipes to form cross bars 15 cm (6 inches) from the ground and 5 to 10 cm from the top. Orange plastic fencing 1.2 m (4 foot) snow fence (Model #191049, Tenax Corporation, Baltimore, MD) was fixed to the corner posts and horizontal cross bars with zip ties. A crude gate was made on one side of each square fence. Four of the plants that occurred immediately outside these fences were randomly chosen for monthly multi-leaf gas exchange measurements and harvest.

Site environmental characteristics were monitored at Boot study with similar weather station equipment used at the greenhouse. Monthly vegetation surveys using a 1 meter radius sampling frame were conducted inside each fenced plot. Percent cover by species was visually estimated at each survey date. Data management was in keeping with the methods outlined in Appendix 2. Hitchcock and Cronquist (1973) served as the basis of nomenclature and identification with Pojar and McKinnon (2003) serving as a supplementary aid.

#### 4.2.5 Gas Exchange Measurements

Gas exchange measurements were assessed using a portable LI-COR 6400 infra-red gas analyzer (LI-COR Biosciences, Lincoln, NE) with a red/blue LED light source (Model # 6400-02B). Instantaneous carbon assimilation rate (net photosynthesis:  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ), transpiration rate ( $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ), and water use efficiency ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1} \cdot \text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ) were measured. Measurements were taken between the hours of 0800 and 1230 hrs. Flow rate of the system was set at  $400 \mu\text{mol s}^{-1}$ . Light intensity within the leaf cuvette was set at  $1500 \mu\text{mol m}^{-2} \text{ s}^{-1}$ . This saturating intensity was determined from the photosynthetically active radiation (PAR) sensor (Model # S-LIA-M003, Onset Computer Corporation, Bourne, MA) at Boot that had recorded values from June to September (2007 to 2009) during the morning hours in excess of  $1500 \mu\text{mol m}^{-2} \text{ s}^{-1}$ . Incoming air into the LI-COR was scrubbed of ambient  $\text{CO}_2$  and injected at  $400 \mu\text{mol mol}^{-1}$  by the  $\text{CO}_2$  mixer (Model #

6400-01). Relative humidity and temperature were allowed to vary (i.e. desiccant was bypassed and the temperature regulation features of the 6400 were not used). Black foam gaskets were used on the leaf chamber. For more information on these settings and methods, please refer to the LI-COR manual (LI-COR 2004).

Infrared gas analyzer outputs were checked against a known amount of CO<sub>2</sub>, pure nitrogen (i.e. CO<sub>2</sub> free air), and a dew point generator to better ensure accurate outputs (measures of zero and span for CO<sub>2</sub> and water vapor). Minor adjustments of less than 2 ppm CO<sub>2</sub> and 1% H<sub>2</sub>O were needed. System checks (matching) outlined in the manual were followed on a daily and hourly basis throughout each sampling date.

Prior to measuring with the LI-COR 6400, a mature full-sun leaf was selected near the middle of plants at the greenhouse and field sites. A small piece of yarn was tied to the petiole of this leaf for identification purposes. Due to the rapid growth rates, this leaf did not remain in the middle of the plant and in some cases died as the season progressed. When a measurement leaf died, a new leaf was chosen (toward the apical meristem) with the criteria that it was a mature full-sun leaf. This was done on a case by case basis for all study plants.

On each measurement date, a new set of random numbers was used to determine the order in which the blocks/replicates would be sampled. All plants were assessed biweekly from early-June to early-September in an alternating fashion between the two sites. Seven sampling dates were achieved for each site. At each measurement, the leaf cuvette was clamped onto the measurement leaf such that 2 cm of leaf extended beyond the internal edge of the gasket with the central midrib running down the long axis of the rectangular opening. A measurement was not recorded until the coefficient of variation was below 1.5%. This generally meant that the leaf was exposed to the conditions in the cuvette for 90 to 120 seconds. Based on the maximum time needed for a leaf to stabilize to cuvette conditions, 128 single measurements was the highest number that could be made between 0800 to 1230 hrs.

Measured plant leaves may not have always filled the chamber due to small size or deeply dissected lobes. The LI-COR 6400 was programmed to assume each

leaf completely filled the chamber (i.e. 6 cm<sup>2</sup> of leaf area) when a measurement was being made. This difference between reality and the machine's assumption meant that a correction needed to be made in order to compare species on an equal basis.

Without a correction, there was a possibility of introducing gas exchange differences that may be due to sampling methodology rather than actual species differences. A photographic leaf correction procedure was developed to adjust photosynthetic and transpiration rates. A 2 cm x 3 cm window was cut in a rectangular piece of 3.175 mm thick (1/8 inch) hardboard 1.5 cm from the upper right corner (see Appendix 4b). A second board was attached with fiber tape, similar to a book binding. The front surfaces of half the front board and all of the back board were painted with flat white paint to minimize reflected light. Flat black paint was used to make a 2 cm x 3 cm rectangle 1 cm below the opening cut on the front board. Lastly, a mark on the back board 2 cm below the edge of the opening in the front board serving as a line-up aid.

This photoboard was then gently clamped onto a leaf in the same orientation that the photosynthetic measurements were taken with the LI-COR 6400. Only the portion of the leaf which was positioned within the cuvette at the time of measurement was visible in the cutout window of the photoboard. Individual pictures were taken with a Canon PowerShot A610 camera (Canon U.S.A. Inc., Lake Success, NY). The camera's flash was used (set at +1/3) for all pictures to minimize shadows and each plant was identified with a dry erase marker on the opposite half of the front board. Image J (version 1.45), an image analysis program made available by the National Institute of Health (NIH 2004), was used to count the number of pixels associated with the leaf within the window and the black painted rectangle. A correction factor was developed by dividing the number of pixels associated with the measured section of leaf by the pixels associated with the black reference rectangle. All photosynthetic and transpiration rates were divided by the correction factor associated with each leaf. This step increased the gas exchange rates to the level that would have been observed had the cuvette been completely full at the time of measurement. All photosynthetic and transpiration rates presented in this paper have been corrected in this manner.

It is acknowledged that leaf surface area may occur in three dimensions as a result of wavy leaf margins, pubescence, and surface roughness. Techniques to handle this additional level of complexity have not been found. In fact, only one study has been located that utilized a similar technique to capture the two-dimensional leaf area within the LI-COR 6400 leaf cuvette. Hill et al. (2006) used a spare chamber gasket to outline the leaf section of knapweed and other co-occurring grass species held in a LI-COR 6400 cuvette. A photograph and image processing software were used to determine leaf area (Hill et al. 2006). Some alternate methods used by researchers include scanned images of entire leaves (Brock and Galen 2005) or tracing plant parts onto paper that was scanned in a leaf area meter (Galen et al. 1993). More commonly, correction methods are not presented or discussed despite plants that have leaves which may not completely fill a leaf cuvette (Allred et al. 2010; Gulías et al. 2003; Hollinger 1987).

#### 4.2.6 Multi-leaf Gas Exchange and Biomass Partitioning

Once per month in June, July, August, and September, two plants per species and irrigation treatment (greenhouse,  $n=16$ ) or one plant per replicate of each species (field,  $n=16$ ) were randomly selected for multi-leaf gas exchange measurements. Plants specified for this purpose occurred in positions C and D at the greenhouse and outside the fences in the field. In early June, colored pieces of yarn were tied to the petioles of expanded full-sun leaves that occurred relatively equidistant along the stem of all plants (Figure 4.1). Marked leaves began with the lowest and extended to the highest leaf that could be measured with the cuvette on the LI-COR 6400. Gas exchange measurements were made following the procedures outlined above with the exception that they occurred on multiple leaves on each of these randomly selected plants. At minimum, this meant that three measurements were taken per plant. As the plants grew, new mature leaves were marked with yarn generally keeping to the distances set in early June. The same photographic corrections were used to adjust gas exchange rates that were observed when the leaf cuvette was not completely filled.

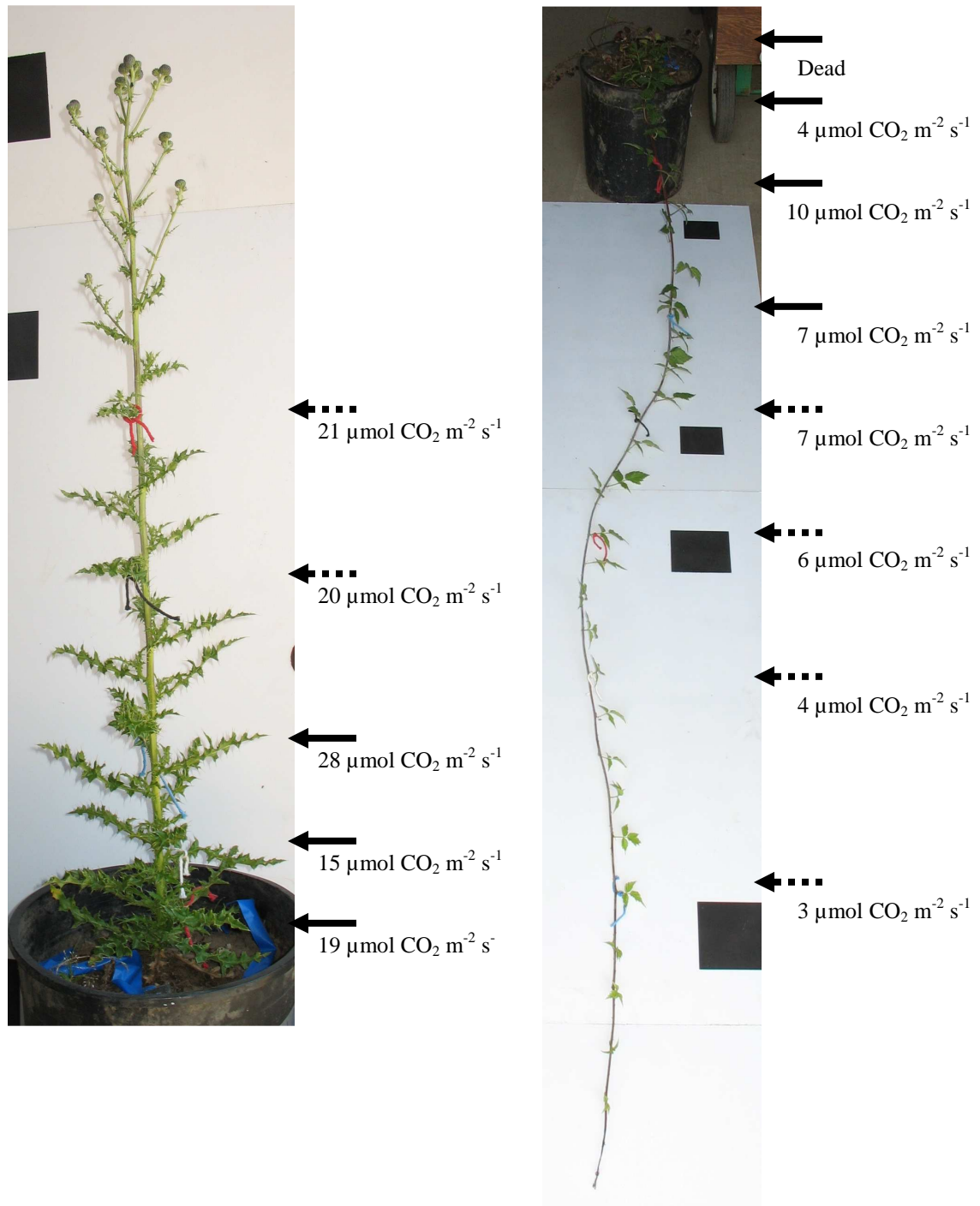


Figure 4.1: Example CIAR (left) and RUUR (right) plants measured for multi-leaf gas exchange on 8 July 2010 at the greenhouse. Solid arrows were the initial leaves identified in June and those with dotted arrows were added prior to this sampling date. Photosynthetic rates (net assimilation) by position have been included.

Following gas exchange measurements, plants were taken to laboratory facilities for detailed biomass measurements (field plants were dug up and brought back in a cooler with ice). Marked leaves measured for gas exchange were cut from the stem and placed adaxial (upper) surface down on a Visioneer® OneTouch scanner (Model 9420 USB, Visioneer Inc., Pleasanton, CA) that had a plastic transparency sheet on the scanner bed. This sheet had two 5 cm x 5 cm flat black reference squares painted in opposite corners. A sheet of 100 lb vellum paper (Strathmore Artist Papers™, Neenah, WI) was placed on top of the leaves and the scanner lid closed with modest pressure while an image was taken (two leaves at a time). One-sided leaf area was then determined with the Image J software using procedures outlined above with one additional step, the ratio of leaf pixels to reference square pixels was multiplied by 25 cm<sup>2</sup> (the area of the painted black squares) to provide the leaf area. These leaves were then placed in labeled envelopes to be dried with the remaining plant parts. The rest of the plants were dissected into the various morphologic components; inflorescence (including the peduncle, fruits, and/or seeds), leaves, stems, and roots. Roots were gently washed free of soil. These morphologic components were placed in separate paper bags, dried at 66°C (151°F) for 48 hours, and weighed. Specific leaf area was calculated as the one-sided leaf area (cm<sup>2</sup>) divided by the dry weight (grams).

#### 4.2.7 Soil Moisture

The same Hydrosense TDR probe used to determine irrigation schedules was used to assess volumetric soil water on the seven dates that photosynthesis was measured at both the greenhouse and field sites. Soil water was assessed vertically 5 to 10 cm from the point of contact between the plant and soil. This sensor requires data to be compared with known soil moisture measurements in order to provide calibrated values specific to soil conditions. Two calibration procedures specific to the greenhouse and field were used.

For the greenhouse, three additional pots were “planted” from excavation locations across the Boot study area on each date that the four plant species were

transplanted (a total of 12 pots). The same transplanting procedures were used to pack soil into the 14 liter (3.7 gallon) pots with one exception, no plants were added. These pots were strictly for the purposes of developing a dataset that could be used for calibrating soil moisture values observed in the greenhouse plants. These calibration pots were labeled and brought back to a greenhouse at Oregon State University. Simple water diffusers were constructed out of 12 clean 1.10 kg (2 lb 7 oz) plastic coffee containers that had four 2 mm holes drilled equidistant around the bottom. One diffuser was placed on top of each pot and 1 L of water was added two or three times daily for a period of two weeks. The infiltration of 1 L of water was slowed to a period of 15 to 20 minutes aiding in the thorough wetting of the soil contained in each pot.

Pot capacity, analogous to field capacity, was a necessary benchmark for accurate calibration equations. An overnight test was conducted on 18-19 May 2010 after the two week wetting period. One clean empty 7.6 L (2 gallon) pot was placed upside down in each of 12 5-gallon plastic buckets. The calibration pots were then placed on top of this clean pot and again, 1 L of water was added to the diffuser at 1700 hrs on 18 May 2010. At 0800 hrs on 19 May, the water that collected inside the 5-gallon paint bucket was poured into a graduated cylinder. Each was found to be near pot capacity as between 950 and 1000 ml of water were measured.

Following this test, the TDR probe was used to take a soil moisture measurement between 5 and 10 cm from the center of the pot (a similar location as to where the measurements were taken in the potted plants). Each pot was then weighed. These TDR and weight measurements were conducted on an approximate weekly basis from 19 May to 7 and 10 September (16 measurement dates) at which time the soil was removed from the pot, placed in labeled aluminum pans, dried at 66°C (150°F) for 48 hours, and reweighed. After subtracting the weight of the pot, the known weight of dry soil allowed the calculation of gravimetric water, bulk density (from earlier measurements of the volume of soil in each pot), and volumetric water content at each measurement date (Brady and Weil 2002).

Field calibration was done using eight randomly selected locations that coincided with half of the plots and one of the plants occurring outside the fences at Boot. A small hole was excavated to expose a vertical face. The Hydrosense TDR probe was used to take a vertical measurement of soil moisture 10 to 15 cm back from the cut-face of the small hole. A soil core was then taken horizontally centered at 10 cm depth using an AMS core sampler with removable sleeves and a slide hammer (AMS Inc., American Falls, ID). Soil cores were labeled, placed in a resealable plastic bag, and brought back to laboratory facilities at Oregon State University. Soil was then removed from the sleeve, weighed, dried for 48 hours at 50°C (122°F), and reweighed. Volumetric soil water was calculated using the data from these cores. This process was repeated at each of the seven sampling dates in 2010. While field capacity could not be directly tested under these conditions, it is assumed to be close to this value due to the 141 mm of precipitation received by the site from 15 May to 15 June 2010 (data provided by the weather station network outlined in Chapter 2).

### **4.3 Statistical Analysis**

#### **4.3.1 Season-long Gas Exchange Measurements**

SAS version 9.1 (SAS Institute, Cary, NC) was used for all analysis. Sites were analyzed separately with the greenhouse having 32 experimental units in a factorial randomized complete block design and the field having 16 experimental units in a complete randomized design. Analyses were conducted on the means calculated by date for each measurement parameter utilizing the two season-long measurement plants occurring in each experimental unit. At the greenhouse, these were the plants in positions A and B while in the field it was the two plants growing inside the constructed fences. The PROC MIXED function in SAS was used for analysis of gas exchange data using procedures outlined in Littell et al. (1996). A first order autoregressive [AR(1)] covariance structure was used to model the increased correlation in error terms between observations closer in time (Littell, et al. 1996). Assumptions of normality, linearity, and constant variance were examined on the

residuals and no transformations of the data were required. Unless otherwise noted, a significance level of  $\alpha=0.05$  was used for all analysis.

Repeated measures ANOVA was used to test for the effects of species (four species), irrigation (two levels), and date (seven dates) on the photosynthetic and transpiration rates as well as water use efficiency at the greenhouse. A split plot in time design was used to partition the sums of squares and incorporate two error terms, one associated with the basic factorial structure of the study and a second associated with the sub-plot factor of time. Species, irrigation, and date were treated as fixed effects in the model while blocks were random. All interactions among fixed effects (three first order and one second order) were included in the models for each measurement parameter. The field site had two treatment levels, species and date, both fixed effects. Replicates in the field were treated as random and again a split plot in time design was used to assess species based on one error term while time and time x species were based on a second. See Appendix 5 for the ANOVA tables, expected mean squares, and the autoregressive structures used. Statistical references included Federer (1955), Hicks (1964), Steele and Torrie (1960), Anderson and McLean (1974) as well as Clewer and Scarisbrick (2001).

#### 4.3.2 Multi-leaf Gas Exchange and Biomass Partitioning

Monthly means of multi-leaf photosynthetic and transpiration rates were calculated by site, irrigation level (greenhouse only), species, and position on the plant. Specific leaf area means by study site and irrigation treatment are presented. Biomass partitioning data was computed by dividing the weight of the plant part (e.g. inflorescence) by the total plant weight. Leaf mass fraction included the weight of leaves used to calculate specific leaf area. Means of the biomass partitioning data were calculated for a site, species, and irrigation level (greenhouse) providing the basis for graphical analysis. The sample size on any survey date was restricted to 16 (four plants per species per month) due to the amount of time required to conduct the

monthly multi-leaf gas exchange as well as the limited number of plants that could be destructively harvested.

Monthly vegetation survey results were used to calculate summed cover values (i.e. summation of cover values for all species found) as well as divide the dataset into six growth habit components following procedures outlined in Appendix 2.

Environmental variables temperature, humidity, and photosynthetically active radiation (PAR) are presented by site and the hour when gas exchange measurements began and ended providing the range of weather characteristics observed during the sampling period (0800 to 1300). Daily maximum and minimum values also are shown. The greenhouse PAR sensor drifted during the years of service and was incapable of recording values over  $915 \mu\text{mol m}^{-2} \text{s}^{-1}$  during 2010. The data from the sensor appeared to portray relative differences correctly such that a cloudy day had lower PAR values when compared to a sunny day. PROC REG was used to compare the greenhouse PAR readings (independent) with the field PAR readings (dependent) across the 24 measurements (one per hour) that occurred on nine common measurement dates from 22 June to 3 September. The best fitting equation was  $\text{PAR} = 34.095 + (2.962 * \text{GH}) - (0.0010 * \text{GH}^2)$ , where PAR is the calibrated photosynthetically active radiation and GH is the greenhouse value. The equation had an  $R^2$  of 0.9244 and was used to calibrate all greenhouse PAR sensor readings.

#### 4.3.3 Soil Moisture

Simple linear regression procedures were used to compare the Hydrosense TDR soil moisture values (independent) with the soil moisture values (dependent) provided by the calibration pots (greenhouse) and soil cores (field) using the PROC REG function in SAS. A quadratic term was included in the model similar to results presented by Czarnomski et al. (2005) and manufacturer's instructions. Figure 4.2 presents a scatter plot of the data by study site, the resulting calibration equations, and adjusted  $R^2$  for each. The site specific linear regression equations explained 95% of the variation in the greenhouse and 86% of the variation in the field. All Hydrosense

TDR soil moisture values taken on measurement dates were then calibrated using the equations specific to a site. For the purposes of simplicity, these calibrated volumetric soil moisture values will be referred to as soil moisture throughout the paper.

Mean soil moisture values were calculated by experimental unit for each sample date. These means were then summed across the seven sampling dates independently by site to form a cumulative soil moisture value. This method (cumulative soil moisture value) was chosen as irrigation water had been added as needed throughout the season to achieve the various treatment regimes (greenhouse) making “date” unrepresentative of any seasonal effect. A similar challenge occurred with the field data. While date and species could be considered treatment levels in a repeated measures analysis, these plants were growing in a complex vegetation community where the seasonal weather had the potential to change from year to year. The factorial design at the greenhouse enabled the testing of irrigation and species effects as well as the interaction of the two treatment levels. In the field, these cumulative soil moisture values were analyzed as a complete randomized design. Assumptions of normality, linearity, and constant variance were assessed on the residuals and no transformations were required.

#### 4.4 Results

*Summary: A significant date by species interaction at both sites indicated that photosynthetic rates peaked at species specific levels near the end of June and early July then decreased differently as the season progressed. Transpiration remained relatively consistent across the season despite soil water declining below  $0.25 \text{ m}^3 \text{ H}_2\text{O}/\text{m}^3$  soil in the drought treatment (greenhouse) and in the field. All study species maintained high water use efficiency rates until late-July when it decreased below  $5 \mu\text{mol CO}_2 \cdot \text{mmol H}_2\text{O}^{-1}$  for the remainder of the season. Irrigation treatments employed at the greenhouse were effective at creating statistically different soil water conditions but had little impact on gas exchange rates. Multi-leaf gas exchange measurements as well as biomass partitioning data demonstrated how these species*

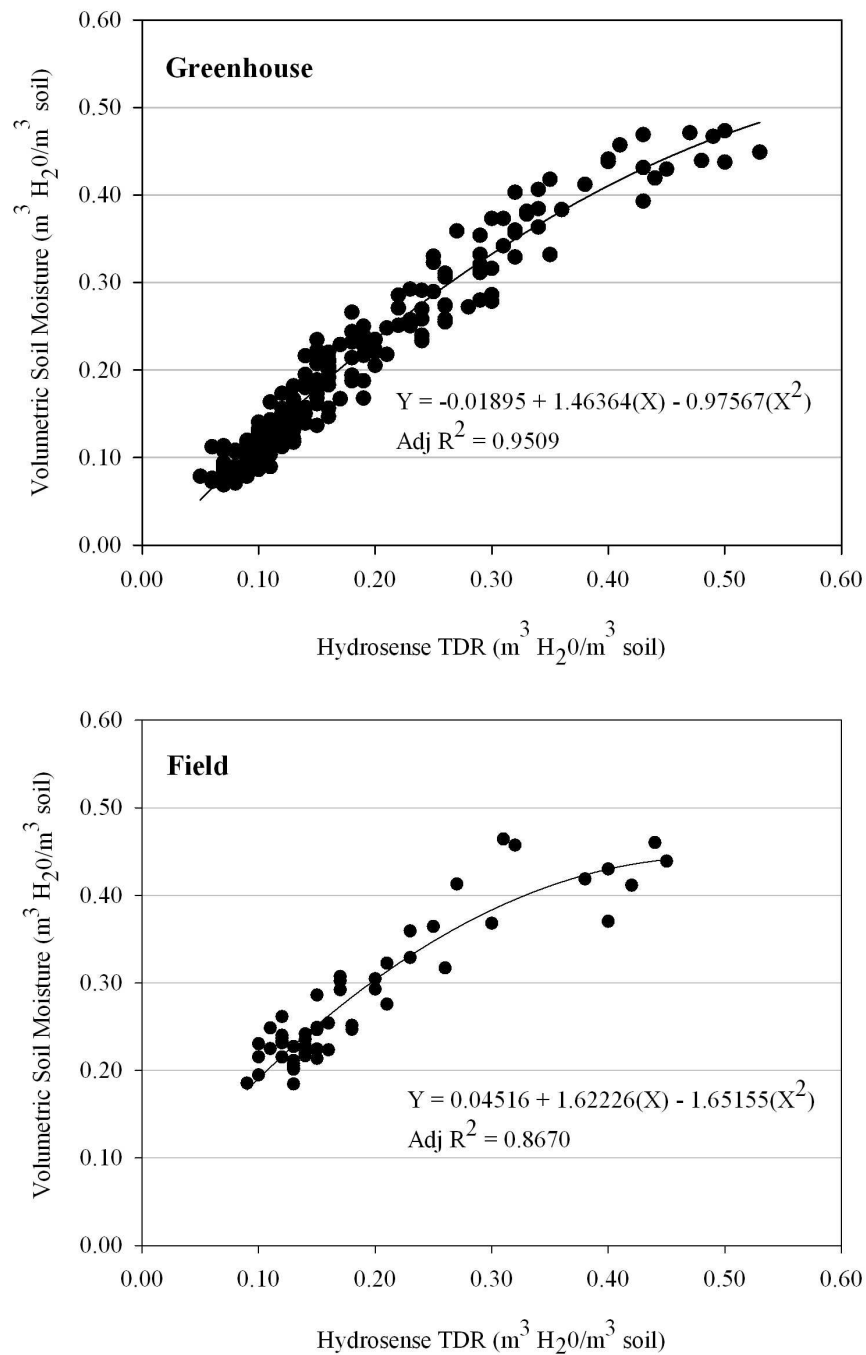


Figure 4.2: Scatter plots and regression equations developed through the calibration experiments unique to the greenhouse (upper) and field (lower) study sites.

*mature and shunt resources to actively growing tissues. During the initiation of flowering through seed dispersal, the highest rate of gas exchange tended to occur at the top of CIAR, CIVU, and SESY. RUUR had much more consistent rates of gas exchange along the extending canes which grew up to three meters. Specific leaf area was highest during June and declined for all species as lower leaves senesced. Plants included in the study showed various physiologic and morphologic mechanisms used to adjust to seasonal development and resource availability in order to produce tissues that would perpetuate the species. It was not possible to detect a difference among the species use of soil water.*

#### 4.4.1 Gas Exchange (Objectives 1 and 2)

Photosynthetic rates were significantly affected by an interaction between species and date at both the greenhouse and field sites (Table 4.1). This indicated that species reached different maximum rates of carbon dioxide assimilation early in the growing season and as time progressed, decreased at different rates (Figures 4.3 and 4.4). At an alpha of 0.05, the two levels of irrigation employed at the greenhouse did not have an effect on photosynthetic rates of the species tested. CIAR was capable of photosynthesizing at  $31 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  (greenhouse) and  $27 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  (field) in the later part of June coinciding with the bolt phase of growth. As inflorescence was determined through flowering and seed dispersal, photosynthetic rate steadily decreased until stabilizing at approximately  $15 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  and  $10 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  in the greenhouse and field, respectively. CIVU was photosynthesizing at 20 and  $21 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  through the end of June at the greenhouse and field, respectively. It then decreased below  $10 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  in late-July at the greenhouse and the end of August in the field. Flowering for this species did not begin until late-July with seed dispersal occurring in the later half of August. SESY reached a photosynthetic peak of 24 and  $25 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  in early-July at the greenhouse and field, respectively. From this point on, leaf photosynthetic

Table 4.1 Repeated measures ANOVA tables presenting Type III effects for photosynthetic and transpiration rates as well as water use efficiency by site. Asterisk indicates significance at  $\alpha=0.05$  level.

Photosynthetic Rate	Greenhouse	Effect	Num DF	Den DF	F Value	Pr > F
		Irrigation	1	21	3.14	0.0908
		Species	3	21	46.19	<0.0001 *
		Irr*Spp	3	21	0.55	0.6568
		Date	6	144	182.97	<0.0001 *
		Irr*Date	6	144	0.53	0.7858
		Spp*Date	18	144	13.62	<0.0001 *
		Irr*Spp*Date	18	144	0.29	0.9981
	Field	Effect	Num DF	Den DF	F Value	Pr > F
		Species	3	9	2.75	0.1048
		Date	6	72	22.56	<0.0001 *
		Species*Date	18	72	2.62	0.0020 *

Transpiration Rate	Greenhouse	Effect	Num DF	Den DF	F Value	Pr > F
		Irrigation	1	21	3.70	0.0681
		Species	3	21	28.50	<0.0001 *
		Irr*Spp	3	21	0.85	0.4813
		Date	6	144	6.89	<0.0001 *
		Irr*Date	6	144	0.57	0.7565
		Spp*Date	18	144	7.23	<0.0001 *
		Irr*Spp*Date	18	144	0.23	0.9996
	Field	Effect	Num DF	Den DF	F Value	Pr > F
		Species	3	9	0.94	0.4606
		Date	6	72	13.06	<0.0001 *
		Species*Date	18	72	3.23	0.0002 *

Water Use Efficiency	Greenhouse	Effect	Num DF	Den DF	F Value	Pr > F
		Irrigation	1	21	0.28	0.6042
		Species	3	21	13.20	<0.0001 *
		Irr*Spp	3	21	2.18	0.1207
		Date	6	144	38.36	<0.0001 *
		Irr*Date	6	144	1.12	0.3514
		Spp*Date	18	144	0.97	0.4936
		Irr*Spp*Date	18	144	0.39	0.9884
	Field	Effect	Num DF	Den DF	F Value	Pr > F
		Species	3	9	8.27	0.0059 *
		Date	6	72	74.80	<0.0001 *
		Species*Date	18	72	4.88	<0.0001 *

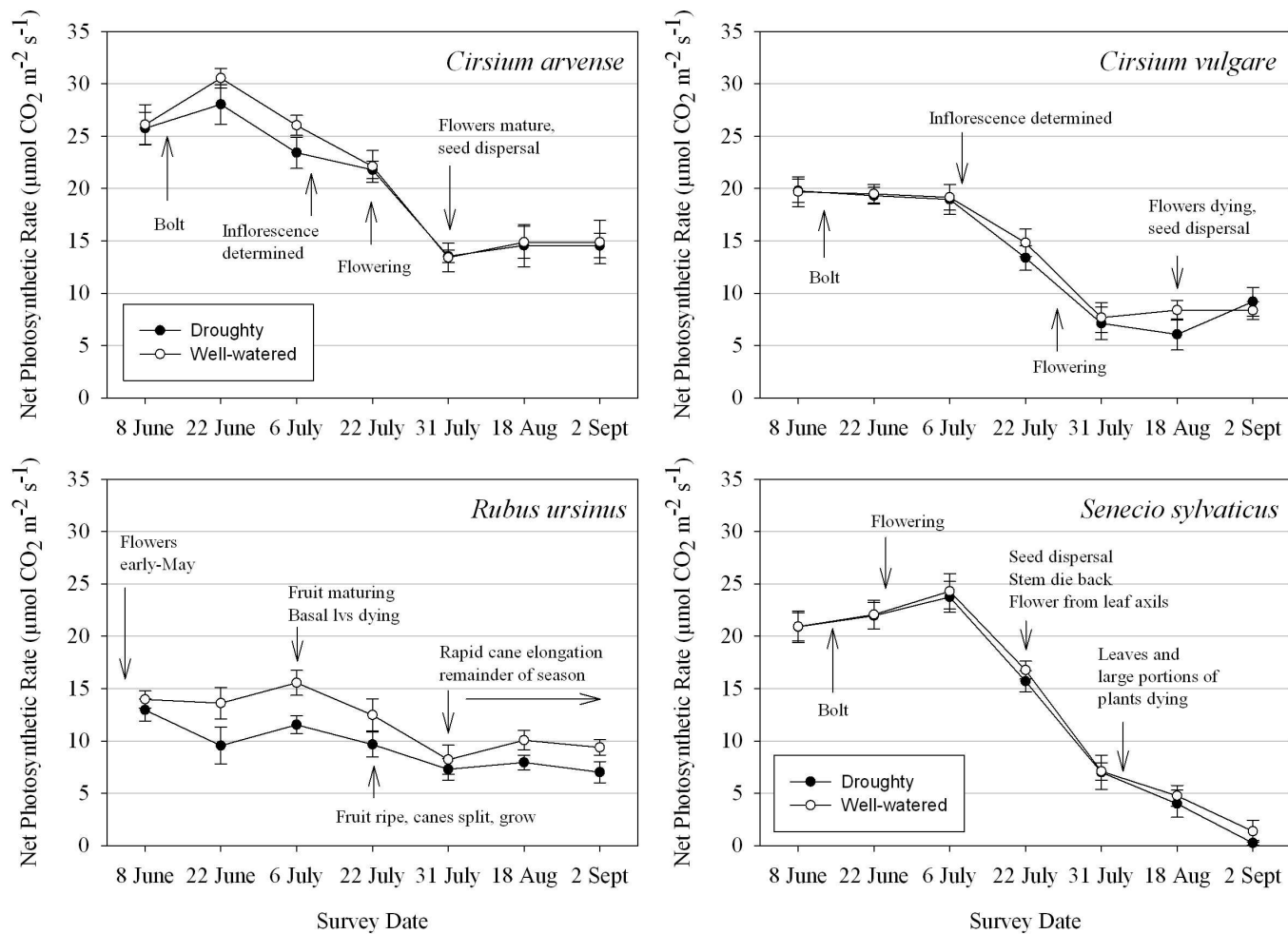


Figure 4.3 Greenhouse net photosynthetic rate (μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) by species and irrigation level from 8 June to 2 September. Approximate stage of phenologic development has been included. Standard errors for a species are calculated by irrigation level over replication.

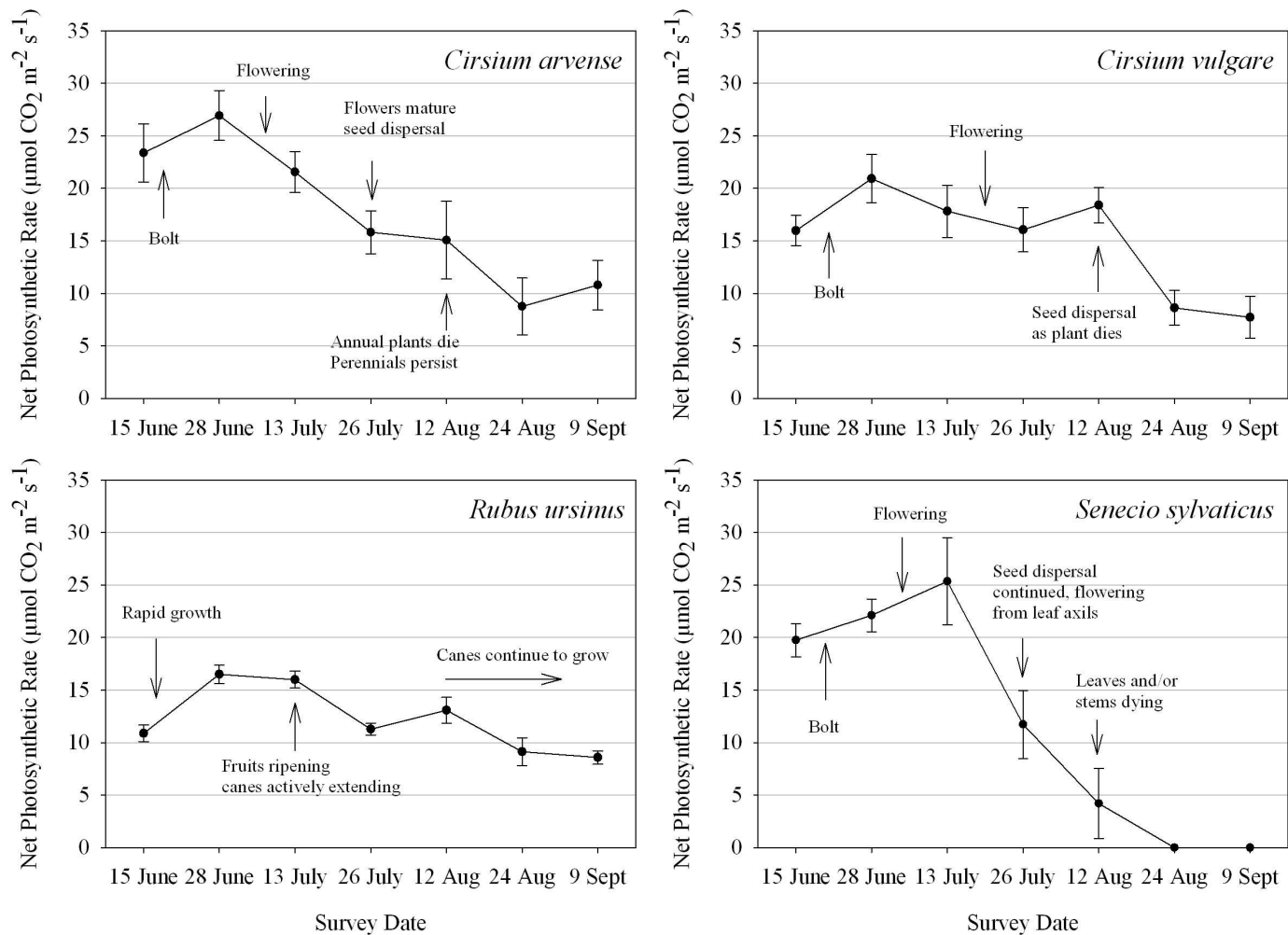


Figure 4.4 Field net photosynthetic rate ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) by species from 15 June to 9 September. Approximate stage of phenologic development has been included. Standard errors for a species are calculated by date over replication.

rates decreased as the plants flowered, dispersed seed, and began to senesce by the end of August in the field or early-September at the greenhouse. RUUR maintained more consistent rates of photosynthesis across the season and ranged from a high of 16 and 17  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  at the greenhouse and field (respectively) in late-June/early-July to below 10  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  in early-September.

A significant species by date interaction was also found for the transpiration rates observed at the greenhouse and field sites (Table 4.1). Similar to the photosynthetic rate, this interaction indicated that these species transpired water differently across the growing season (Figures 4.5 and 4.6). The two levels of irrigation did not result in different transpiration rates at an alpha of 0.05. It is noteworthy that plants in the field on 13 July 2010 had reduced transpiration rates when compared to the remainder of the season on that site. CIAR transpired water at rates from 3.7 to 5.5  $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$  in the greenhouse and from 1.2 to 4.8  $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$  in the field. CIVU had transpiration rates that remained consistent between 2.9 and 4.3  $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$  in the greenhouse. In the field, a peak of 4.1  $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$  was observed on 28 June. The lowest transpiration rate at 1.3  $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$  was found on 13 June and rates near 2.5  $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$  in late-August and early-September. SESY had the highest transpiration rate of the four species included in the study. In late-June (field) and early-July (greenhouse), transpiration rates of 5.7  $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$  in the well-watered greenhouse setting and in the field were observed. After these peak rates, SESY decreased transpiration until reaching nearly zero as the plants senesced. Much like the photosynthetic rate, the transpiration rates of RUUR were low and consistent across the season when compared to the other species. RUUR transpiration rates for both irrigation treatments ranged between 1.4 and 3.0  $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ . RUUR plants in the field had higher transpiration rates (compared to the greenhouse) with a peak of 3.6  $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$  in late-June and low of 2.0  $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$  on 13 July and 9 September.

Water use efficiency (WUE) was significantly different among the species and dates at the greenhouse (Table 4.1). Irrigation level did not affect WUE and none of

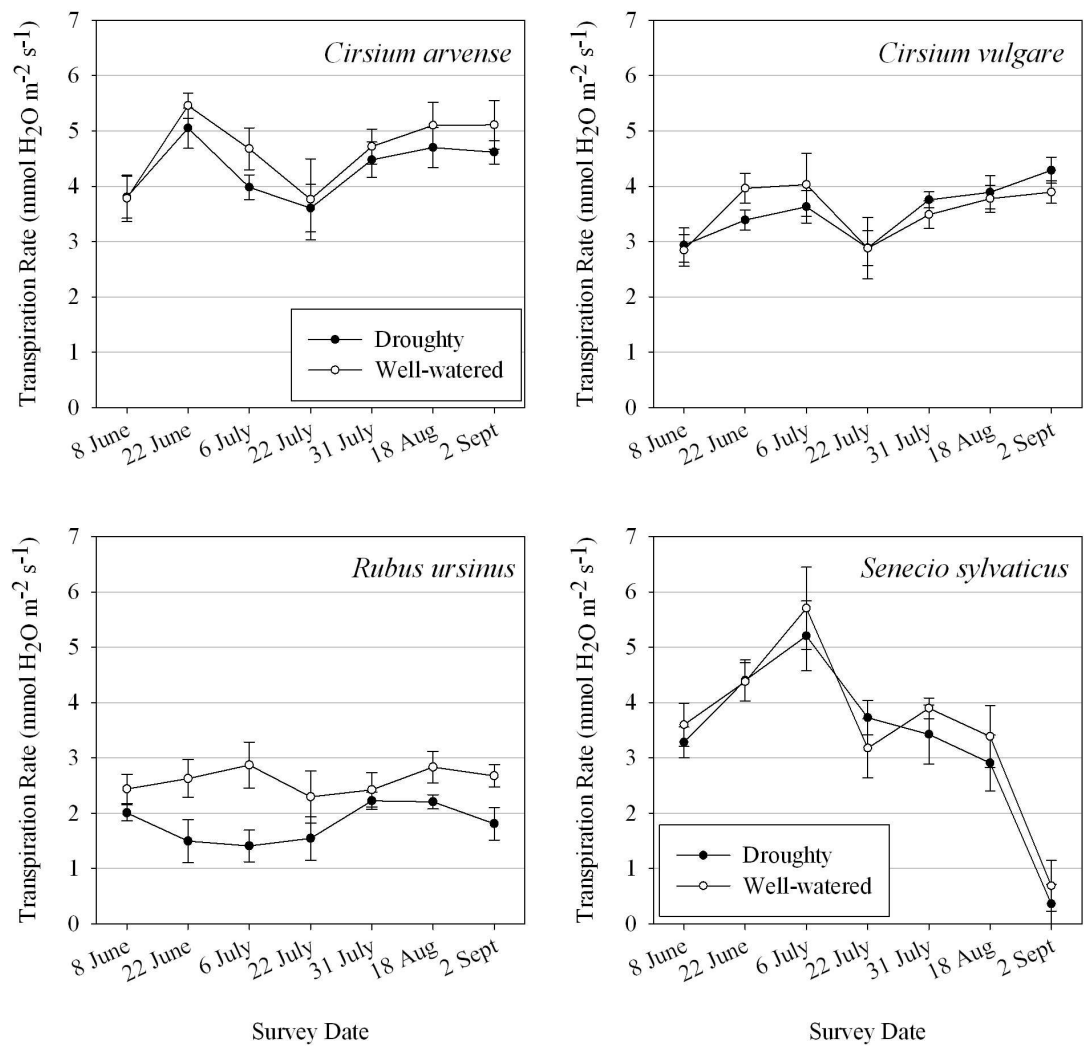


Figure 4.5 Greenhouse transpiration rate (mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>) by species and irrigation level from 8 June to 2 September. Standard errors for a species are calculated by irrigation level over replication.

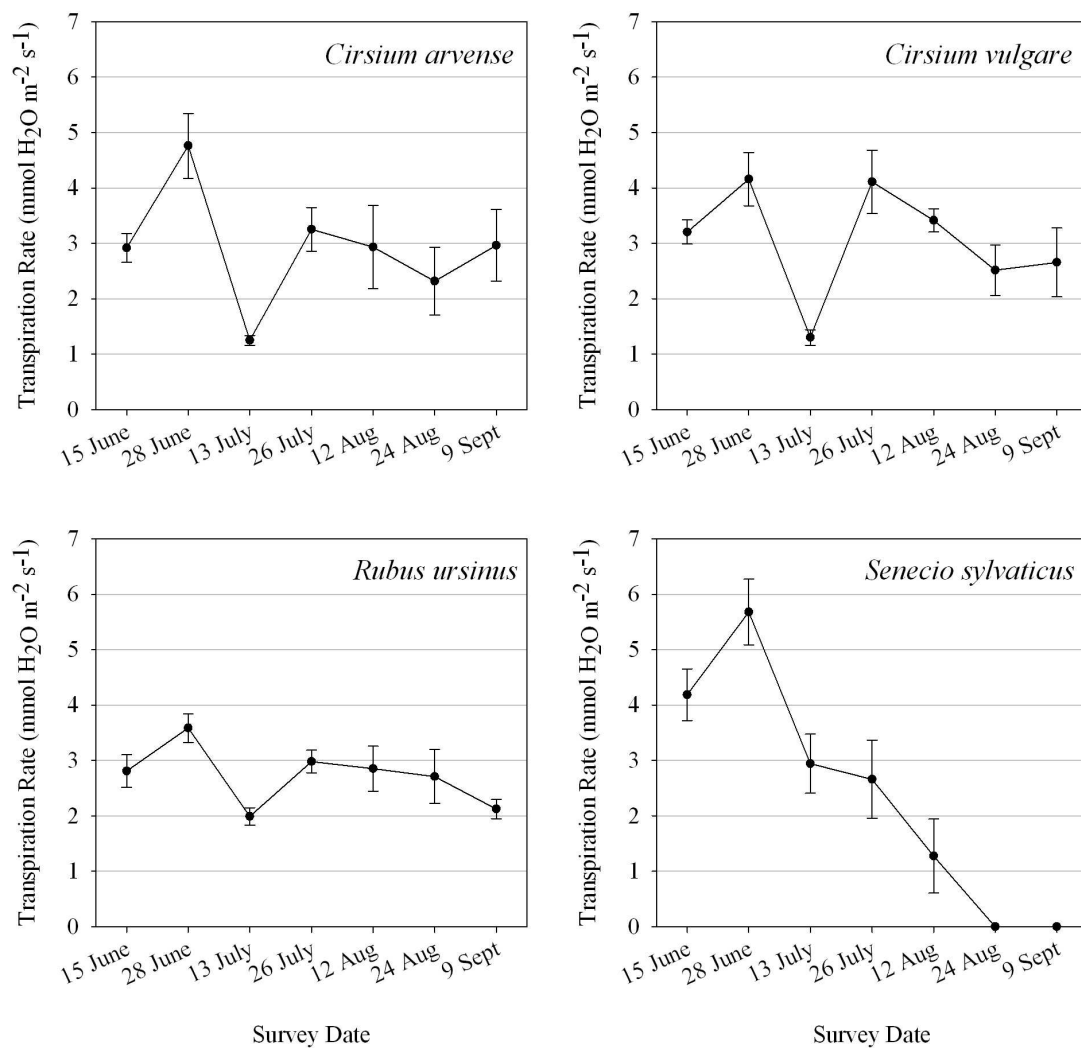


Figure 4.6 Field transpiration rate (mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>) by species from 15 June to 9 September. Standard errors for a species are calculated by date over replication.

the interactions were significant at the greenhouse. As a brief explanation, WUE is a calculated ratio of carbon dioxide fixed to water lost at the time of measurement. For example, a WUE of 6 indicated that for every six micromoles of carbon dioxide that was assimilated, one millimole of water was released (per square meter per second). This value indicates the ability of a species to regulate the gas exchange process. In the greenhouse, all four species maintained higher WUE until 22 July after which time, efficiency rates declined to species specific levels (Figure 4.7). CIAR was above  $5.7 \mu\text{mol CO}_2 \cdot \text{mmol H}_2\text{O}^{-1}$  until 22 July after which time it decreased to  $3.0 \mu\text{mol CO}_2 \cdot \text{mmol H}_2\text{O}^{-1}$ . CIVU maintained WUE between  $7.0$  and  $5.0 \mu\text{mol CO}_2 \cdot \text{mmol H}_2\text{O}^{-1}$  up to 22 July then decreased below  $2.2 \mu\text{mol CO}_2 \cdot \text{mmol H}_2\text{O}^{-1}$  for the remainder of the season. SESY showed a relatively consistent decline in WUE across the season beginning at  $6.5 \mu\text{mol CO}_2 \cdot \text{mmol H}_2\text{O}^{-1}$  and ending near zero on 2 September. RUUR growing under the drought treatment regime had minor improvements in WUE in the first half of the growing season. WUE increased to  $8.8 \mu\text{mol CO}_2 \cdot \text{mmol H}_2\text{O}^{-1}$  in this treatment while those in the well-watered condition remained closer to  $6.0 \mu\text{mol CO}_2 \cdot \text{mmol H}_2\text{O}^{-1}$ . From 31 July to the end of the season, RUUR in both irrigation treatments had WUE below  $4.3 \mu\text{mol CO}_2 \cdot \text{mmol H}_2\text{O}^{-1}$ .

WUE in the field was significantly affected by an interaction between species and date (Table 4.1). The low transpiration rates observed on 13 July had a pronounced effect on the WUE observed (Figure 4.8). On this date, WUE of  $17.4$  and  $14.0 \mu\text{mol CO}_2 \cdot \text{mmol H}_2\text{O}^{-1}$  were observed for CIAR and CIVU, respectively. SESY and RUUR were not as dramatically affected with WUE of  $9.0$  and  $8.3 \mu\text{mol CO}_2 \cdot \text{mmol H}_2\text{O}^{-1}$ , respectively. With the exception of the 13 July date, the overall trajectory of season-long WUE for the study species showed similar trends in the field when compared to those from the greenhouse. CIAR had a peak WUE of  $8.0 \mu\text{mol CO}_2 \cdot \text{mmol H}_2\text{O}^{-1}$  and declined to approximately  $2.7 \mu\text{mol CO}_2 \cdot \text{mmol H}_2\text{O}^{-1}$  at the end of August. WUE of CIVU was nearly  $5.2 \mu\text{mol CO}_2 \cdot \text{mmol H}_2\text{O}^{-1}$  until 12 August then decreased to  $2.2 \mu\text{mol CO}_2 \cdot \text{mmol H}_2\text{O}^{-1}$  on the last measurement date.

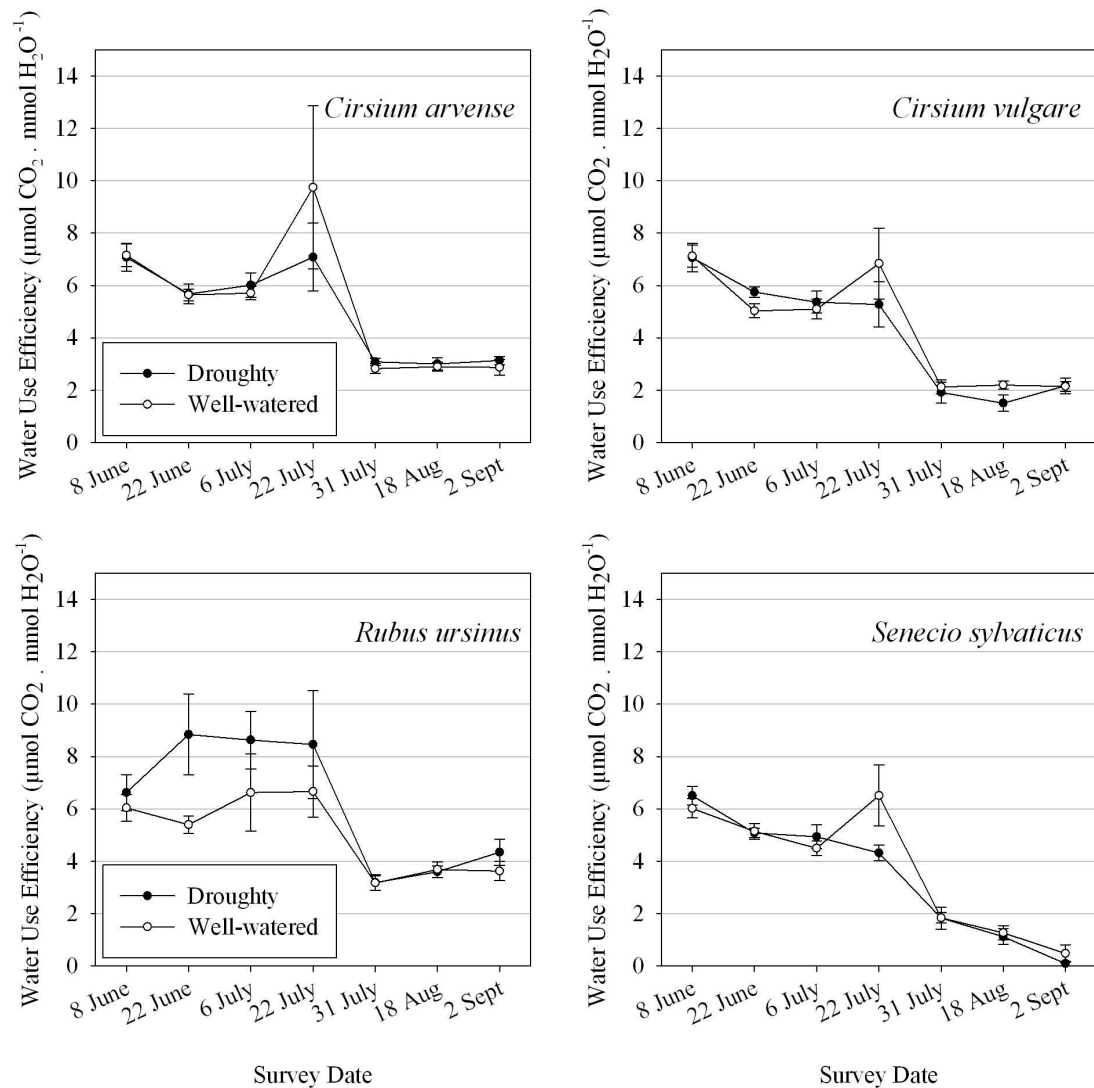


Figure 4.7 Greenhouse water use efficiency ( $\mu\text{mol CO}_2 \cdot \text{mmol H}_2\text{O}^{-1}$ ) by species and irrigation level from 8 June to 2 September. Standard errors for a species are calculated by irrigation level over replication.

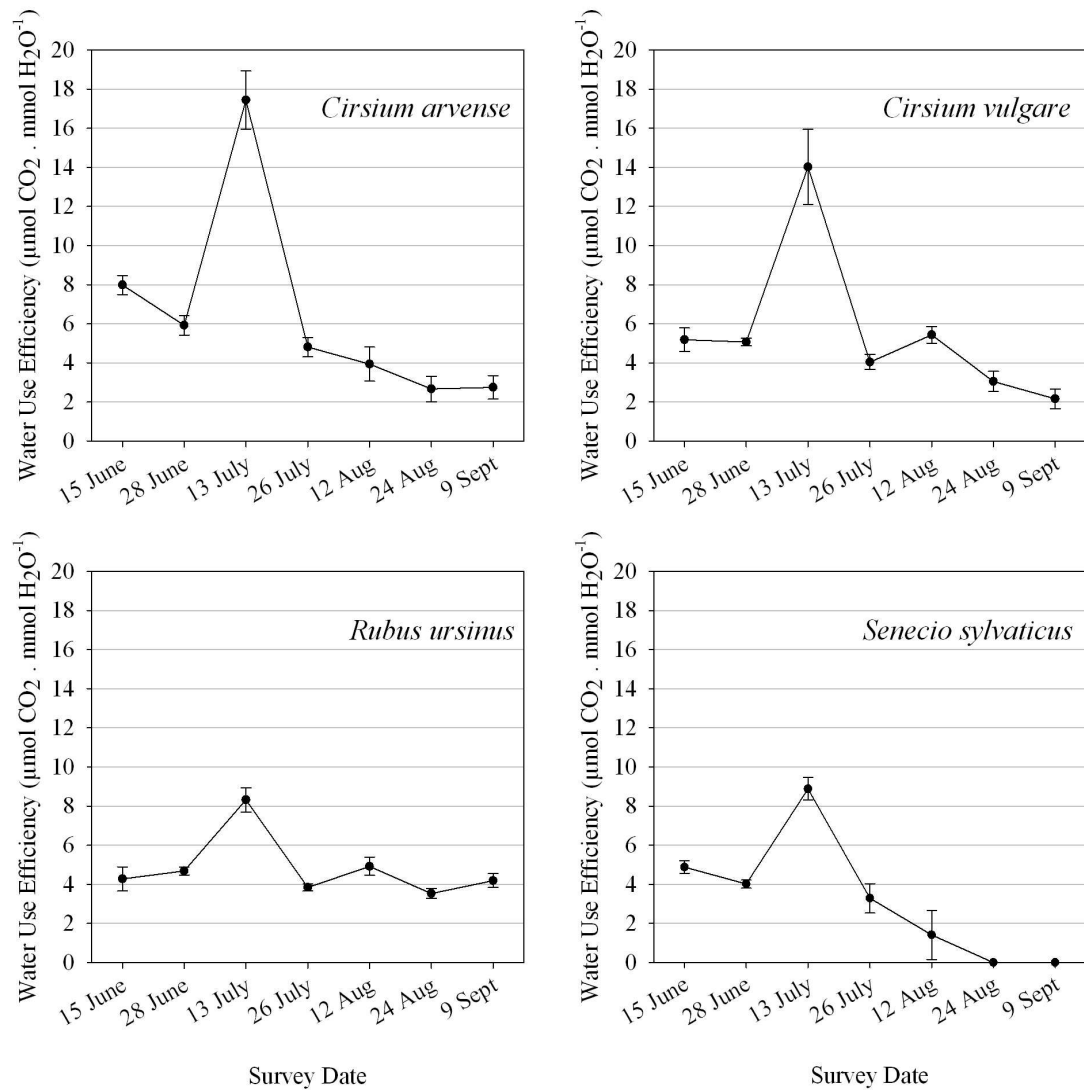


Figure 4.8 Field water use efficiency ( $\mu\text{mol CO}_2 \cdot \text{mmol H}_2\text{O}^{-1}$ ) by species from 15 June to 9 September. Standard errors for a species are calculated by date over replication.

SESY showed a consistent decline in WUE from  $4.9 \mu\text{mol CO}_2 \cdot \text{mmol H}_2\text{O}^{-1}$  in mid-June to zero on 24 August when the plants were either dead or had no leaves to measure. In the relatively undisturbed conditions of the field, WUE of RUUR remained between 4.9 and  $3.5 \mu\text{mol CO}_2 \cdot \text{mmol H}_2\text{O}^{-1}$  the entire season.

#### 4.4.2 Multi-leaf Gas Exchange and Biomass Partitioning (Objective 3)

At both sites, CIAR, CIVU, and SESY in June had photosynthetic and transpiration rates that were relatively similar from leaf to leaf on an individual plant (Figures 4.9, 4.10, 4.11). This time frame coincided with the bolt stage where shoot growth predominated and flowering had not yet begun. Photosynthetic rates of CIAR ranged from 16 to  $24 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ , CIVU from 11 to  $20 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ , and SESY from 12 to  $24 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ . Transpiration rates showed similar consistent trends with values ranging from 2.8 and  $3.5 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$  for CIAR and 1.9 to  $3.2 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$  for CIVU. SESY transpiration rates were vertically consistent but, depending on site and hence sampling date, the rates ranged from 1.5 to  $4.6 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ . In June, growth activity for RUUR was primarily building new leaves (Figure 4.12). Photosynthetic and transpiration rates were highest on leaves close to the root system and decreased as measurements were taken along the cane toward the apical meristem. Photosynthetic rates ranged from 18 down to  $7 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  with transpiration rates between 2.8 and  $2.0 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$  for this species.

For CIAR, CIVU, and SESY, July was marked by inflorescence determination and/or flowering (Figure 4.9, 4.10, and 4.11). The highest photosynthetic and transpiration rates observed in the study were during this month and tended to increase with increasing height of measured leaves. Alternately said, the lower leaves on these plants, those present in the spring and early-summer, were often at reduced gas exchange rates and, in many cases, were beginning to senesce at this point in the season. In the field, SESY was observed to have photosynthesis rates that differed between the top and bottom of the plant in excess of  $18 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  in the greenhouse and  $23 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  in the field. Among the live leaves for this

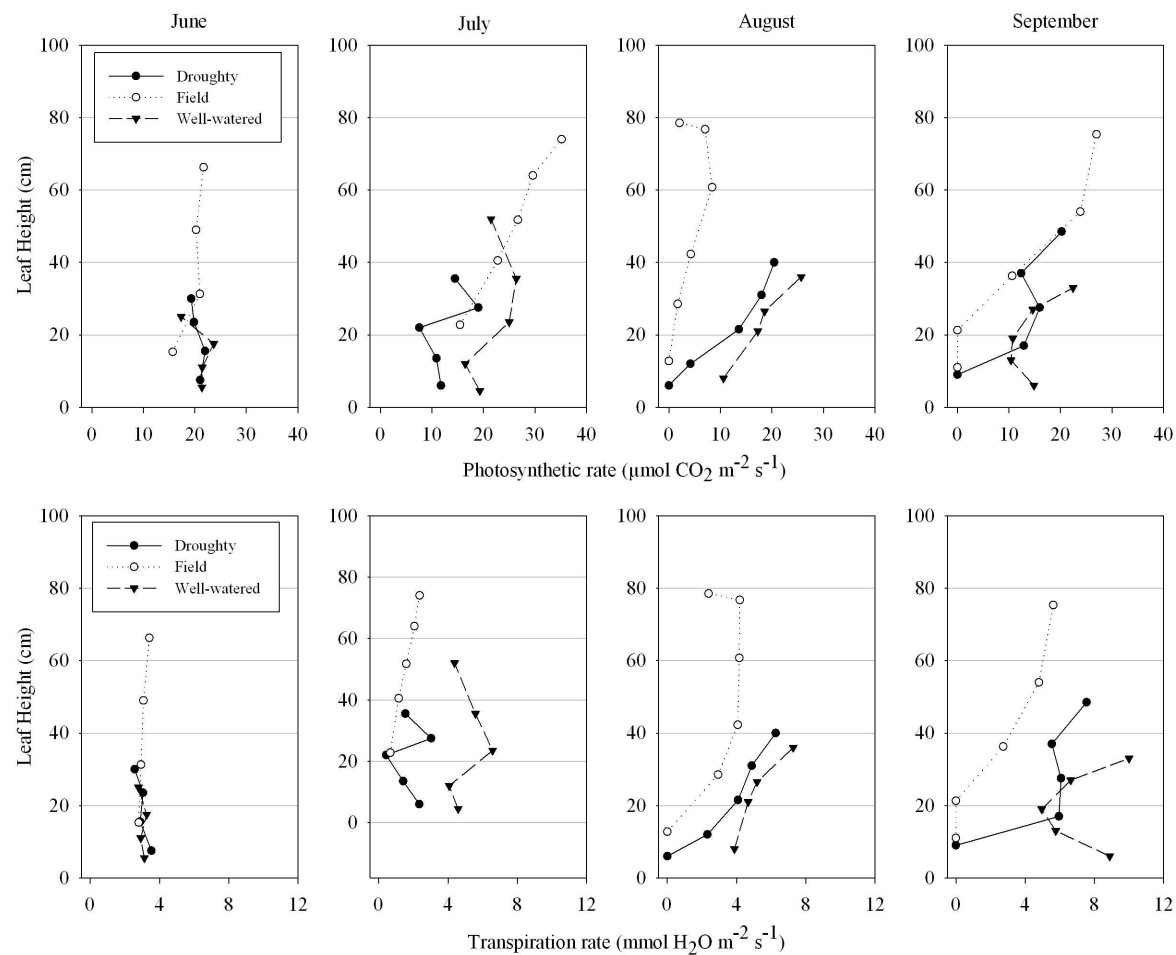


Figure 4.9 Mean photosynthetic ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) and transpiration ( $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ) rates by site, position, and month for *Cirsium arvense*. Means calculated based on the 16 plants sampled on each date (four per species). At the greenhouse, these four plants were divided based on the two levels of irrigation supplied (i.e. two plants compose each data point for the droughty and well-watered treatments).

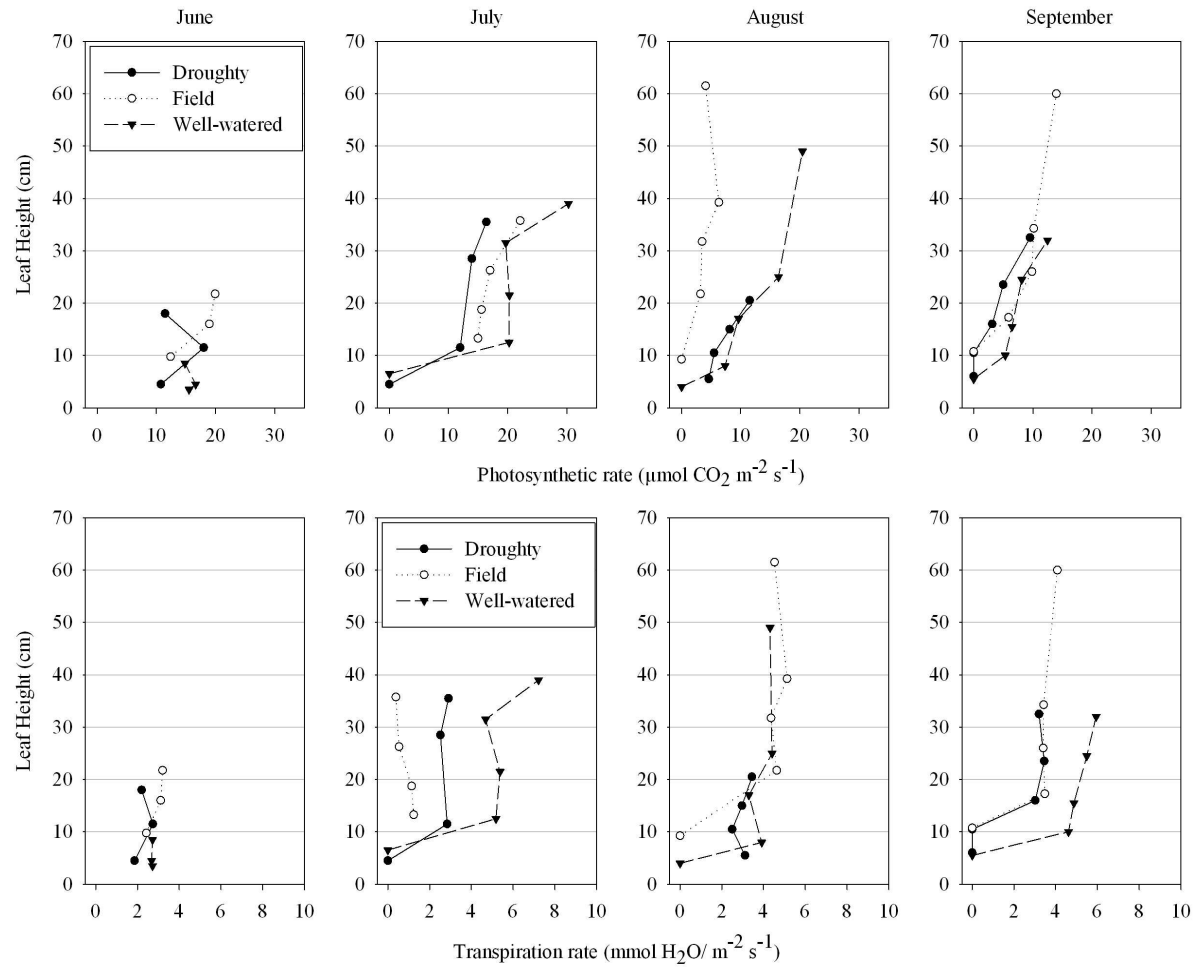


Figure 4.10 Mean photosynthetic ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) and transpiration ( $\text{mmol H}_2\text{O} \text{ m}^{-2} \text{ s}^{-1}$ ) rates by site, position, and month for *Cirsium vulgare*. Means calculated based on the 16 plants sampled on each date (four per species). At the greenhouse, these four plants were divided based on the two levels of irrigation supplied (i.e. two plants compose each data point for the droughty and well-watered treatments).

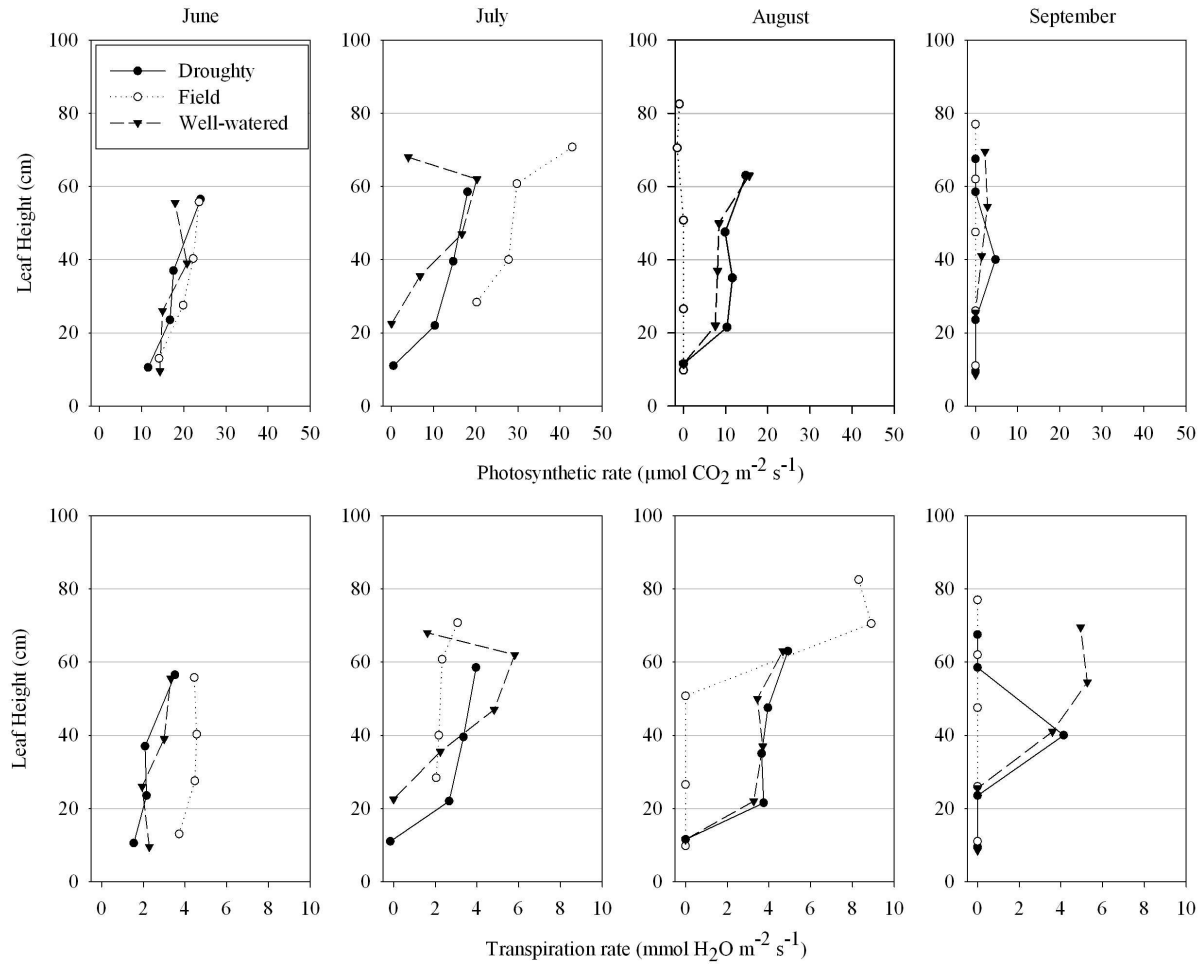


Figure 4.11 Mean photosynthetic ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) and transpiration ( $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ) rates by site, position, and month for *Senecio sylvaticus*. Means calculated based on the 16 plants sampled on each date (four per species). At the greenhouse, these four plants were divided based on the two levels of irrigation supplied (i.e. two plants compose each data point for the droughty and well-watered treatments).

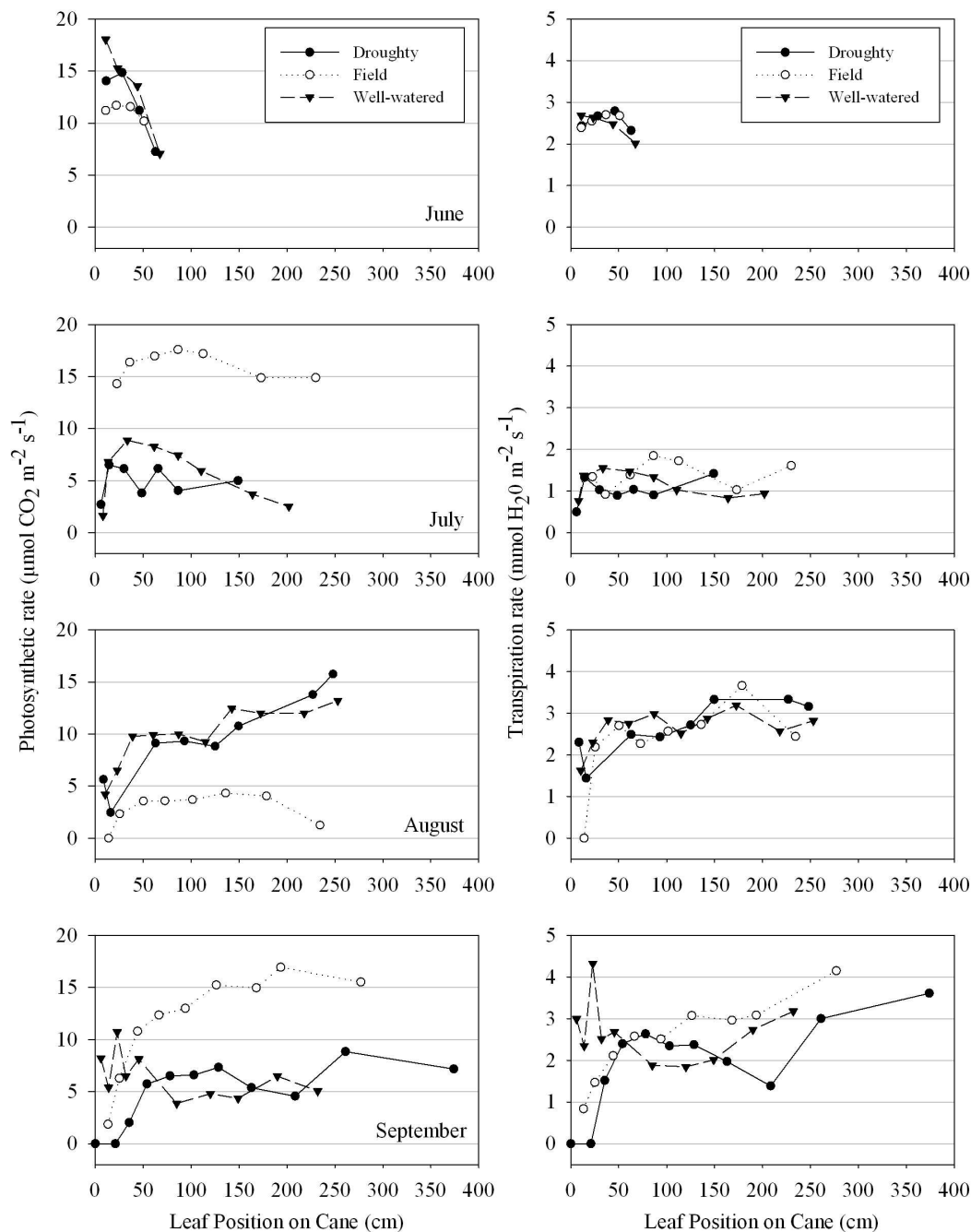


Figure 4.12 Mean photosynthetic ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) and transpiration ( $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ) rates by site, position, and month for *Rubus ursinus*. Means calculated based on the 16 plants sampled on each date (four per species). At the greenhouse, these four plants were divided based on the two levels of irrigation supplied (i.e. two plants compose each data point for the droughty and well-watered treatments).

species, transpiration rates were between 2.0 and 5.8 mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> during this time. Upper leaves of CIAR had photosynthetic rates that went as high as 26 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup> in the greenhouse and 35 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup> in the field. Photosynthetic rates on leaves near the ground were lower by as much as 10 and 20 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>. Transpiration rates ranged vertically as much as 0.4 to nearly 6.6 mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> in the greenhouse and from 1.0 to 2.4 mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> in the field. On some CIVU plants, the lowest leaves measured in June were dead in July, while those higher on the plant could be photosynthesizing at rates separated by as much as 10 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>. Among the live leaves on CIVU in July, transpiration rates were separated by less than 2.1 mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>, but reached a peak rate of 7.2 mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> in the well-watered greenhouse setting. RUUR leaves closest to the root system with high photosynthetic rates in June were found to have lower photosynthetic rates when compared to those occurring farther along the extending canes (Figure 4.12). Photosynthetic rates reached a peak near the middle of the cane at 9 or 18 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup> (greenhouse and field, respectively) and decreased toward the actively growing tip. Transpiration rates for RUUR were as low as 0.5 mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> for leaves closest to the root system, but remained more consistent between 0.9 and 1.8 mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> along the remainder of the cane.

Seed dispersal and lower leaf senescence was common for the herbaceous species in August. RUUR, the native perennial in the study, continued to rapidly extend canes through this month. Again photosynthetic and transpiration rates tended to be the highest at the uppermost leaves measured for CIAR and CIVU (Figure 4.9 and 4.10). At both sites, SESY plants were disseminating seed and portions of the plants were dying. Measurements occurred on the 1 August (greenhouse) and 16 August (field) and at this point in the season, especially in the field, only a few upper leaves were alive which were found to have negative assimilation rates (i.e. they were respiring CO<sub>2</sub>). RUUR showed similar trends in gas exchange as observed the month prior with low rates of photosynthesis and transpiration on leaves near ground-line,

consistent rates along the cane, and site dependent increases or decreases as measurements progressed toward the apical meristem.

While some of the lower leaves of CIAR and CIVU were dead in the month of September, these plants were still actively exchanging carbon dioxide and water in the upper leaves. The majority of SESY plants were dead by this time in the growing season and only a small number of measurable leaves remained (Figure 4.11). RUUR continued to extend canes and while those spring leaves may have died or been at a reduced capacity, relatively consistent gas exchange rates were observed along the length of the canes which grew over 3 meters during the study period (Figure 4.12).

Specific leaf area (SLA) is a common measure of the effort a species places on the production and/or maintenance of photosynthetic area. Lower numbers indicate a larger investment in photosynthetic area with the change in the ratio of one-sided surface area to leaf weight. The results presented in Table 4.2 show that all species regardless of site or irrigation treatment had the highest SLA early in the season then progressively decreased until the final harvest in September. In June during the bolt phase of growth, SESY had the highest SLA between  $183$  and  $207 \text{ cm}^2 \cdot \text{gram}^{-1}$ . RUUR had a SLA of  $141$  to  $157 \text{ cm}^2 \cdot \text{gram}^{-1}$  and the two thistle species had the lowest SLA observed with CIVU between  $120$  and  $127 \text{ cm}^2 \cdot \text{gram}^{-1}$  and CIAR between  $90$  to  $106 \text{ cm}^2 \cdot \text{gram}^{-1}$ . Despite the declining SLA across the season, the order of decreasing SLA from  $\text{SESY} > \text{RUUR} > \text{CIVU} > \text{CIAR}$  was still present at the harvests occurring in August and September.

Biomass partitioning data presented in Figure 4.13 demonstrate the various tissue investment strategies utilized by these four species. Inflorescence mass fraction included all flowering parts and/or seed/fruit produced. CIAR placed between  $0.11$  and  $0.19$  of the overall biomass effort toward inflorescence while CIVU invested  $0.16$  to  $0.24$ . SESY placed the most effort of the four species into the production of flowering parts with up to  $0.38$  of the mass fraction in the greenhouse and  $0.19$  in the field. Flowers were not harvested for RUUR as this process occurred prior to the beginning of this study. Fruit, however, was collected on a few plants in July and

Table 4.2 Mean specific leaf area ( $\text{cm}^2 \cdot \text{g}^{-1}$ ) by growing condition and month. Standard errors are calculated by the number of leaves sampled per irrigation level (greenhouse) and date (field) over replications. GH stands for greenhouse and is followed by the irrigation level.

<i>Cirsium arvense</i>	Site - condition	June	SE	July	SE	Aug.	SE	Sept.	SE
	GH - Droughty	105.9	6.4	87.7	5.5	79.7	7.6	70.1	3.7
	GH - Well-watered	104.0	5.6	85.3	6.6	96.8	6.3	91.5	4.4
	Field	89.8	3.8	86.0	3.7	74.3	2.1	66.2	4.3
<i>Cirsium vulgare</i>	Site - condition	June	SE	July	SE	Aug.	SE	Sept.	SE
	GH - Droughty	127.0	9.1	90.9	5.2	101.2	6.7	78.8	10.6
	GH - Well-watered	120.5	9.3	84.7	6.0	77.2	4.6	71.6	3.9
	Field	120.2	5.5	108.0	5.5	95.2	3.5	78.6	3.0
<i>Senecio sylvaticus</i>	Site - condition	June	SE	July	SE	Aug.	SE	Sept.	SE
	GH - Droughty	183.1	8.9	231.8	12.8	162.3	15.5	135.2	.
	GH - Well-watered	207.0	14.6	200.9	19.7	177.9	8.9	154.0	19.7
	Field	205.0	10.2	147.2	9.1	111.7	5.5	.	.
<i>Rubus ursinus</i>	Site - condition	June	SE	July	SE	Aug.	SE	Sept.	SE
	GH - Droughty	141.8	14.3	133.0	13.4	125.6	5.1	111.7	3.3
	GH - Well-watered	157.1	16.4	168.5	15.8	119.0	5.9	114.7	5.2
	Field	140.6	4.8	134.6	5.3	126.6	5.2	113.6	1.5

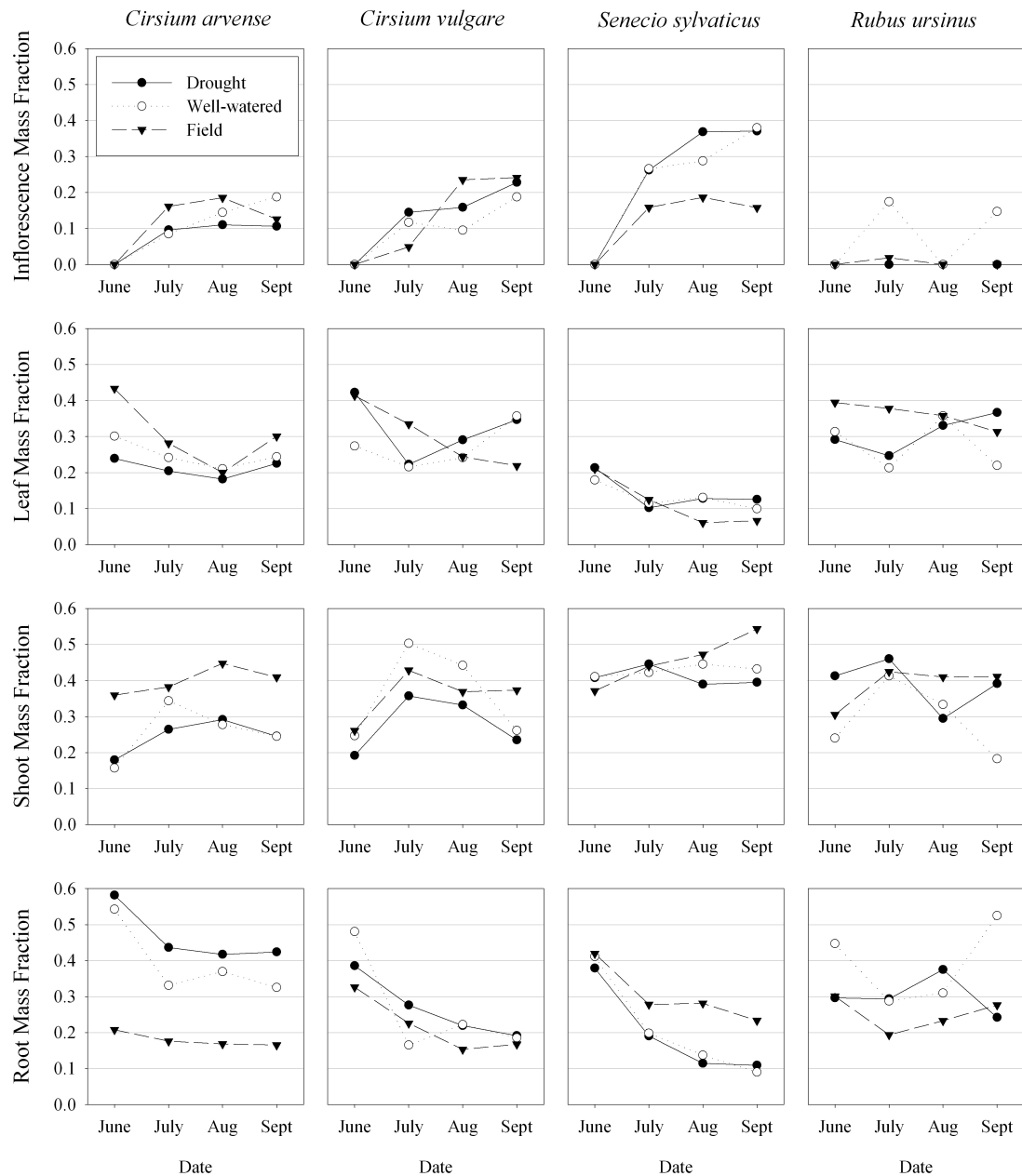


Figure 4.13 Biomass partitioning of the four study species presented by inflorescence, leaf, shoot, and root mass fraction and month. Four plants of each species were sampled for a given month ( $n=16$  total). At the greenhouse, these four plants were divided into the two irrigation treatments so that only two plants per species and irrigation level were sampled. Biomass fraction was calculated as the oven dry weight of the component divided by the total oven dry weight of the plant.

September resulting in a maximum observed inflorescence mass fraction of 0.17. Leaf mass fraction was generally highest for CIAR, CIVU, and SESY during June and remained a relatively consistent investment for RUUR. After this time, CIAR leaf mass fraction was observed to be between 0.18 to 0.30 and CIVU ranged from 0.22 to 0.36. SESY had up to 0.21 of its mass in leaves during June, but decreased to below 0.12 from July to September. RUUR, on the other hand, maintained a more consistent leaf mass fraction at 0.21 to 0.39 across the season.

Shoot mass fraction constituted a higher proportion of the biomass for all four species. As the season progressed, the three herbaceous species showed an overall increase in shoot mass fraction while RUUR increased until July then either remained stable or decreased. In June, CIAR had the highest observed root mass fraction at over 0.50 in the greenhouse and while this decreased in relation to the other aspects of growth, it represents a consistent input of photosynthate as the rates stabilized at an average of 0.38 in the greenhouse and 0.17 in the field. Across the season, SESY and CIVU placed a decreasing effort into the biomass of root systems declining below 0.22 and 0.28, respectively. The root systems of RUUR were observed to be of high importance to the species as biomass remained at an average 0.31 of the mass fraction across the measurement period.

#### 4.4.3 Soil Moisture (Objective 4)

The irrigation levels employed at the greenhouse resulted in different soil moisture conditions between the two treatments (Table 4.3). No differences in the soil moisture utilization by the four study species were detected at either study site. Soil water stayed above  $0.31 \text{ m}^3 \text{ H}_2\text{O}/\text{m}^3$  soil across the measurement dates in the well-watered treatment (Figure 4.14). Limiting the addition of water according to the droughty treatment allowed soil water to decrease below  $0.25 \text{ m}^3 \text{ H}_2\text{O}/\text{m}^3$  soil in the initial two week period between the first and second measurement dates. It was maintained at this low level for the remainder of the experiment reaching the lowest value of  $0.18 \text{ m}^3 \text{ H}_2\text{O}/\text{m}^3$  soil on 18 August. Soil moisture in the field was marked by

Table 4.3 ANOVA results of the cumulative analysis of soil moisture by study site. Asterisk indicates significant treatment effects for the irrigation applied in the greenhouse at  $\alpha=0.05$ .

Soil Moisture		Source	DF	Type III SS	Mean Sq	F Value	Pr > F
Greenhouse		Block	3	0.1591	0.0530	2.21	0.1167
		Irrigation (Irr)	1	4.7236	4.7236	197.04	<0.0001 *
		Species (Spp)	3	0.0422	0.0141	0.59	0.6300
		Irr*Spp	3	0.0892	0.0297	1.24	0.3202
Field		Species	3	0.0439	0.0146	1.00	0.4253

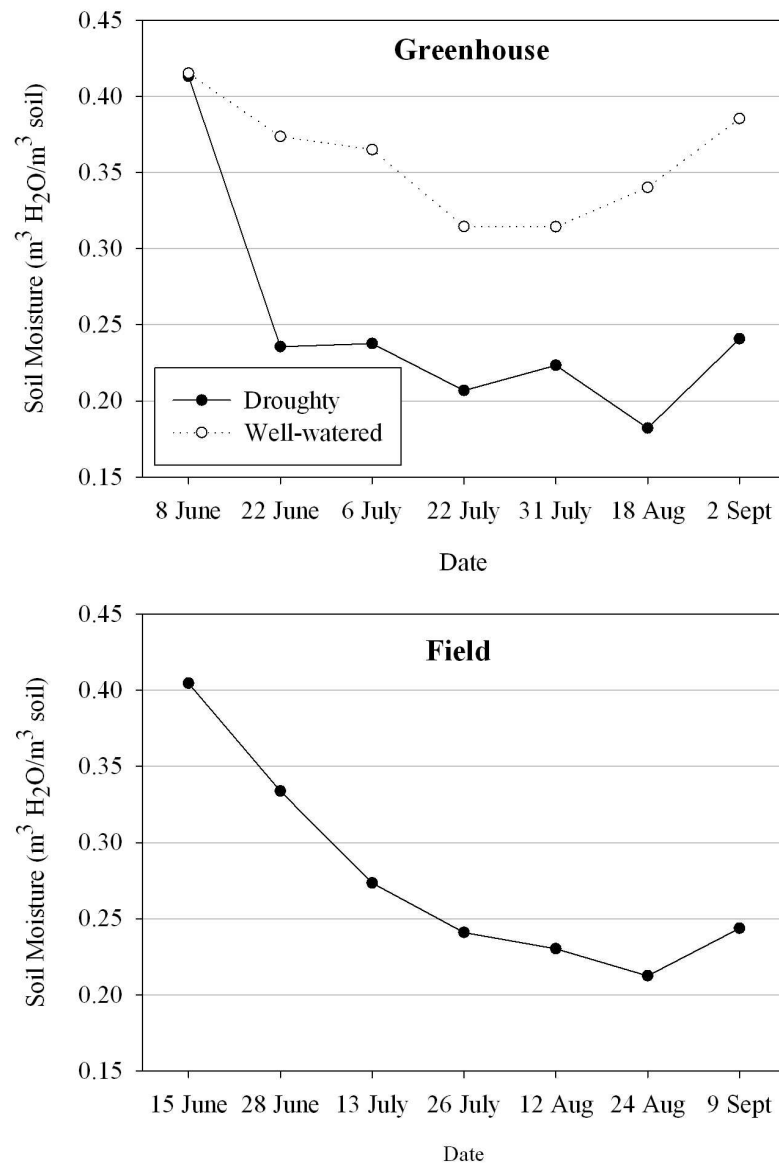


Figure 4.14 Mean soil moisture by date and irrigation treatment in the greenhouse (upper panel) and in the field (lower panel).

a steady depletion across the sample dates. The site was presumed to be near field capacity at the first measurement date when a value of  $0.40 \text{ m}^3 \text{ H}_2\text{O}/\text{m}^3$  soil was recorded. From 15 June to 9 September, 18 mm of precipitation was received by the site with 14 mm of this amount occurring from 30 August to 9 September. Soil moisture during this droughty period gradually diminished below  $0.25 \text{ m}^3 \text{ H}_2\text{O}/\text{m}^3$  soil near the end of July and reached the lowest observed value of  $0.21 \text{ m}^3 \text{ H}_2\text{O}/\text{m}^3$  soil on 24 August. Rain events occurring in late-August and early-September began replenishing soil moisture as shown by the increase to  $0.24 \text{ m}^3 \text{ H}_2\text{O}/\text{m}^3$  soil.

#### 4.4.4 Vegetation Community Development and Weather

Vegetation survey results indicated one year after herbicide use on the site, summed cover climbed from 55% on 7 May to 90% on 12 July (Figure 4.15). On the 12 July survey, 60% of the cover in this vegetation community was composed of 43 herbaceous forb species. On 23 August, summed cover decreased to 58%. This was mainly due to a 27 percentage point decrease in the abundance of forbs which had declined to 33%. Overall vine/shrub species (*Rubus* spp.) were the second most abundant growth habit and increased in abundance from 5 to 13% as the season progressed. Smaller numbers of fern, graminoid, shrub, and tree species were found on the site. Individually these growth habits were less than 8% of the observed cover.

Figure 4.16 presents the temperature, relative humidity, and photosynthetically active radiation observed on each measurement date. Indicated by the grey arrows, temperature and light level tended to increase during the sampling period while relative humidity tended to decline. Daily minimums and maximums bracketed the environmental conditions under which the gas exchange measurements were made. Conducting scheduled season-long measurements will increase the likelihood that weather patterns will affect a study spanning a three month time period. This was especially apparent with the results from the field on 13 July. A malfunction with the LI-COR 6400 IRGA was highly unlikely due to the consistency of the data before and after this point as well as the calibrations and systems checks regularly employed. It is

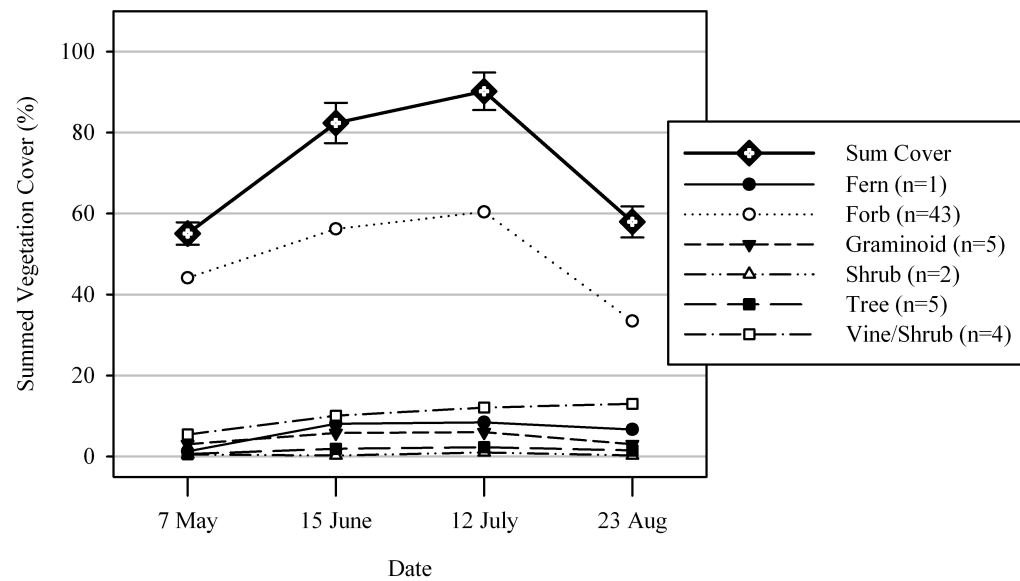


Figure 4.15 Vegetation survey results across the 2010 season. Summed vegetation cover (upper panel) is presented in the top line along with the six growth habits (fern, forb, graminoid, shrub, tree, vine/shrub) that make up this cover value.

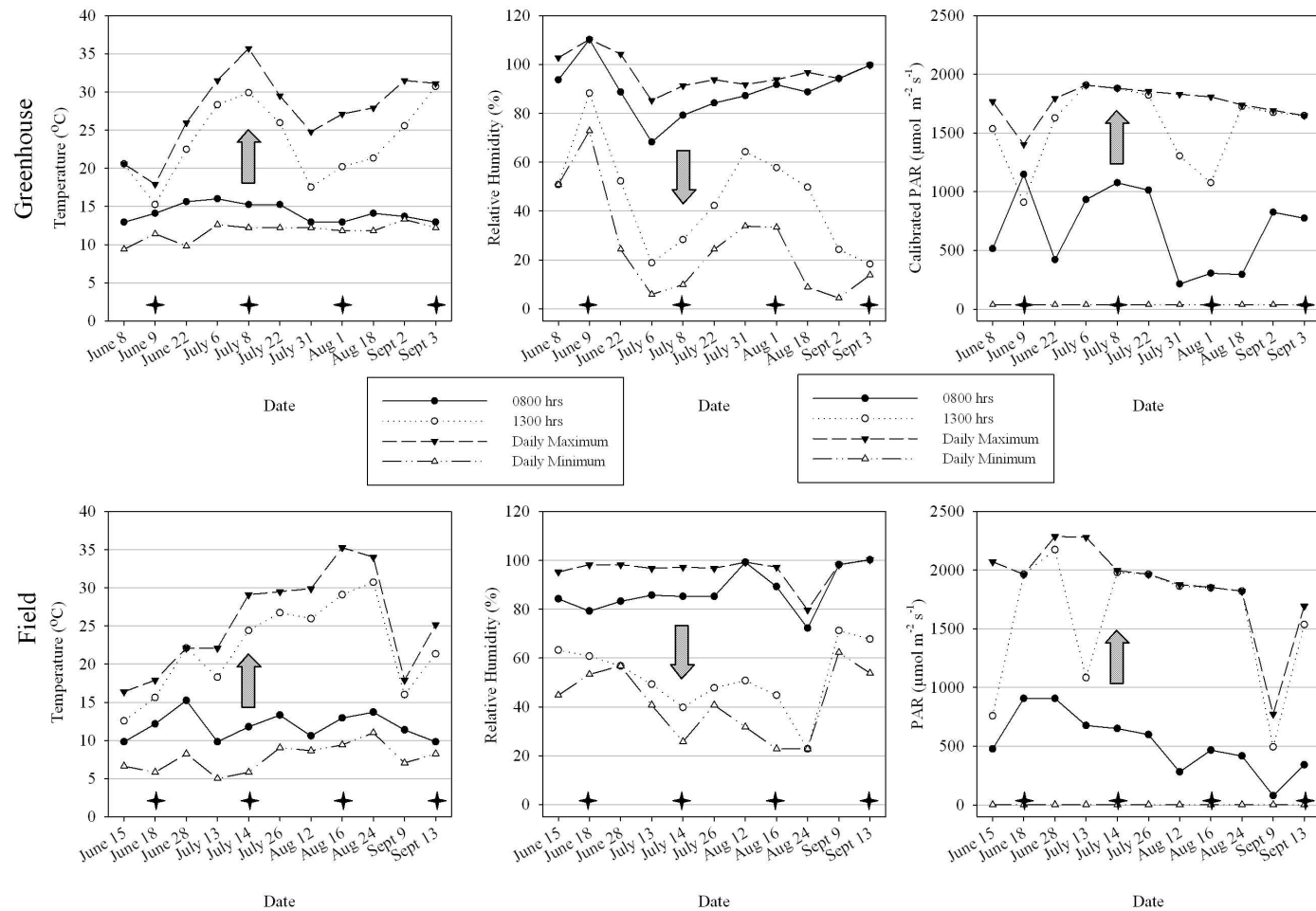


Figure 4.16 Temperature ( $^{\circ}\text{C}$ ), relative humidity (%), and photosynthetically active radiation ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) at the greenhouse and field sites for each gas exchange measurement date. Grey arrows indicate the direction of change in each environmental parameter from 0800 to 1300 hrs. The four monthly sampling dates associated with multi-leaf gas exchange and harvest are denoted with stars. Refer to methods section for the calibration used to adjust greenhouse PAR sensor values.

suspected that cool overnight conditions and low evaporative demand may have enabled CO<sub>2</sub> assimilation to proceed unhindered, minimized transpiration rates, and led to the high WUE observed on this date. Overnight air temperatures decreased to 5°C at 0500 hrs and began rising after this point reaching 10°C at 0800 hrs and 18°C at noon. Relative humidity decreased from 86% to 46% during the same time period. Cloudy conditions prevailed through the morning as shown by the low difference between PAR measured at 0800 and 1300 hrs on this day. VPD at the start of sampling was 0.17 Kpa and increased to 1.11 Kpa at the end of the measurement period. The clouds broke shortly after the measurements were finished and the maximum PAR recorded was over 2200  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . If comparisons in future studies are to be made over shorter periods of time, these kinds of weather phenomenon will be important to consider.

## 4.5 Discussion

### 4.5.1 Gas Exchange, Community Growth, and the Use of Water

The gas exchange rates (Objective 1) showed a strong tie to the perceived development of the vegetation community and are the first time these plants have been studied across a season in a regenerating PNW forest. The highest rates of gas exchange were recorded in June and early July, a time of year marked by active vegetative growth which coincided with a rise in abundance of the developing plant community. When compared to published rates of gas exchange (Larcher 2003), these photosynthetic and transpiration rates appear to be above-average for herbaceous species. However, Nkurunziza et al. (2010) reported photosynthetic rates of *C. arvense* as high as 30  $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$  at a saturating light intensity (1500  $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$ ) in a glasshouse in Denmark. McDowell (2002) presented photosynthetic capacity of *R. ursinus* can be as high as 15  $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$  by studying assimilation rates in relation to varying amounts of internal leaf CO<sub>2</sub> concentration (A/Ci curves) growing in three PNW Coast Range locations. Fioretto and Alfani (1988) studied 14 plants in the genus *Senecio* endemic to areas on or near the African continent (plus one

native to California) and found that certain species had the ability to use both C3 and CAM photosynthetic pathways. *S. sylvaticus* was not studied and gas exchange measurements were not made in a manner that would be directly comparable to the current study (Fioretto and Alfani 1988). Future research results will be needed to confirm the high photosynthetic rates of CIVU and SESY, species whose gas exchange characteristics have not been reported in the literature.

The well-watered treatment at the greenhouse demonstrated that diminishing soil water was not the primary reason for the decline in photosynthetic activity as the season progressed beyond mid-July (Objective 2). Soil water was above 31% in this treatment, a level that was greater than what was observed under field conditions. Despite this amount of soil water availability, none of the species in this treatment maintained high rates of photosynthesis across the season. Irrigation treatments had little impact on gas exchange rates and it is presumed that the difference between well-watered and droughty conditions was not enough to slow or arrest this process. It is proposed that other physiologic cues such as developmental state, changes in the internal balances of plant hormones, seed production, and/or environmental conditions such as diminishing light levels and temperature fluctuations could be responsible for this reduction in photosynthetic capacity (Fraser and Bidwell 1974; Harrington et al. 1994; Leakey et al. 2004; Larcher 2003).

Morning transpiration rates remained at relatively consistent levels despite diminishing soil water availability and appear unaffected by the droughty conditions observed in this study. SESY was the exception to this trend, predominantly due to rapid flower production and senescence unique to this annual species. The induced drought at the greenhouse was more extreme than that observed in the field as soil water declined faster and stayed in this drier state for nearly one additional month. The lack of decrease in transpiration rates across the season coupled with the declining photosynthetic activity, resulted in WUEs that decreased from the end of July to early-September.

It has been shown that transpiration rates do not necessarily decrease until certain species specific thresholds are reached. Cotton (*Gossypium hirsutum*) maintained high rates of transpiration two days into an intense drying cycle, where as the related weed velvetleaf (*Abutilon theophrasti*) showed immediate reductions to transpiration when exposed to the same conditions (Patterson 1988). Sorghum (*Sorghum bicolor*) was to be capable of maintaining high rates of transpiration until soil water decreased below 40% of the plant available water fraction (Nable et al. 1999). Sugarcane (*Saccharum* spp.), on the other hand, decreased transpiration (and growth) almost immediately as the fraction of plant available water declined from 100%. Lecoœur and Sinclair (1996) showed that normalized transpiration rates of field pea (*Pisum sativum*) were unchanged until soil water decreased below 50% of the fraction of water available to the plants.

The methods used to study these species are important to consider when discussing gas exchange rates. A combination of season-long study plants and multi-leaf gas exchange measurements were required to balance the goal of studying the developmental processes at work in these early-seral species with the need to account for leaf senescence patterns and the variability that can occur within a plant. Measurement leaves associated with season-long study plants (those in positions A and B or within the fences) had to be moved to a healthy mature full sun leaf. Continuing to statically measure lower leaves that were senescing would provide an unrepresentative view of the gas exchange capabilities of an otherwise healthy plant. The measurements presented in this paper are thus at the plant level. Studies often use a single mature full sun leaf for short-term highly-detailed photosynthetic measurements (e.g. Brock and Galen 2005) or restrict multi-leaf gas exchange measurements for the purposes of season-long analysis to tree species (Ow et al. 2010; Weng et al. 2005; Misson et al 2006; Limousin et al 2010; Xu and Baldocchi 2003; Wilson et al 2000).

Gas exchange measurements taken on multiple leaves provided a more complete understanding of plant-level physiologic mechanisms used to adapt to

seasonal development changes and drought (Objective 3). After the vertically consistent gas exchange rates during the bolt phase, CIAR, CIVU, and SESY began shunting photosynthetic activity to the upper most leaves. Kisiaki et al. (1973) found that the highest rates of photosynthetic activity of two month old tobacco plants increased with increasing height and reached a peak at leaf 25 and 27 (counting upward from the first dicot leaves). Rawson (1979) studied sunflower development reporting that gas exchange measurements were highest in the upper leaves of the plant in support of the determinate inflorescence. CIAR, CIVU, and SESY also have determinate inflorescence and it is possible that photosynthetic activity shunted toward the upper leaves supported this reproductive effort similar to results presented by Kisiaki et al. (1973) and Rawson (1979).

The declining SLA across the season and senescence of lower leaves may indicate a morphologic mechanism utilized by these species to support flowering. Taking into account the sampling methods used, the decrease in SLA revealed that these species placed relatively little effort into early season leaves when compared to those higher on the plant (Feng et al. 2008; Baruch and Goldstein 1999). These lower leaves with high SLA enable rapid carbon fixation, growth, and an increased ability to begin the process of invading new habitats (Baruch and Goldstein 1999; Gulías et al 2003; McDowell 2002). These high rates of gas exchange on lower leaves did not last suggesting that their importance was served in a relatively short period of time. In the presence of seasonal development and declining soil water, lower leaves may have been sacrificed in favor of those higher on the plant with a lower SLA that could continue to support the flowering event.

Gas exchange activity shunted higher on a plant as well as decreased photosynthetic area enable plants to balance developmental needs and resource availability (Gordon et al. 1999; Hill and Germino 2005; Kisiaki et al. 1973; Parsons et al. 1981). When lower leaves of *Phaseolus vulgaris* (the common bean) were cut, a 9% increase in net photosynthetic rate of the remaining upper leaves was found (Meidner 1970). Meidner (1970) postulated this boost in photosynthesis was partly

due to an increase in the proportion of cytokinins in the remaining leaves. Hill and Germino (2005) demonstrated that as drought persisted, *Centaurea maculosa* (knapweed) began redirecting photosynthetic activity from basal rosette leaves early in the season toward smaller cauline leaves on the stem and finally to the photosynthetic stem tissues. Allred et al. (2010) reported declines in SLA in the presence of drought for two plants, *Ambrosia psilostachya* (forb) and *Andropogon gerardii* (grass), native to tall grass prairies in North America. *Pteridium aquilinum* and *Calluna vulgaris* growing in a controlled setting in the United Kingdom were found to reduce shoot growth and hence lower transpirational demand in the presence of drought (Gordon et al. 1999). During a two year drought in California during the mid-1970's, *Arctostaphylos viscida* sacrificed up to 90% of the branch area in order to maintain the long-term survival of a plant (Parsons et al. 1981).

Further support of this concept of shunting physiologic activity has been reported for *Senecio sylvaticus* which utilizes a fibrous root system to capture the majority of nutrient resources necessary for annual growth early in the season. These resources are then redistributed to leaves and inflorescence through the course of development (van Andel and Vera 1977; van Andel and Jager 1981; Antos and Halpern 1997). While nutrient content of the biomass components were not measured in the current study, the biomass partitioning data of SESY and CIVU support this concept with the consistent decline in root mass fraction and overall increase in emphasis placed on above-ground growth.

At later dates in the season, it was observed that the leaves and occasionally tops of the plants were dead and the only remaining live tissues were stems. Gas exchange of the stems was not measured in the current study, but each of these species may have the ability to photosynthesize from this tissue. Stems, carpels, and even flowers can support a portion of the assimilation required for plant growth (Aschan and Pfanz 2003; Bazzaz et al 1979; Fioretto and Alfani 1988; Galen et al 1993; Hill and Germino 2005; Nilsen et al. 1993). Future research into the capacity of stems for the four study species may be required to quantify the contribution of this plant part to

the overall carbon budget of these plants. Research will also need to be conducted across years and other species to confirm these physiologic and morphologic trends within these study species occurring in young PNW forests.

While it was possible to detect trends in soil moisture on the sites, the Hydrosense TDR probe was not capable of detecting species differences in soil water usage (Objective 4). Soil water in horizons near the surface have been shown to dry to low levels in the presence of certain plant species that possess different abilities to exploit this resource (Gordon et al. 1999; Hill et al. 2006). By taking measurements vertically, the sensor averaged the soil moisture over the length of the probe, in this case 20 cm. This provided a quantification of soil moisture in the upper soil horizon(s), but did not necessarily characterize the entire volume of soil exploited by the plants or how this may change with depth (Bates and Hall 1982). Irrigation water at the greenhouse was supplied by one centrally located drip emitter and this application method could have reduced the ability of the Hydrosense TDR sensor to detect species differences. The vegetation community growing with the study plants in the field made it difficult to detect which plants were utilizing soil water. A different study design, no irrigation, and sensor readings taken horizontally could all be used in future studies to understand if individual species use of soil water can be detected.

#### 4.5.2 Tissues Perpetuating a Species

The physiologic and morphologic results presented in this study illustrate different strategies used by these species to produce perennating tissues or those plant parts that will survive and perpetuate the species (Harper and Ogden 1970). SESY invested the largest portion of its resources into the production of seed, similar to species that avoid drought (Bell et al 1979). Bell et al. (1979) found investment in roots declined as reproductive effort increased during the winter and early spring for eight annual species growing in the Mojave Desert, all species that produced seed prior to the extreme summer drought period. In addition to the primary determinant

flowering event, SESY flowered in smaller secondary flushes from peduncles that extended out of leaf axils (personal observation). A similar observation was made by Harper and Ogden (1970) who studied *Senecio vulgaris* and found that the species invested about 21% of the net energy budget toward the production of seed. It was proposed that these secondary flowering events constituted a rejuvenating system allowing a plant to grow and reproduce following damage.

CIVU is a biennial and spends the first year in a rosette stage storing carbohydrates in its tap root (Michaux 1989). In the second season, CIVU withdraws carbohydrates from this taproot as well as a large amount of site resources (Randall and Rejmánek 1993) in the initial stages of bolting, leaf production, and flowering. The perennating tissue of this plant is the seed as individual plants die upon the completion of flowering. These second-season morphologic traits of CIVU were illustrated with the presented biomass partitioning data. CIAR placed a lower effort into floral production (when compared to CIVU) and a consistent investment in roots. Both of these tissues will perpetuate CIAR in future growing season(s). The consistently lower amount of root mass fraction for CIAR observed in the field was primarily due to the digging methods and difficulty extracting the root mass when compared to the ease of the process with a potted plant.

RUUR plants invested in both above and belowground growth. When compared to the length of the canes, gas exchange measurements varied little from the point of contact with the ground toward the apical meristem. Biomass partitioning data presented here contrast slightly with those provided by McDowell and Turner (2002) who reported that the biennial canes spend the initial year elongating and the second year flowering and producing fruit. Whether it was through seed germination or resprouting following herbicidal degradation, RUUR plants included in this study had canes less than 1 meter in length, indicating that they had indeed grown in 2009. In keeping with McDowell and Turner (2002), the species flowered in 2010. This effort (including fruit production) was small, however, as the plants spent the majority of the study period growing vegetatively. It was possible that the act of digging, root

disturbance, response to the vegetation management regimes, or perhaps flowering may have triggered a change in the internal hormonal balance stimulating vegetative growth in 2010 (Kramer and Kozlowski 1979). An alternate and perhaps simpler explanation for the lack of sexual reproduction may be that the highly palatable fruit of RUUR was eaten by wildlife between sampling periods.

#### 4.5.3 Competition, Physiology, and Silvicultural Perspectives

In the early stages of forest stand development, these four study species utilize the discussed physiologic and morphologic mechanisms to access site resources and compete with planted tree seedlings. Photosynthetic rates and biomass partitioning data demonstrate the ability to fix carbon, grow, and produce perennating tissues according to unique species patterns. High transpiration rates and low water use efficiencies during a time of year with low soil water availability indicated that certain plants continued utilizing this resource after the essential developmental processes were finished. When these developmental and water use patterns are compared to those of conifer trees, a physiologic basis for how these plants compete for resources during the early period of forest establishment can be demonstrated.

These four plant species did not show the same response to declining water availability as those found by other researchers for conifer species. Havranek and Benecke (1978) reported that transpiration of Swiss pine (*Pinus cembra*) declined immediately when exposed to drying soils. In the presence of drying soils, Douglas-fir (*Pseudotsuga menziesii*) transpiration rates were immediately reduced (Lopushinsky and Klock 1974). Photosynthetic rates for Douglas-fir remained unchanged so long as xylem water potential was above a species specific threshold of -1.0 MPa (Brix 1979). Once this xylem water potential threshold is breached, assimilation rates and morphologic development of Douglas-fir seedlings can be negatively impacted (Brix 1979; Dinger and Rose 2010). The four study species, on the other hand, were either able to avoid drought stress by completing their lifespan within a time of year when resources were relatively plentiful (SESY), modestly adjust

water use efficiency in the early-season (RUUR), or tolerate drought by shunting resources to actively growing tissues (CIAR, CIVU, and RUUR). Regardless of the strategy, each of these study species utilized water during the season and may contribute to the inhibition (Connell and Slayter 1977) of the crop species.

These data illustrate, from a uniquely physiologic perspective, a fundamental reason why vegetation management regimes are applied in tandem using both pre- and post-planting applications of herbicides during the early year(s) of forest establishment. Species like RUUR are often controlled by the application of a fall site preparation using chemicals sprayed prior to planting as time must elapse allowing herbicidal activity to degrade and Douglas-fir trees to grow unhindered. The application of a spring release following a fall site preparation is designed to maintain low amounts of competitive cover by reducing herbaceous species that germinated in the late-winter and early-spring. CIAR, CIVU, and SESY are examples of common species targeted through the application of this silvicultural treatment.

Given the data from this study, a priority ranking system of competitive ability may be desired to aid in the judicious use of these regimes and their chemical components. The challenge with this approach is that the outcome of the ranking system would depend greatly on the parameter used as the criteria. As an example, if this competitive ranking was based on the maximum rate of photosynthesis, it would be  $CIAR > SESY > CIVU > RUUR$ . If the basis for comparison was changed to a calculation of transpiration based on leaf area across the season, the order would shift to  $RUUR > CIAR > CIVU > SESY$  (calculation not shown). Thus, a ranking system with any real field applicability would require site specific abundance data and research that includes resource thresholds these plants need to survive and grow.

Economic and ecologic efficiencies that may result from the integration of physiologic data into silvicultural prescriptions will depend greatly on the composition of the vegetation community. Logically, if high amounts of CIAR invade a site, the high photosynthetic rate, rapid growth, investment in sprouting root tissues, as well as continued transpiration despite drying soil conditions would make it a priority for

targeted control. CIVU has been shown to be more competitive with ponderosa pine (*Pinus ponderosa*) in the second year of its lifespan when compared to the first (Randall and Rejmánek 1993). The transpiration rates observed in this study in conjunction with this work by Randall and Rejmánek (1993) indicate that it may be possible to delay treating a site until the spring of the second year, if this species was present in large amounts. RUUR may invade a site due to its presence prior to harvest and is best targeted with an initial fall site preparation. SESY has an erratic, intense, and ephemeral invasion strategy but, if the abundance was high (e.g. over 20%) in the late-winter or early-spring, controlling this species may be necessary to avoid the high use of soil resources in the spring and early-summer.

#### **4.6 Conclusions and Management Implications**

Two study sites, carefully controlled conditions, standardized measurements, and some new techniques enabled data to be collected across a season on plants that have been previously unreported in forest regeneration literature. Detailed physiologic information on gas exchange as well as biomass partitioning has shown how these species grow during a season and in a region marked by a prominent summer droughty period. While the study plants are only four species among a complex assemblage of plants, their behavior in the current study illustrated fundamental physiology of gas exchange and how resource availability affected this process. The selected species represent subtle differences in what management prescriptions may be used to minimize negative effects on planted tree seedlings.

Moore et al. (2007) asserted that information focused on the goal of increasing management efficiencies are needed to maintain a strong forest industry in the PNW. The authors noted that there has been an overall decrease in the number of regional studies designed to achieve this objective in the last 20 years. Continued work using designed ecophysiological studies such as this one will be needed to provide better understanding of the fundamental mechanisms of competition across different yearly weather patterns, plant species, and sites in the PNW. It is from this detailed

level of understanding that best management practices will continue to be refined enabling the targeted control of species accounting for both the economic and ecologic aspects of efficacy (Zimdahl 1988).

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## CHAPTER 5.0

### SYNTHESIS OF DISSERTATION

Harvesting a forest creates a disturbance that dramatically alters light conditions, water availability, and scarifies surface soil promoting the development of early-seral vegetation according to secondary successional trends. When a new stand of rapidly growing trees is a desired outcome, vegetation management regimes are often employed to interrupt these successional processes and favor crop trees. Land harvested in the late-spring and summer presents a unique challenge. Harvesting operations may finish at nearly the same time that early establishment activities would normally begin making common management strategies seem out of phase with the vegetation community. A land manager may choose to delay forest establishment introducing a fallow year in the hopes of improving long-term forest growth. A side by side study was conducted to compare six potential responses to this situation. After statistically adjusting for the initial size of seedlings planted across two years, trees planted following a fallow year were smaller than those planted immediately after harvest. While seedlings were found to be growing at the same rate in height and diameter, size differences three years from harvest indicated that each growth year was important. Sulfometuron methyl added to a fall site preparation tank-mix did not have a negative affect on seedling growth. Delaying the establishment of the next stand simply lengthened the amount of time associated with the early stage of forest development unless weather patterns and site characteristics combined to thwart reforestation efforts.

Equally important to the tree growth is understanding the tradeoffs associated with the vegetation community's response to silvicultural regimes. Fall site preparations in conjunction with spring release treatments during the first year reduced cover below 10% irrespective of the year applied. Adding sulfometuron methyl to the fall site preparation tank-mix did not statistically reduce competition in the year

following application when compared to a similar treatment regime without the chemical. In the year(s) following treatment, herbaceous plants were quick to colonize. Woody/semi-woody plants were slower, but steadily increasing in abundance. Applying only a spring release treatment may minimize the number of treatments, but it did little to control the development of woody/semi-woody plant species. Depending on physical stature and proximity to crop trees, these plants could become a hindrance in future years due to continued competitive interference.

Collecting spatially explicit graphical data on plant community development was made possible through the use of ground-based stem mapping procedures and pixilated vegetation survey maps of experimental plot conditions. Stem mapping procedures were used to develop relative positional data during a time of forest growth with unhindered line-of-site. Tree data can then be updated for the life of the stand and visual images of conditions presented through the Stand Visualization System developed by the USDA Forest Service. Utilizing features of the experimental units themselves, coarse whole-plot vegetation surveys were conducted and compared to more traditional survey methods. Results showed a close fit between the two methods of presenting forest plant communities and graphics made in Sigmaplot® clearly showed plant community dynamics. Future avenues for this research could include aspects of forest planning and carbon modelling, spatial analysis at various scales, as well as the study of community ecology and site resource use.

Of great importance to the continued understanding of the dynamic principles involved in the management of early-seral environments is detailed physiologic information on weedy plant species. The effect of competition for limited site resources has been well-quantified for forest tree species, but little regionally relevant work has been done to define the seasonal physiology of common competitive species. Investigating autecologic strategies from the perspective of season-long gas exchange as well as biomass development aided in the understanding of how *Cirsium arvense*, *Cirsium vulgare*, *Rubus ursinus*, and *Senecio sylvaticus* successfully colonize disturbed forest sites in a region marked by a pronounced summer drought period.

Species achieved various peaks in CO<sub>2</sub> assimilation rates prior to mid-July, but decreased differently as seasonal development progressed. Transpiration rates declined for SESY after the annual species flowered while the remaining species showed more consistent rates across the season despite droughty conditions. The combination of multi-leaf gas exchange and biomass development data show that these species have physiologic and morphologic abilities to shunt activity to plant parts that will perpetuate the species even in the presence of diminishing soil water. Overall, these results demonstrated a physiologic basis for competition as well as a rationale for applying vegetation management regimes.

The studies contained in this dissertation have sought to expand the current state of silvicultural knowledge by characterizing specific competitive mechanisms during early forest establishment. Developed methods as well as the results produced from these studies assist with the description of the complex interactions that occur among species within PNW forests. Evaluating tradeoffs associated with silvicultural decisions has provided practical decision support tools that account for both treatment efficacy and the need to minimize environmental costs. Alternate methods of quantifying and presenting forest plant communities with spatially explicit data opens new avenues of ecologic research. Describing the fundamental physiology used by certain plants to colonize open conditions in PNW forests has just begun.

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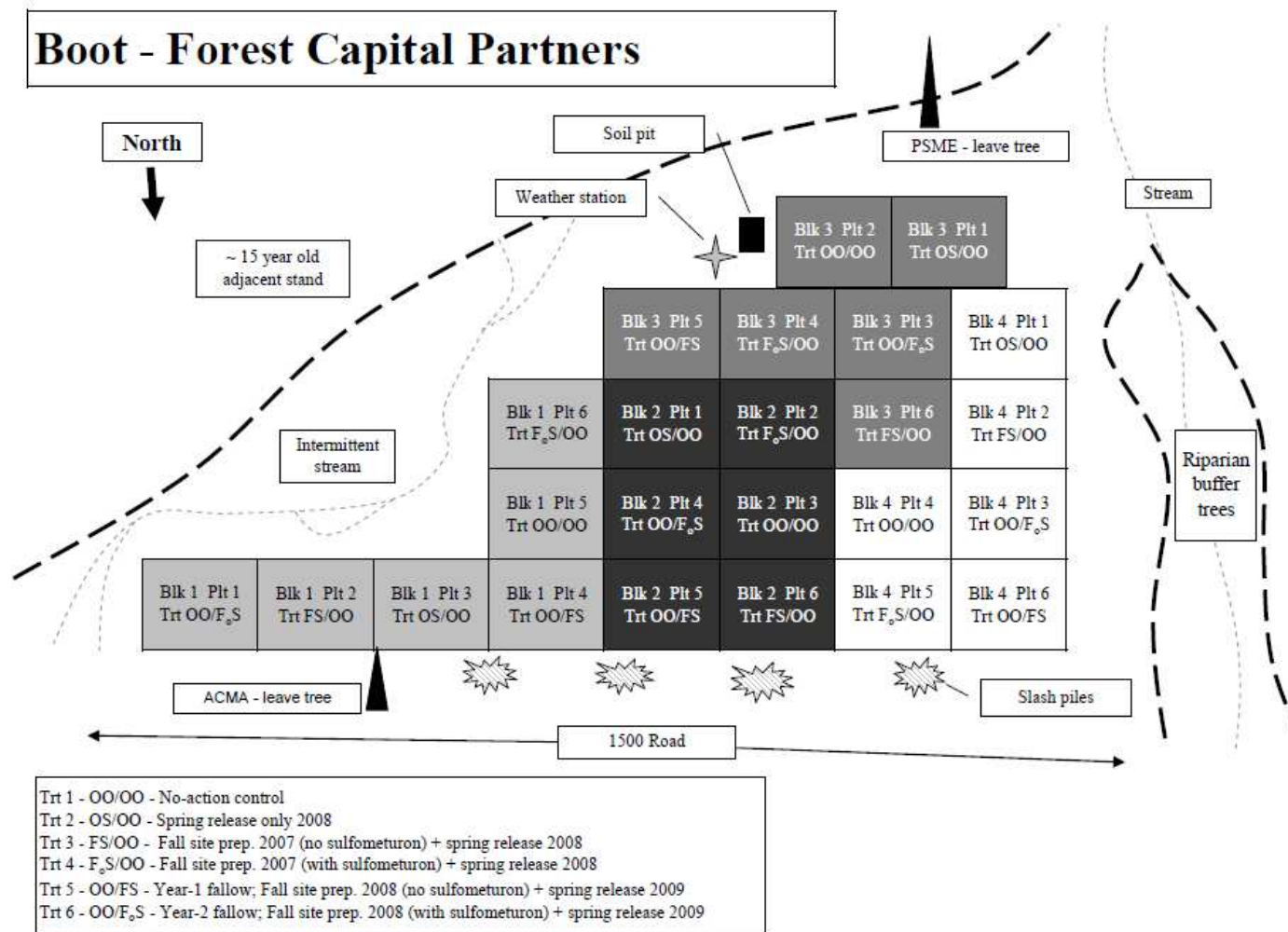
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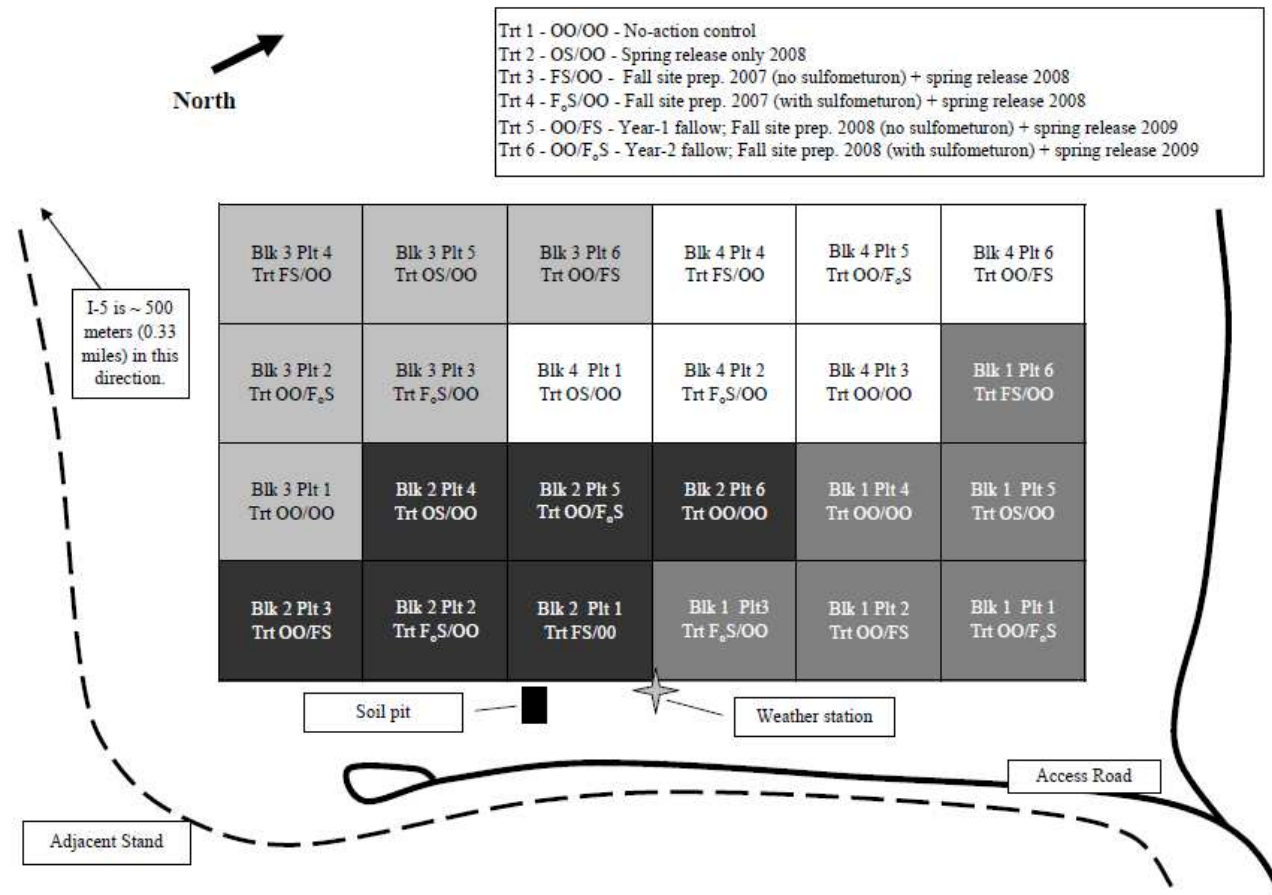
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## **Appendices**



Appendix 1a – Boot study site map.

## Jackson Mast - Lone Rock Timber Co.



Appendix 1b Jackson Mast site map.

## Appendix 2 – Instructions and code for vegetation data management

*Summary: In order to compare vegetation communities which have a variety of species present, it becomes necessary to have a common “dummy” dataset. Vegetation survey data is used to create a common species list unique to a site. A “dummy” dataset is created that inserts this species list into each subplot. The real cover values for each plant species are then inserted in a separate process. Plants not found in the vegetation survey are given zeros (see Step 12). This “dummy” dataset is created using Microsoft Excel and Access then merged with the actual vegetation survey in SAS. This “dummy” dataset can be updated as surveys are done over successive years. There may be other techniques for doing this kind of work, but I am unaware of a single publication describing how to do this process or why certain components are important.*

*Credit for the initial framework of this process belongs to my good friends Michelle Buonopane (USDA Forest Service, botanist) and Lee Rosner (former VMRC Research Assistant). Over the years, I have continued to work with this process and develop additional approaches to analyze vegetation communities. The original instructions fit on one piece of paper and may have been forever lost (In 2008, Michelle asked me to write down what I remembered and send it to her). The detailed instructions that follow are designed to walk the reader through the process.*

Step 1: Conduct the vegetation survey. It is easiest to learn and use the four letter species codes common to USDA protocols. The first two letters indicate the genus while the second two represent the species. For example, *Cirsium vulgare* is CIVU.

Step 2: Enter the data into an Excel spreadsheet. Be sure the data is in columns with unique names in the first row. Access and SAS require data to be organized this way.

<u>Block</u>	<u>Plot</u>	<u>Subplot</u>	<u>Species</u>	<u>Cover</u>	
1	2	3	TOTAL	85	
1	2	3	CIVU	35	
1	2	3	RUUR	20	...etc.

Note: It is important to have clean data. This means only data is in the columns and there are no extras (see Step 5). Calculations or notes in other columns need to be in a different worksheet or file. The best technique is to actually delete the columns to the right of your data and below (if anything was added to any other cells) so that there is nothing and never was anything in these columns (see step 6 below). Neither program “sees” comments in cells so this is an acceptable way to write notes. Also, if a comment column is used, do not put spaces within a cell. Certain programs like Access and SAS can have difficulties importing these kinds of data.

Step 3: Open Microsoft Access and create a new file by clicking on the “create a new file” link then “blank database” (both of these are on the right portion of the screen). Name and save the database as prompted. After this point, there is no real way to “save” the work you do in Access. The program is set up to do it automatically so any time a database is imported, it will save the changes when the program is closed.

Step 4: A small window (the main dialogue box) will open up that has Tables, Queries, Forms, etc. down the left-hand side. Right-click on the “Tables” button and select “Import...” This opens up a secondary screen designed to help identify the file to be imported. The default is to accept Access files so change the “Files of type:” at the bottom to “.xls.” Use the browse feature to select the file and click “Import.”

Step 5: A secondary screen will come up titled “Import Spreadsheet Wizard.”

1. Select the appropriate Excel worksheet, click next at the bottom of the screen.
2. It will then ask if the first row contains column headings (which it should) so check this box if it is not already checked. Hit next.
3. The next screen allows certain “fields” (aka columns) to be skipped. This is where in Step 2, adding extra information can add extra columns. The program recognizes columns that have or once had data. Click and skip them, if needed. When finished, click next.
4. It will now ask if it should “add a primary key.” Click no then next.
5. Name the file in a simple logical manner. For example, if the survey was done in July 2008 at Boot, one idea would be BTjuly08. Click “Finish” and the program will say it has finished importing file X. Click ok.

At this point, the only window open should be the main dialogue box. Double click on the imported file name and it should pop up allowing the data to be seen. If it looks good, proceed with step 6 below. If something went wrong, highlight the file name (left click once) then right-click on it and select “delete.” Start over with the Import wizard (Step 4 above).

Step 6. A query allows the creation of a new dataset from existing data imported into Access. Two datasets will be made using this function; a plot list and a species list. The plot list creates unique address labels for each vegetation survey. For example, the block number would be analogous to the state, plot (or treatment) would be the city, and the vegetation subplot is the street address. So if I have four blocks, six treatment plots in each block, and four subplots in each treatment plot, I should have 96 different “addresses.”

### Make a plot list

1. Left-click on queries (just below Tables on the left-hand side of the dialogue box), left click on New (at the top of the main dialogue box), and a small dialogue box pops up. “Design View” should be highlighted and hit “ok.”
2. Two dialogue boxes open simultaneously “Query” in the back and “Show Table” in front. “Show table” is asking what dataset(s) should be used (at this point there should be only one but there will be more in the future). In the “Show table” window double click the file name (or highlight and click add). This will open a list of column headings inside the Query window. Click “close” in the “Show table” window. The Query window will now be active.
3. Double click each of the features in the “address” to put them in sequential order on the lower half of the query window. This might be “Block” then “Plot” then “Trt” then “Subplot.” If attributes were named differently or a different experimental design was used, follow the address analogy.
4. On the main toolbar of Access there is a black sigma symbol ( $\Sigma$ ). Clicking this button creates a “sum” that tells Access each address is to remain unique. Click the sigma (if it is not clicked, it will provide the entire dataset).
5. Click the “Query” tab on the top toolbar and scroll down to “Make Table Query...” A new dialogue box pops up and asks for the name of the new table. Name it something simple like “plot\_list” and hit ok. Note: by default, the box should say ‘current database’ which is correct).
6. The last step to make a plot list is to click the red exclamation point which is the “run” button telling Access to run the query just designed. It is located to the left of the sigma on the upper tool bar. The program will explain the number of rows about to be pasted into the new table. Basic math is a good check of this number (e.g. 4 blocks x 6 treatments x 4 subplots = 96 rows).

To avoid confusion, it is best to close the query window. Doing so will bring up a message that says, “Do you want to save changes to the design of query ‘Query1’?” Click no and go ahead and close it. Then click on the Tables button (left-hand side) and open the new dataset you created to make sure it looks right (this is one method that can be used to spot data entry errors).

### Make a species list.

1. Open a new query (query, new, design view) and double click the vegetation survey you imported in the secondary dialogue box (it should go away leaving only the larger query dialogue box active).
2. Double click ONLY “species” and hit the sigma ( $\Sigma$ ) symbol. Again, this is telling Access you want each species to remain unique. Go up to the “Query” tab and scroll down to “Make Table Query...” Name it “species” and click ok. Click the red exclamation point, close the query, and look at the table.
3. This is where checking datasets for errors becomes easy. Look down this species list and make sure each species code is correct. For example, there is no species with the code RUUr2. It is probably a typographical error for

RUUR. Normally you can spot errors quickly with this comparison. When found (notice it is not an “if” as typographical errors are common), open the original dataset in Excel and search for that specific mistake (In Excel: on the top toolbar click the Edit tab, scroll down to “Find...”, type in the WRONG species code (e.g. RUUr2), click find. It is a good idea to look at the original paper datasheets and make sure a typo is really a typo before anything is changed. Correct all of the errors in the Excel file, save, and close it.

If any corrections were made to the Excel version of the vegetation survey, the survey and species list in Access will need to be deleted (perhaps even the plot list if this had a mistake as well). Delete the incorrect versions in Access and re-import the corrected vegetation survey. Then remake the necessary datasets following the procedures above. Check over the new versions of these datasets and if needed, repeat the editing process (it can take several cycles to hammer out mistakes). Think of it as an iterative process that develops patience. ☺

Note: It is not necessary to close Access while cleaning datasets in Excel.

Step 7. Export the species list from Access as an Excel spreadsheet (name it species.xls). To do this, right click on the “species” database (the species list) and scroll down to “export...” Change the “Save as type” to the latest version of Excel, select a place to save it, and name it. Hit “Export” and open up the file using Excel.

Note: This is when more information on each species can be added. It creates the foundation for the depth of dummy datasets. Make two versions of this form on two separate Excel worksheets and name them “details” and “dummy.” The first, “details,” is used as a reference that includes all the species names spelled out, family, diagnostic characteristics, etc. The second, “dummy,” is the one that will be used in Access to create the dummy dataset. Add columns of information that will be helpful (to both if this is preferred but definitely the second one “dummy” that will be used in Access). It will have the species codes plus information like “Habit” (F=forb, Fe=fern, etc) or “Duration” (A=annual, B=biennial, etc). Look this information up carefully and consider what was found during the vegetation survey. Insert all the information required into distinct columns.

Step 8. Copy the entire dataset (all the columns of information in the worksheet made for Access) and paste it below the same number of times that the experiment has replicates. If there are four replicates (i.e. blocks) on the site, there will be four repeats of this data. Add a new column to the left of this data and type “Block” into the first cell (do not type in the quotes). In the cell below “Block,” type a 1 and drag it down to the end of the first species list. At the beginning of the second repeat of the species list type in a number 2 and drag it down to the bottom of this species list. Repeat until all of your replicates of the species list have an associated block number. If all is well, save this Excel spreadsheet and close it.

Step 9. Back in Access, import the species.xls dataset with all the plant information that was just created (remember: Tables, import, and follow the wizard). Name this dataset something like “spp\_detail” so it is known that this is the one with all the extra information. Open the file and make sure it is correct.

Step 10. The right data is now in Access that can be used to make a dummy dataset.

1. Go to Queries, new, design view, click ok.
2. Double click on “plot\_list” **first** then double click on “spp\_detail.” Close the “show table” window. The query dialogue box will now have two windows on the left side of the upper half. The first is the plot list and the second is the detailed species list.

Carefully follow these next steps:

3. In the plot\_list box, double click **block** then **plot** then **treatment** then **subplot** (in that order and from that box only; adapt as needed for the study design).
4. From the spp\_detail box, double click **species** then each heading corresponding to the additional information (it may look something like **origin**, **duration**, **habit**). **DO NOT CLICK BLOCK FROM THIS BOX!**
5. Left click and hold block in the “plot\_list” and drag it over to the block in the “spp\_detail.” Do not worry if it is not lined up perfectly, just drag block FROM the “plot\_list” TO the “spp\_detail.” When the left click is released, a small line will appear as a link between the two boxes.
6. Place the cursor over that line and right click (it is sensitive so be precise). Select join properties. This brings up the “Join Properties” dialogue box. It should say left table “plot\_list”, right table “spp\_detail”, and that the left and right column names are both “block.” Select #2 and hit ok. As I understand it, this is telling Access that the information from plot\_list is to be joined with that from spp\_detail using block as the common element between the two. A small arrow head will appear on the line after #2 is selected showing that indeed, the two boxes are linked.
7. Go up to Query on the top tool bar, scroll down to Make Table Query..., and name the query something like “Dummy08.” Hit ok.
8. Do a little math before hitting the red exclamation point. As an example, if there are 96 addresses and 102 species in the species list, there should be 9792 lines in the new “dummy” dataset that will be created. Go up to the red exclamation point and click it (do NOT hit the sigma first, that is only for the creation of a plot and species lists). A box will pop up that says, “you are about to paste 9792 row(s) into a new table” if this is correct, click ok. If not, figure out what is wrong and correct the problem (normally this means going back through the steps above).
9. Go ahead and close the query.

Step 11. Export the dummy dataset as an Excel file to a designated folder with a unique name. Close Access, take a deep breath, and smile. Few people know how to do this and you are now one of them.

Occasionally data will be taken on a study site over the course of many years. The species found at the beginning of the experiment will not necessarily be there in the years that follow. Other species will show up as the time progresses. It may be desirable to make one all-inclusive species list for that site. The easiest way to do this is to take the corrected vegetation surveys (the versions that have been checked using the Access “species” database) and put them into ONE Excel spreadsheet. For example, the vegetation survey data at Boot would start with Sept07 and pasted immediately under it would be July08 then July09, etc. Add a column to the left and put in the appropriate date for each survey. In an effort to be clear, this means columns will look like this:

<u>Date</u>	<u>Block</u>	<u>Plot</u>	<u>Subplot</u>	<u>Species</u>	<u>Cover</u>
Sept07	1	2	1	Total	85
...					
July08	1	2	1	Total	100 ...etc.

Save this file as something like “Boot\_vegsurvey\_all.xls” and import the file into Access. Then follow the above procedures to create a total species list. This all-inclusive species list will provide the total number of unique species on that site. This is another great way to catch data entry errors.

Step 12. Open a SAS workbook. I have included the code that used to work with all of this vegetation data. This is by no means exhaustive, but it may serve as a foundation that can help get analysis started.

### Importing Data

There are many ways to import data. I prefer to use the import wizard in SAS due to the size of these files. Every once and a while, SAS does not like Excel files so try saving them with a “.prn” extension and import them using the “Infile” code below. It is important to remember that the “input” statement must specify which columns need to be included. SAS assumes everything is numbers and will not import columns if it is not told which have letters using a dollar sign (e.g. Trt is a letter so it is Trt \$). I print almost every step in the process. Turn this off by preceding each proc print with an asterisk.

```
Data BT_July08;
infile 'C:\VMRC\Dinger Thesis\PhD Dissertation\Chapter 02 -
Delayed\Data\July08_veg_survey.prn' firstobs=2;
input Blk Plt Trt $ Species $ Cover; run;
*proc print data=BT_July08; run; quit;
```

The code that follows is developed using the Import Wizard (File, Import, and follow the instructions). I save this code in a separate file, open it, copy the code, and paste it into my SAS window. Import both the vegetation survey and the dummy dataset.

```
options nodate linesize=75 formdlm='.';
PROC IMPORT OUT= WORK.BT_July08
  DATAFILE= "C:\VMRC\Delayed Planting Study\Boot\
Vegetation Surveys\03_BT_Veg_Survey_July_2008.xls"
  DBMS=EXCEL REPLACE;
  SHEET="July08$";
  GETNAMES=YES;
  MIXED=NO;
  SCANTEXT=YES;
  USEDATE=YES;
  SCANTIME=YES; RUN;
proc print data=BT_July08; run; quit;

PROC IMPORT OUT= WORK.dummy08
  DATAFILE= "C:\VMRC\Delayed Planting Study\Boot\
Vegetation Surveys\BT_dummy08.xls"
  DBMS=EXCEL REPLACE;
  SHEET="BT_dummy08";
  GETNAMES=YES;
  MIXED=NO;
  SCANTEXT=YES;
  USEDATE=YES;
  SCANTIME=YES; RUN;
proc print data=dummy08; run; quit;
```

### Sorting Data

Prior to merging data, a sort is required. Data in spreadsheets is not always in an order that is logical to the computer software so sorting based on the “by” category allows SAS to put everything in numerical and alphabetical order. IMPORTANT: the two files that are to be merged must be sorted in the same order!

```
proc sort data=BT_July08;
  by block plot trt subplot species;
proc sort data=Dummy08;
  by block plot trt subplot species;
```

### Merging Data

The first line (data) is a step telling SAS the name of a new file that is being created. The merge statement names which files that are going to be joined while the “by” statement tells SAS how they will be merged. The vegetation survey has all of the data while the dummy is a rigid framework that allows comparison across different plant communities. The “if then” statement is telling SAS to enter a zero everywhere there is no data. When a species that was in the dummy dataset does not show up in the vegetation survey, SAS automatically enters a period then changes it to a zero (due to this code).

```
data BT_July08_merge;
  merge BT_July08 Dummy08;
  by block plot trt subplot species;
  if cover =. then cover=0; run;
proc print data=BT_July08_merge; run; quit;
```

### Creating Summed Cover

As a part of vegetation survey protocols, the VMRC always takes total cover out of 100%. Plant community development often leads to overlapping vegetation making “summed cover” a more representative measure of the community especially after several years of development. Summed cover is simply the cover associated with each species found in a subplot added together. It can exceed 100%.

This first code is telling SAS “for each subplot I want all the species cover values added together but exclude total, stump, and logs.” During the vegetation surveys, data is often collected on a stumps or logs that take up a significant portion of a subplot. For the purposes of developing “summed cover,” these components of the site are not included. Note: the SAS code for “not equal to” looks like ^= ‘TOTAL’.

```
proc sort data=BT_July08_merge;
  by block plot trt subplot;
proc means data=BT_July08_merge noprint;
  by block plot trt subplot;
  title 'July08 sum cover';
  var cover;
  where (species ^= 'TOTAL') and (species ^= 'STUMP')
  and (species ^= 'LOGS');
  output out=July08_sum_cover sum=sum n=n;
proc print data=July08_sum_cover; run; quit;
```

The next step is to sort the sum cover file that was just created and ask SAS to provide a mean for the experimental unit. In this case, SAS is providing the mean of the four subplots (made in the step above) associated with each experimental unit on the site (there will be 24 numbers in this case).

```
proc sort data=July08_sum_cover;
  by block trt;
proc means data=July08_sum_cover noprint;
  by block trt;
  var sum;
  output out=July08_avg_sumcover mean=mean stderr=se n=n;
proc print data=July08_avg_sumcover; run; quit;
```

### Creating a dataset based on a specific criteria

One interesting aspect of analyzing summed cover is that the number can be regrouped into various categories depending on the needs of the project. In other words, the parts add up to the whole. The next example demonstrates how information included on plant habit (forb, fern, graminoid, shrub, tree, or vine/shrub) can be used to understand

how the treatments have influenced plant community development based on these categories. Note: this code is designed to make a figure. If analysis is to include a randomized complete block design, the code would need to include “block” in the by statement.

```
proc sort data=BT_July08_merge;
  by trt species habit;
proc means data=BT_July08_merge noprint;
  by trt species habit;
  var cover;
  output out=BT_July08_hab mean=mean stderr=se n=n;
proc print data=BT_July08_hab; run; quit;
```

Based on the six growth habits, the code is creating a mean cover for each species that could have occurred in each treatment (n=16, 4 subplots in 4 treatment plots for this example). The second part is summing these mean species cover values based on the six growth habits. This is done by the “where” statement and the sum=sum code in the output line.

```
proc sort data=BT_July08_hab;
  by trt habit;
proc means data=BT_July08_hab noprint;
  by trt habit;
  var mean;
  where habit='F' or habit='G' or habit='Fe' or habit='V/S' or
habit='S' or habit='S/T' or habit='T';
  output out=BT_July08_h sum=sum stderr=se n=n;
proc print data=BT_July08_h; run; quit;
```

### The most abundant species

Sometimes it is valuable to be able to find what species are the most abundant in a particular treatment regime or across the site. The first portion of code creates a mean cover for each species that is strictly diagnostic in nature. Then when this dataset is printed, the code is specifying “only print species where the mean is greater than 2 and NOT total, stump, or logs.” This is an iterative process. Start with a mean greater than 1 and see how big the list is. Increase the number until you get a satisfactory number of species. For example, many people want the “top ten” while others just want the top three. Write these species codes down.

```
proc sort data=march_merge;
  by trt species;
proc means data=march_merge noprint;
  by trt species;
  var cover;
  output out=march_top_spp mean=mean stderr=stderr n=n;
proc print data=march_top_spp;
  where mean>2 and (species^='Total') and (species^='stump') and
(species^='logs');
run; quit;
```

Now that the top species are known, it is possible to come up with means for these plant species. The following code creates that mean based on the species found through the process above. The codes are written in quotes. Here are some helpful suggestions: Boolean logic is important so pay attention to the difference between ‘and’ and ‘or’, SAS is not case sensitive BUT, if something is put in single quotes (e.g. ‘Total’), SAS is extremely case sensitive.

```
proc sort data=BT_July08_merge;
  by trt species;
proc means data=BT_July08_merge noprint;
  by trt species;
  var cover;
  where species='SARA' or species='POMU' or species='RUUR' or
species='SESY' or species='PREM' or species='CIVU'
(species^='Total') and (species^='STUMP') and
(species^='LOGS');
  output out=July08_top_spp mean=mean stderr=stderr n=n;
proc print data=July08_top_spp; run; quit;
```

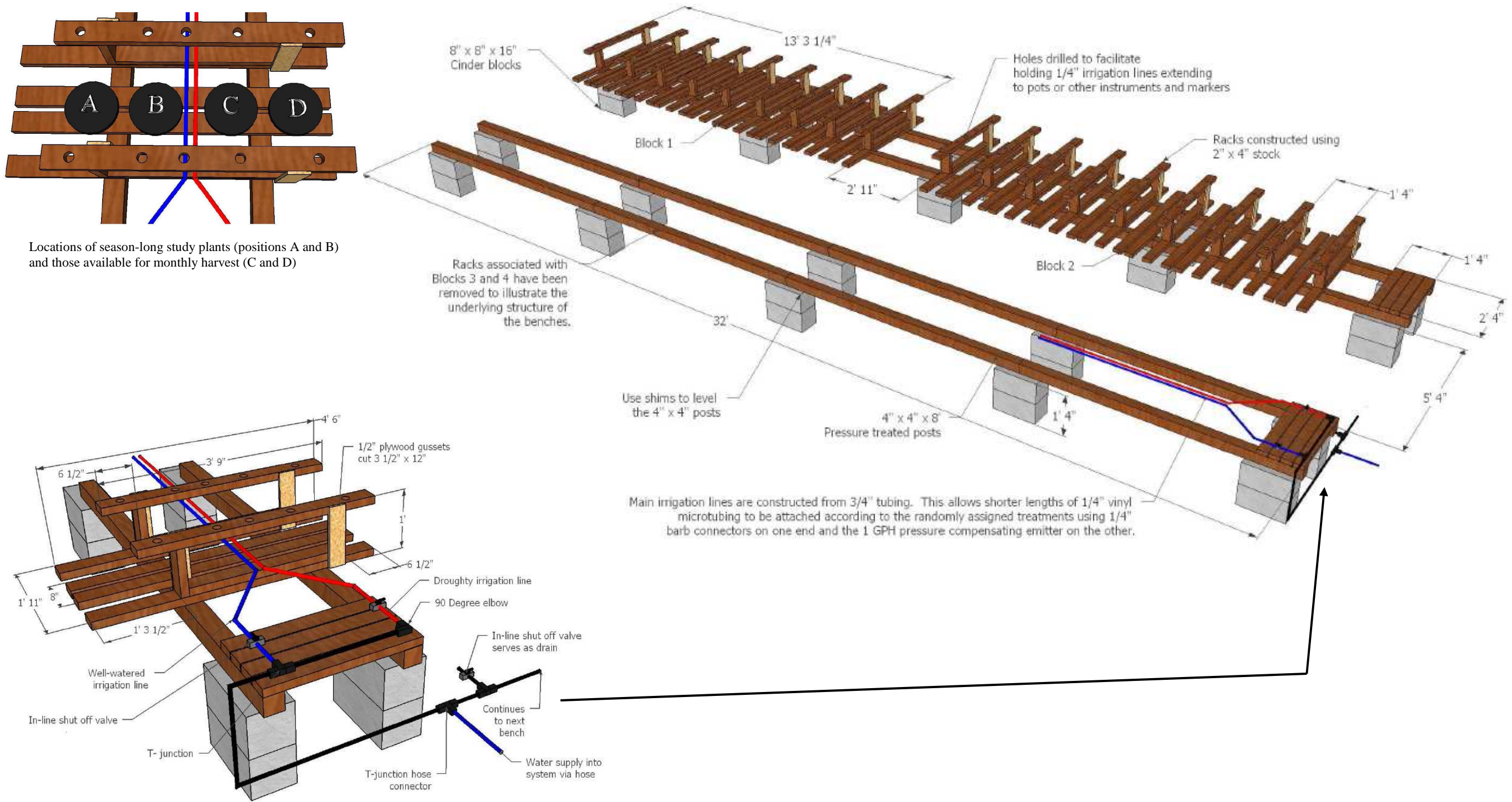
I hope this tutorial and explanation help.

Appendix 3 – Aerial photographs of Boot provided by Google Earth.

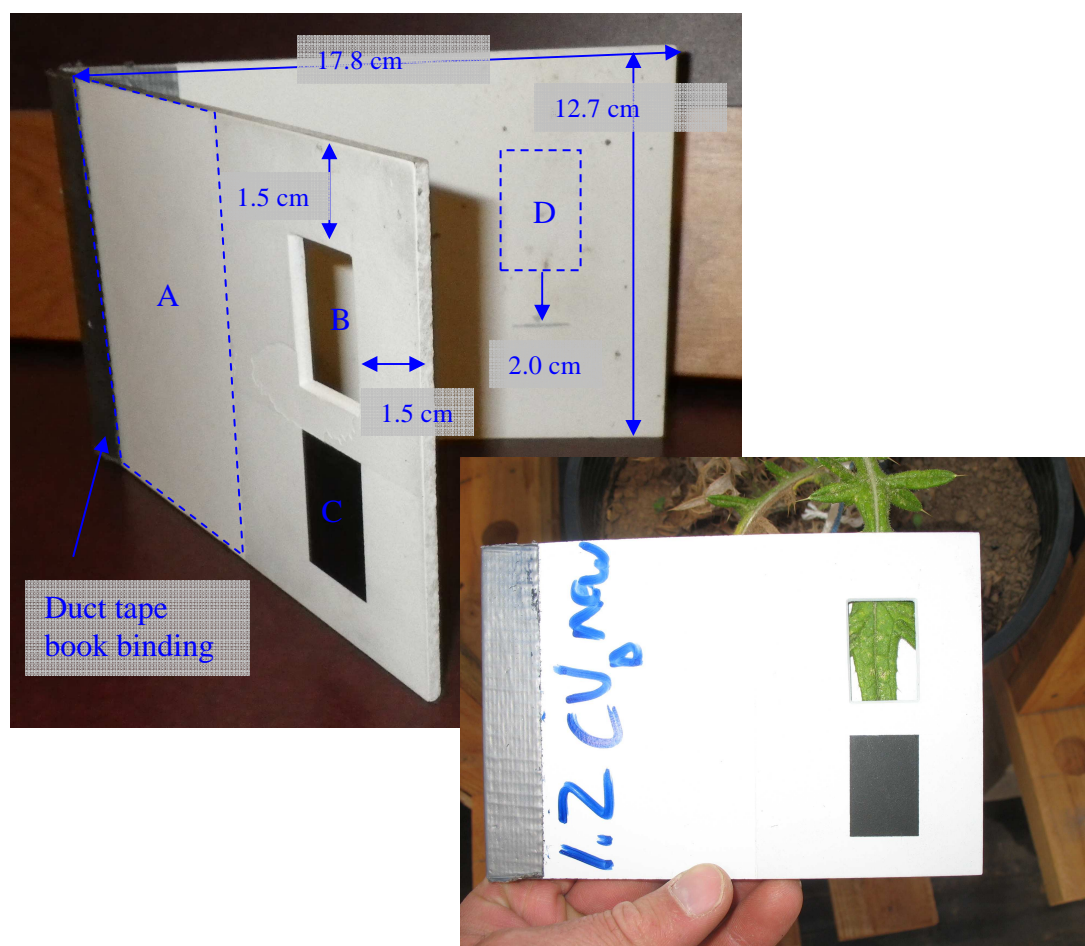


Note: The overall study site (above) and the 2 m x 2 m pens constructed to protect the season-long weeds (below) are plainly visible.

Appendix 4a - Bench construction plans



## Appendix 4b - Photo board plans



Note: The hardboard used in the construction of this instrument had a coating which allowed dry erase markers to be used. Upper Image: Part A was simply left unpainted while all other parts were coated with the flat white paint to minimize light reflection from the camera flash. Part B was the 2 cm x 3 cm cut out necessary for the segment of leaf to be viewed which was measured with the LiCor 6400. Part C corresponds with the 2 cm x 3 cm flat black painted area used by the Image J software as the “known” area. Part D was simply where part B lines up with the back board. A line was drawn 2 cm below this area to aid in the consistent capture of the same segment of leaf that was measured with the Licor 6400. Lastly, the two boards were joined using fiber tape (duct tape) to form a book-style binding which allowed easy pivoting. Lower Panel: This picture was used by Image J software to assess leaf area. Hand written code indicates a kind of address unique to this leaf. The plant was located in Block 1 Plot 2, *Cirsium vulgare*, position D, and was a new leaf (selected because the lower leaf had died).

Appendix 5 – Chapter 4 analytic approach. Repeated measures ANOVA tables with expected mean squares as well as the covariance structure used for analysis.

Model			
$Y_{ijkl} = \mu + \rho_i + \alpha_j + \beta_k + (\alpha\beta)_{jk} + \delta_{ijk} + \gamma_l + (\alpha\gamma)_{il} + (\beta\gamma)_{kl} + (\alpha\beta\gamma)_{jkl} + \varepsilon_{ijkl}$			
Greenhouse	Source		
	df		Expected Mean Squares
	Whole-plot	Block $\rho_i$	3 $\sigma^2 + 56\sigma_\rho^2$
		Irrigation $\alpha_j$	1 $\sigma^2 + 7\sigma_\delta^2 + 112(\Sigma \alpha_j^2 / 1)$
		Species $\beta_k$	3 $\sigma^2 + 7\sigma_\delta^2 + 56(\Sigma \beta_k^2 / 3)$
		Irr x Spp $(\alpha\beta)_{jk}$	3 $\sigma^2 + 7\sigma_\delta^2 + 28(\Sigma (\alpha\beta)_{jk}^2 / 3)$
		Error (a) $\delta_{ijk}$	21 $\sigma^2 + 7\sigma_\delta^2$
	Split-plot	Date $\gamma_l$	6 $\sigma^2 + 32(\Sigma \gamma_l^2 / 6)$
		Irr x Date $(\alpha\gamma)_{il}$	6 $\sigma^2 + 16(\Sigma (\alpha\gamma)_{il}^2 / 6)$
		Spp x Date $(\beta\gamma)_{kl}$	18 $\sigma^2 + 8(\Sigma (\beta\gamma)_{kl}^2 / 18)$
		S x I x D $(\alpha\beta\gamma)_{jkl}$	18 $\sigma^2 + 4(\Sigma (\alpha\beta\gamma)_{jkl}^2 / 18)$
		Error (b) $\varepsilon_{ijkl}$	144 $\sigma^2$

Autoregressive covariance [AR(1)]
$$\Sigma = \sigma^2 \begin{pmatrix} 1 & \rho & \rho^2 & \rho^3 & \dots & \rho^6 \\ \rho & 1 & \rho & \rho^2 & \dots & \rho^5 \\ \rho^2 & \rho & 1 & \rho & \dots & \rho^4 \\ \rho^3 & \rho^2 & \rho & 1 & \dots & \dots \\ \dots & \dots & \dots & \dots & \dots & \dots \\ \rho^6 & \rho^5 & \rho^4 & \dots & \dots & 1 \end{pmatrix}$$

Model			
$Y_{ijk} = \mu + \rho_i + \alpha_j + \delta_{ij} + \beta_k + (\alpha\beta)_{ik} + \varepsilon_{ijk}$			
Field	Source		
	df		Expected Mean Squares
	Whole-plot	Rep $\rho_i$	3 $\sigma^2 + 28\sigma_\rho^2$
		Species $\alpha_j$	3 $\sigma^2 + 7\sigma_\delta^2 + 28(\Sigma \alpha_j^2 / 3)$
		Error (a) $\delta_{ij}$	9 $\sigma^2 + 7\sigma_\delta^2$
	Split-plot	Date $\beta_k$	6 $\sigma^2 + 16(\Sigma \beta_k^2 / 6)$
		Date x Spp $(\alpha\beta)_{jk}$	18 $\sigma^2 + 4(\Sigma (\alpha\beta)_{jk}^2 / 18)$
		Error (b) $\varepsilon_{ijk}$	72 $\sigma^2$

Autoregressive (my definition): a random process that can best be described by a weighted sum of previous values. A first order process is one where the immediately previous value has an increased effect on the current value.

