SEED DORMANCY AND GREENHOUSE PROPAGATION OF ARROWLEAF BALSAMROOT (*Balsamorhiza sagittata*) AND SILVERLEAF PHACELIA (*Phacelia hastata* var. *hastata*)

by

Charissa Maria Bujak

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DEDICATION

I would like to dedicate this thesis to my family, Thomas and Carolyn Bujak, and Kasimir, Zoe, and Joseph Bujak, and my fiancae, Edward Barge, as they have offered nothing but encouragment and support in all my academic and personal pursuits.

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ABSTRACT

Native plant material, defined by the United States Forest Service, includes all indigenous terrestrial and aquatic plant species that evolved naturally in a defined ecosystem. Native plant material is important for horticultural and restoration purposes; however, propagation protocols for many Montana native plant species remains unknown. This study addressed the following: greenhouse propagation, seed dormancy classification, and seed-dormancy release treatments for arrowleaf balsamroot (*Balsamorhiza sagittata*) and silverleaf phacelia (*Phacelia hastata* var. *hastata*). Additionally, an assessment was completed to better understand current challenges, successes of the Montana native plant market.

In this study gibberellic acid (GA3) significantly increased final germination of arrowleaf balsamroot to 81% ± 2%. Utilizing the Baskin and Baskin (1998, 2004) seed dormancy classification scheme, arrowleaf balsamroot seed dormancy was classified as: 1) nondeep physiological dormancy type 2, 2) intermediate physiological dormancy type 2, and 3) deep physiological dormancy type 1 and 3. Following the Schewienbacher (2011) reclassification scheme, arrowleaf balsamroot seeds were indicative of physiological deep dormancy type 3. In the greenhouse, fertilizer rates up to 200 mg nitrogen/L (20-10-20 NPK of Jack's Professional Peat Lite Special ™) resulted in positive shoot growth but beyond 100 mg nitrogen/L reduced the root-to-shoot value. The Ray Leach Cone-tainer™ was an effective container type.

For silverleaf phacelia, 87% ± 5% germination was achieved within four days with scarification for at least 90 seconds. After 1 year of storage at 5 ± 1^{0}C, following the Baskin and Baskin (1998) classification scheme, silverleaf phacelia seed dormancy was classified as 1) nondeep physiological dormancy type 1, 2, and 5, and 2) intermediate physiological dormancy type 1 and 2. Addressing nondeep physiological dormancy type 5 would be most effective in breaking seed dormancy. In the greenhouse, fertilizer rates beyond 50 mg nitrogen/L (20-10-20 NPK of Jack's Professional Peat-Lite Special) resulted in positive shoot growth but beyond 50 mg nitrogen/L reduced root mass. The 4-inch square pot was an effective container type.

A survey of 30 Montana native plant growers indicated a demand for native plants in the Montana nursery industry. However, more work needs to be completed to develop and stabilize the native plant market.

INTRODUCTION/ LITERATURE REVIEW

Utilization of native plant material may help promote the conservation of resources and human health. It has been suggested that the use of native plant material is beneficial, as it may: 1) promote ecological stability through increased biodiversity and preservation of local genotypes, 2) reduce herbicide and pesticide use by hosting beneficial insects, and 3) create native pollinator habitat (Potts et al 2002; Meyers et al. 2005; Erikson 2008). However, more research is necessary to investigate the validity of arguments promoting the utilization of native plant material based on their being more adaptive, requiring lower maintenance, and being more resistant to pests (Potts et al. 2002).

Nonetheless, within the last century, the demand for native plant material has increased due to the rising popularity of the "green industry" and an increased focus on the promotion of sustainable horticulture practices (Peppin et al. 2010). Green industry professionals have predicted increased demand, potentially creating an unfulfilled market niche within the floriculture industry (Potts et al. 2002). In addition, recent changes to federal and state policy requiring utilization of native plants in revegetation activities has helped raise demand (Richards et al. 1998; PEO 1999; SCEO 1999; Erikson 2008; Peppin et al. 2010). Restoration and preservation of disturbed and natural areas is currently the primary application of native plant products (Potts et al. 2002).

Production of native plant materials for the nursery industry remains limited. In a survey of nursery and landscape professionals in Florida, Colorado, and the Southwestern United States, the following limitations were identified: propagation difficulties, lack of

education and knowledge of native plants, increased labor and production expenses, and lack of demand (Potts et al. 2002; Brzuszek and Harkess 2009; Peppin et al. 2010; Pe´rez et al. 2010; Kauth and Perez 2011). Additionally, the need to assess the role and economic importance of the native plant market within the floriculture industry was identified (Peppin et al. 2010; Pe´rez et al. 2010). To improve the potential of native plants in Montana's floricultural industry, further research needs to be completed on greenhouse propagation and seed dormancy of native species. Furthermore, a baseline assessment of the current Montana native plant market must be completed.

The purpose of this project was to research greenhouse production techniques, seed dormancy mechanisms, and seed dormancy-breaking treatments for two Montana perennial native plant species; arrowleaf balsamroot (*Balsamorhiza sagittata*) and silverleaf phacelia (*Phacelia hastata* var. *hastata*). Additionally, in order to establish a baseline assessment of the current obstacles and successes of the Montana native plant market, a survey was completed of 30 current Montana native plant nurseries and growers.

Literature Review

Seed Dormancy

Seed dormancy is defined as a barrier to germination of a viable seed, even under seemingly favorable growing conditions (Hilhorst 1995; Li & Foley 1997; Finch-Savage and Leubner-Metzger 2006; Bewley 2013). Dormancy in seeds is thought to be an adaptive trait, evolutionarily obtained through adaptation to changes in climate (Hilhorst,

1995; Vleeshouwers et al. 1995; Li & Foley 1997; Baskin and Baskin 2004; Fenner & Thompson 2005; Bewley et al. 2013). Dormancy may be present when seeds are released from the mother plant (primary dormancy) or induced by unfavorable environmental factors (secondary dormancy), or seeds may cycle seasonally in between a dormant and non-dormant state (conditional dormancy) (Bewley 1997).

A dormant seed may have to undergo physiological or morphological changes, or a combination of both in order for germination to occur (Bewley et al. 1999). Barriers to germination may be induced endogenously or exogenously. Causes of endogenous barriers include: 1) undifferentiated embryo, 2) immature embryo 3) chemical inhibitors, and 4) physiological constraints (Bewley et al. 2013). Caused of exogenous barriers involve the seed coat including: 1) interference with water uptake 2) interference with gas exchange 3) prevention of the exit of inhibitors from the embryo, and 4) mechanical constraints (Bewley et al. 2013).

Although dormancy caused by attributes of the embryo or the seed coat are categorized as separate forces, researchers have noted the need to look upon the cause of seed dormancy in a "whole seed" view (Finch-Savage and Leubner-Metzger 2006) in which dormancy may be imposed separately by characteristics of the covering tissue, the embryo, or from both the embryo and covering tissues simultaneously or successively (Baskin and Baskin 2004; Bewley et al. 2013). Dormancy release occurs when specific exogenous or endogenous factors driving dormancy are characterized or overcome (Baskin and Baskin 2004).

Seed Dormancy Classification

Nikolaeva (1967) was one of the first scientists to publish a substantial classification scheme for seed dormancy in 1967. Martin (1946) developed a morphological classification for seed structure, which can be used to identify seed type based on the size and structure of the embryo and endosperm. Baskin and Baskin (2007) further revised the Martin (1946) seed structure classification system. Baskin and Baskin (1998, 2001; 2004) also further revised and reorganized the Nickolaeva (1967) scheme into a hierarchy of classes, levels, and sublevels or types (APPENDIX A). Lastly, Schwienbacher (2011) revised the Baskin and Baskin scheme (1998, 2004) (APPENDIX B).

The Baskin and Baskin (1998, 2004) hierarchal classification scheme was adapted from the Nickolaeva (1967) classification scheme, and thus is very similar. Essentially, the Nickoleava systemwas organized into seed-dormancy releasing requirements which reflected endogenous and exogenous dormancy types. Baskin and Baskin (1998, 2004) reorganized this scheme; instead of classifying dormancy types as exogenous or endogenous, dormancy types are organized into 5 separate classes, which are divided into levels, and levels are further subdivided into types. These five classes of seed dormancy are: physiological (PD), morphological (MD), morphophysiological (MPD), physical (PY), and combinational (PY+PD). Physiological dormancy has 3 levels (non-deep, intermediate, and deep) which contain associated types.

Types reflect specific seed dormancy-release requirements. Seeds exhibiting non-deep physiological dormancy may display 5 types of seed dormancy releasing

requirements which are met when: 1) excised embryos produce normal seedlings, 2) GA promotes germination, 3) Cold: moist (0 to 10^0C) or warm: moist (>15^0C) stratification breaks dormancy, 4) seeds may after-ripen in dry storage, and 5) scarification promotes germination (Baskin and Baskin 1998). Seeds exhibiting nondeep physiological dormancy may also exhibit a conditional status of dormancy in which a series of changes in the germination capacity occurs as the seed transitions from primary dormancy into secondary dormancy (Vegis 1973; Baskin and Baskin 1998). Seeds with conditional status may be able to germinate over a broader range of temperatures over time (Baskin and Baskin 1998, Finch-Savage and Leubner-Metzger 2006).

Seeds which display intermediate physiological dormancy exhibit up to 4 types of dormancy-release requirements which are met when; 1) excised embryos produce normal seedling, 2) gibberellic acid (GA) promotes germination, 3) seeds require 2-3 months of cold: moist stratification to break dormancy, and 4) dry storage shortens the time required for cold: moist stratification to break dormancy. Lastly, seeds with deep physiological dormancy may exhibit 3 types; 1) excised embryos produce abnormal seedlings, 2) GA does not promote germination, and 3) seeds require 3-4 months of cold: moist stratification to germinate (Baskin and Baskin 1998, Finch-Savage and Leubner-Metzger 2006).

In morphological dormancy (MD), there are two levels: 1) seeds in which the embryo is differentiated from the endosperm, although it still needs time for development, and 2) seeds with undifferentiated embryos, in which the cotyledon and radicle have not yet visibly formed (Baskin and Baskin 2001). Both levels usually have a

low embryo to seed ratio (E:S) (Forbis et al. 2002). Also, seeds with undifferentiated embryos are usually indicative of a specialized seed structure and classified as micro or dwarf seeds (Martin 1946).

In seeds exhibiting morphophysiological (MPD) dormancy, a combination of morphological and physiological dormancy is displayed; seeds may have either an underdeveloped or undifferentiated embryo, coupled with a low growth potential as exhibited by seeds with physiological dormancy (Baskin and Baskin 2001). Before seeds with morphophysiological seeds germinate, they must develop to the required size, and the physiological block to germination within the embryo must be overcome (Baskin and Baskin 1998).

In seeds exhibiting physical dormancy (PY), the seed is impermeable to water, due to the structural design of the seed coat. To allow for germination, water must penetrate through these impermeable layers and enter the seed. Finally, in seeds with combinational dormancy (PY + PD), the seed exhibits impermeability to water and the embryo has a low growth potential. Thus, for seeds with combinational dormancy to germinate, an opening must be created through the impermeable layers for water to enter the seed, and the physiological block to germination within the embryo must be addressed (Baskin and Baskin 2001, Finch-Savage and Leubner-Metzger 2006).

Overall, the process of classifying dormancy is not always clear (Finch-Savage and Leubner-Metzger 2006). Challenges remain in classifying dormancy to a definite class, level, and type, as the dormancy status of a seed may be constantly changing and may be influenced by numerous factors, such as; current environmental conditions,

previous maternal environment of the mother plant, and natural genetic variation among a population (Finch-Savage and Leubner-Metzger 2006). Seed dormancy is defined as a barrier to germination of a viable seed under favorable conditions, which results in the absence of germination (Hilhorst, 1995; Li and Foley 1997; Bewley 2013). However a dormant seed completing germination is a process, rather than a definite event, with a changing status of the dormant seed as it undergoes the physiological and structural changes needed to complete germination (Finch-Savage and Leubner-Metzger 2006).

Ultimately, seed dormancy is a characteristic of the seed which defines requirements necessary for dormancy-release and germination at a specific time period, and under exposure to specific environmental conditions (Vleeshouwers et al. 1995, Thompson 2000; Fenner and Thompson 2005; Finch-Savage and Leubner-Metzger 2006). Finch-Savage and Leubner-Metzger (2006) suggest a clearly defined physiological dormancy state does not exist, but rather under different conditions there exists different requirements which allow for dormancy-release and the process of germination to occur. Despite this difficulty, the process of dormancy classification remains important for investigation of ecological characteristics and as a tool to identify successful seed treatments for effective propagation protocols for many native plant species.

Propagation of Native Plants

Rapid production of many native plants in a commercially viable setting is limited, due in part, by a lack of knowledge of the growth requirements for these plants (Potts et al. 2002; Lynn et al. 2008; Brzuszek and Harkess 2009; Peppin et al. 2010; Perez et al. 2010). Compared to commercial species, very little is known regarding the

propagation of many native plants in greenhouses (Landis and Simonich 1984). Landis and Simonich (1984) state that native plants may be grown reasonably well in a standard greenhouse environment, using commercial soil-less media mixtures. However, Erikson (2015) states that propagation methodologies for many native plant species are complex and undefined. Similarly, nursery professionals in the Colorado Plateau area have reported difficulties propagating natives in typical nursery conditions due to increased time for production and unknown container-type requirements (Potts et al. 2002).

Landis and Simonch (1984) state that while agricultural crop species have production schedules in which crop planting may be completed within a year, native plants may require up to three years or longer, depending on seed procurement and length of hardening off. Also, native plant production may require the use of multiple facilities to propagate, harden-off and store the native plant material, whereas many commercialized species may only require a single greenhouse. Furthermore, native plants may have different container type and fertilizer requirements than domesticated ornamentals (Cardoso et al. 2007). Cardoso (2007) suggests there is a demand for additional information on the production requirements for container-grown plants native to the Intermountain West region.

Size and shape of container, root control features, cost, and support and handling are all important considerations when selecting a container. These characteristics are important predictors of plant quality and also of economic cost. Characteristics such as the size of the container and ease of shipping and handling greatly impact cost.

Additionally, characteristics such as the depth of the container and root-control features may heavily influence plant quality.

Another area of limited research in the culture of container-grown native plants is the type, rate, and frequency of fertilizer application. Cardoso (2007) suggests that, due to the generally low availability of nutrients in their natural environment, many native plant species may have low fertilizer requirements. However, others have reported that some native herbaceous plant species may respond well to fertilizer concentrations utilized for greenhouse crops (Cardoso et al. 2007).

Ultimately, greenhouse production requirements are species-specific. Liquid fertilizer rates and container type depend on numerous factors, such as; growth characteristics of the specific species, media utilized irrigation frequency, cultural environment, and production schedule (Hartmann and Kester 2011). Further research is needed to understand greenhouse growth requirements and to establish greenhouse production protocols for native plants, specifically related to container-type and fertilizer rate requirements.

Native Plant Market Survey

The native plant market may be poised to develop into a valuable part of the Montana floriculture industry. However, additional information is needed to ascertain the current supply and demand within the Montana native plant market. An initial baseline assessment, identifying successes and challenges for Montana native plant producers, and exploring the role of native wildflowers in current and future trends, is vital to the development and implementation of a niche native wildflower market (Peppins et al.

2010; Kauth and Perez 2011). In summary, availability of native plants is dictated by multiple challenges and may differ regionally, however native plant material grown and sold locally may increase in-state revenue through development of the native plant market niche in the horticultural industry. As of yet, no feasibility study, or even baseline survey, has been implemented to assess the status of the Montana native plant market.

Overall Objectives

Objective 1. Classify the seed dormancy and increase the final germination percentage of two native plant species indigenous to the Intermountain West Region: arrowleaf balsamroot (*Baslamorhiza sagittata*) Pursh Nutt and silverleaf phacelia (*Phacelia hastata* var. *hastata*) Douglas ex Lehm.

Objective 2. Explore species-specific fertilizer rate and container type requirements for greenhouse production of arrowleaf balsamroot (*Balsamorhiza sagittata*) and silverleaf phacelia (*Phacelia hastata* var. *hastata*).

Objective 3. Survey Montana native plant nursery growers to better understand how Montana green industry professionals view the challenges and successes of the Montana native plant market.

CHAPTER 2

SEED DORMANCY OF ARROWLEAF BALSAMROOT

Abstract

With its perennial showy flower, soil-stabilizing taproot, and energy-rich winter foliage, arrow-leaf balsamroot, *Balsamorhiza sagittata*, has several traits lending it to ornamental and conservation applications if it can be propagated successfully and cost efficiently. However, germination percentage remains low due to a complex and unknown seed dormancy classification and an unknown dormancy-release mechanism. In this study gibberellic acid (GA3) (mg/L) followed by cold: moist incubation at 5°C, significantly increased final germination, with a maximum final germination of 81% ± 2%. Utilizing the Baskin and Baskin (1998, 2004) seed dormancy classification scheme to classify arrowleaf balsamroot seed dormancy, seeds exhibit nondeep physiological dormancy, type 2; intermediate physiological dormancy, type 2; and deep physiological dormancy, types 1 and3. Following the Schwienbacher (2011) reclassification scheme, arrowleaf balsamroot most closely exhibits a physiological deep dormancy.

Introduction

Dormancy is an innate characteristic of some seeds preventing germination (the emergence of the radicle from the embryo) even when conditions are favorable for the induction of germination (Baskin and Baskin 1998). Morphological and physiological properties of seeds, and their response to environmental conditions, control the induction

and release of seed dormancy through dormancy-release mechanisms (Vleeshouwers et al. 1995; Geneve 2003; Fenner and Thompson 2005; Baskin and Baskin 2014; Finch-Savage and Leubner-Metzger 2006). The specific properties of seeds which characterize the dormancy-release mechanism include; embryo morphology, permeability to water, capacity to germinate after dry-storage, and physiological growth status of embryo.

Based on the characteristics of these dormancy-release mechanisms, seed dormancy has been classified into five main classes: 1) physiological dormancy (PD), 2) morphological dormancy (MD), morphophysiological dormancy (MPD), physical dormancy (PY) and a combinational dormancy (PY + PD) (Nikolavea 1967; Baskin and Baskin 2004; Schwienbacher et al. 2011). These five dormancy classes have been delineated further, and each class may have additional sublevels and subtypes (Baskin and Baskin 2004). To recognize the importance of after-ripening as a pre-condition or pre-treatment, Schwienbacher (2011) further refined the classification scheme developed by Baskin and Baskin (1998). Physiological dormancy, the most abundant form of all dormancy types, is caused by a low growth potential of the embryo (Finch-Savage and Leubner-Metzger 2006; Heather et al. 2010). The cause of the growth potential of the embryo is the result of complex ABA (abscisic acid) synthesis and GA (gibberellic acid) catabolism signaling which induces and maintains the dormancy state within the embryo (Cadman et al. 2006).

Seed dormancy is common in many plant species and is an important ecological survival mechanism for many native wildflower species. However, seed dormancy may limit cost efficient propagation and production of many native plant species with

commercial potential. There is a lack of information regarding the dormancy-release requirements for many native species (Roemmich et al. 2012). When the seed dormancy release mechanism is not addressed, attempts to propagate native species with complex seed dormancy-release mechanisms may be unsuccessful (Heather et al. 2010). Lack of success entails low germination percentages and erratic germination patterns, decreasing the potential of introducing native plant material into the horticultural market (Heather et al. 2010). Lack of information on the germination requirements of native wildflowers exhibiting seed dormancy may limit the number and type of native species introduced to the horticultural market (Potts et al. 2002; Chambers et al. 2006; Heather et al. 2010).

Arrowleaf balsamroot is an herbaceous perennial plant which grows 30.48 to 60.96 cm in height with a tap-root up to 2.44 meters long with a 4-inch (10.16 cm) diameter; bears multiple long peduncles, has lanceolate leaves, and flower heads have an outer whorl of ray flowers 2.54 to 5.08 cm long, and an inner whorl of disc flowers (USFS 1937; Wasser 1982; Stevens 1985). Its geographical range is Western North America, stretching east as far as the Dakotas and west into California and northwest into Canada (USFS 2008).

Arrowleaf balsamroot is ecologically valuable as winter food source for domestic livestock and wild ungulates (Stanton 1974). The dry herbage has been labelled as "fairly palatable" to multiple classes of domestic livestock including horses, cattle, sheep, and game animals (Wilkins 1957; Stanton 1974). The leaves and flowers, high in dietary fiber and carbohydrates, are consumed by wild ungulates such as mule deer, elk, pronghorns, and bighorn sheep (Wilkins 1957; Stanton 1974). As a long-lived, energy-rich, forage

plant for domesticated livestock, wild range ungulates, and game species, arrowleaf balsamroot has the potential to fulfill a valuable niche as an important native plant forage species for private landowners and federal land management agencies (Wilkins 1957).

Arrowleaf balsamroot is also economically valuable for rehabilitation and restoration of disturbed forested areas (Wasser 1982). In Utah, it has been included in a seed mix directed towards optimizing feed materials for game wildlife. It has a high potential for reclamation of previous oil-shale or coal-mined sites (Wasser 1982). Arrowleaf balsamroot is also an attractive native for roadside revegetation projects, as its showy flower and long taproot provides beautification and soil stabilization (Wasser 1982). It is a drought resistant and fire-resistant species, increasing its potential for the horticultural market in arid and fire-prone environments, such as in the Intermountain Western Region (Staton 1974; Wischnofske 1983; Powell 1994; McWilliams 2002).

Finally, arrowleaf balsamroot is an important part of preservation of the heritage of indigenous people due to its traditional uses as a medicinal plant and as an important source of food. Cheyenne boiled and consumed the roots, stems and leaves (Hart 1976; Stubbendieck et al. 1992). Members of the Salish, Kootenai, and Nez Perce tribe consumed the raw stems of the plant (Hart 1976). Due to its history of utilization by First Nation peoples, arrowleaf balsamroot is included in a tribal nursery handbook developed by the USFS as a potential plant for propagation in modern tribal nurseries (Dumroese 2009).

Due to the potential ecological, economic, and cultural benefits of arrowleaf balsamroot, this native species has a high horticultural potential for production in native

plant nurseries (Chambers et al. 2006). Commercial cultivation and production of arrowleaf balsamroot may fill a vital niche in the Intermountain West region for federal land managers and private landowners.

In spite of its value and economic potential, arrow-leaf balsamroot seeds display low germination percentages and erratic germination, despite exposure to dormancy-releasing treatments (Young and Evans 1979; 1979; Kitchen and Monsen 1996; Chambers et al. 2006). A cold: moist stratification period of 15 weeks at 5°C and a combination of cold: moist stratification with ethylene has been shown to increase germination (Young and Evans 1979; Chambers et al. 2006). However, in these studies, final germination percentage remained at or below 38% (Chambers et al. 2006). This species is considered to exhibit physiological dormancy, however, the level and type of physiological dormancy remains unknown. The lack of information on the exact dormancy-releasing mechanisms, coupled with low final germination percentage has limited the potential for arrowleaf balsamroot introduction into the horticultural market (Chambers et al. 2006).

The objective of this research was to classify the seed dormancy type of arrowleaf balsamroot through the identification of effective seed dormancy-release mechanisms and to increase the final germination percentage germination of arrowleaf balsamroot seeds through overcoming inherent seed dormancy of this species.

Materials and Methods

Seeds were collected at an elevation of 1514.86 meters, in Bozeman, Montana ($45^0$42'35.27" N, $110^0$42'35.27"S $110^0$58'37.73" W) at three separate dates in July 2013 (July 8th, 14th and 27th). Seeds from each collection date were separated and assigned a label based on harvest date. Seeds collected on July 8th were designated as H8 , as H14 on July 14th, and as H27 on July 27th.. When achenes turned a dark brown to black color and fell from the open, dry flower heads, seeds were deemed mature and were collected. Seeds were cleaned through a series of different sized screens and chaff was separated from seed using a South Dakota Seed Blower. Clean seeds were transferred to paper bags for storage.

An initial 2, 3, 5-triphenyltetrazolium chloride (TZ) test to determine viability of was completed within two to four weeks of collection using 4 replicates of 25 randomly selected seeds from a combined, subsample of seeds randomly selected from H8, H14, and H27. Procedures for the TZ test were adapted from the Seed Testing Handbook from ASOCA for species in the Asteraceae family (ASOCA 1993). Seeds were: 1) allowed to imbibe in deionized water overnight, 2) cut at the distal end about 1/3 of the length of the seed, 3) the longer portion of the seed was placed in a glass dish containing 5 mL of 1% TZ solution and, 4) incubated in the dark at 35°C for 5 hours. Seeds were dissected laterally and the embryo examined under magnification (10x). Missing embryos, unstained embryos or embryos stained all or partially black were considered non-viable. Embryos stained dark pink to red were counted as viable.

After the initial TZ test for viability, seeds were exposed to two different storage conditions over a period of 0 to 11 months (Table 1). To test the effect of cold, dry storage (CDS), all seeds were kept dry in separate paper bags labelled by harvest date (H8 for July 8th, H14 for July 14th, and H27 for July 27th) and placed into a seed storage room at 5 ± 1^0C. To test the effect of warm dry storage (WDS), all seeds were kept dry in separate paper bags labelled H8, H14, and H27, and placed into storage at ambient conditions of 21 ± 1^0C.

Initial Seed Tests to Classify Dormancy

A total of six initial germination tests and one experiment on seed structure identification was completed to classify the level and type of seed dormancy and to identify successful treatments to increase the final germination percent of arrowleaf balsamroot. Experiments 1 through 5 were conducted in order to identify and classify the level and type of seed dormancy involved, and their results used to design the final germination experiment to address breaking dormancy (experiment 6). The final germination experiment was utilized to confirm results of the final protocol for increasing final germination and seed dormancy classification.

Unless otherwise noted all experiments followed 8 step standardized protocols for seed preparation: 1) random selection of seeds, 2) surface sterilization of seeds accomplished by soaking seeds in a 15% Clorax and deionized water solution for 10 minutes, 3) sterilization of germination container (cut comb honey container - 4-5/16”x 4-5/16” x 1-3/8” © Pioneer Plastics Inc.) via bleach wash,4) blue blotter paper sterilized in a autoclave at 121°C, at 15 psi for 15 minutes, 5) sterilized seeds placed on blue blotter

paper, 6) blue blotter paper moistened with deionized water or a growth regulator solution, 7) germination container(s) placed into controlled walk-in growth chambers, with cool-white fluorescent lamps, and 8) after the initial treatment, moistened with deionized as needed. Germination was defined when emerged radicle was ≥ 1mm in length.

Unless otherwise noted, all initial seed treatments, were designed as a complete factorial experiment with 3 replicates and 100 seeds per replicate and analyzed as a generalized linear model. Data was analyzed using PROC GLM (SAS University Edition 9.3) with a repeated measures statement to account for the correlation between germination measurements over time. Where only one factor was examined, data was analyzed using a one-way analysis of variance (PROC ANOVA, SAS University Edition 9.3). Means were examined utilizing the least-square means test (LSMEANS SAS University Edition 9.3) and all pair-wise combinations of all factors and levels were then examined with the Tukey-Kramer Honestly Significant Difference test (HSD) at α=0.05 significance level (TUKEY ADJUST SAS University Edition 9.3). Harvest date was not significant and thus the collection date was utilized in initial experiments as replicates. Germinated seeds were counted weekly for 15 weeks and discarded after each weekly count.

Seed Structure Identification. As part of the initial seed classification experiments, the seed classification system of Martin (1946) was used to identify the structure of the seed by examining the embryo under magnification (25x) (Forbis et al. 2002; Finch-Savage and Leubner-Metzger 2006).

Experiment 1: Imbibition. To examine water uptake by intact seeds, imbibition was measured within 2 to 4 weeks of collection time. Four replicates of 25 seeds each were randomly selected and the initial dry mass (Wn) was measured in grams using a Mettler Toledo AG104 balance. After determining the average initial mass, seeds were allowed to imbibe water on moistened blue blotter paper. At periods of 5, 10, 15, 30, 45, 60 and 1080 minutes (18 hours), seeds were taken out, the imbibed tissue weighed (Wi), and the increase in fresh weight after imbibition calculated using the formula [(Wi-Wn)/Wn] x 100.

Experiment 2.1: Cold: Moist Stratification. The effect of cold: moist stratification (CS), in seeds which were after-ripened (dry storage) for 20 days at two storage temperatures (WDS at 21± 1^0C and cold CDS at 4± 1^0C), was examined in a factorial design as seeds were exposed to a control of warm: moist stratification at 15± 1^0C and cold: moist stratification at 4±1^0C.

Experiment 2.2: Cold: Moist followed by Warm: Moist Stratification: The effect of warm: moist stratification followed by cold: moist stratification, in seeds after-ripened (dry storage) for 20 days at two storage temperatures (WDS 21± 1^0C and CDS 5± 1^0C), was examined in a separate complete factorial design in which after-ripened seeds were exposed to warm stratification at 21± 1^0C for 8 weeks followed by 7 weeks of cold: moist stratification at 4±1^0C, with a control at 15 weeks of moist stratification at 4± 1^0C.

Experiment 3: After-ripening Temperature & Time. To examine the effect of after-ripening (dry storage) at two different temperatures (WDS at 21± 1^0C and CDS at

5± 1^{0}C) final germination % was examined at 20 days, 40 days, 80 days, 120 days, and 240 days. To examine the effect of after-ripening over time (between time periods of 20 days, 40 days, 80 days, 120 days, and 240 days), final germination % was examined at 20 days, 40 days, 80 days, 120 days, and 240 days.

Experiment 4: Gibberellic Acid (GA). To examine the effect of gibberellic acid (GA) in seeds after-ripened for 240 days at two temperatures (WDS at 21± 1^{0}C and CDS at 5± 1^{0}C) were exposed separately to four concentrations of GA at 0 (control), 200, 400, 600 mg/L solution. The only active form of GA which was used in this study was GA3 and will generally be referred to as GA throughout the remainder of this study. Seeds were immediately incubated in a growth chamber at 4±1^{0}C.

Experiment 5: Excised Embryos. To examine the germination capacity of excised embryos, 4 replicates of 25 seeds each were removed from H8, H14, and H27, and the embryo excised from seed coat under a dissecting microscope using a scalpel. Excised embryos were then exposed to three different temperatures (4 ± 1^{0}C, 10 ± 1^{0}C, 21 ± 1^{0}C).

Final Germination Test to Classify Dormancy

Experiment 6: Gibberellic Acid (GA). Out of the initial seed treatments to classify dormancy, as evaluated in experiments 1 through 6, GA had the most positive effect on seed germination. A final germination experiment was conducted to confirm dormancy classification and further investigate the dormancy breaking potential of GA. The final

germination experiment was designed in a complete factorial design taking into account the following three factors; 1) harvest date, 2) 11 months (378 days) of after-ripening at two temperatures, and 3) GA. There were multiple levels to each factor; harvest date at 3 levels (July 8th, 14th, 27th), storage temperature at 2 levels (WDS at 21°C, CDS at 5°C), and gibberellic acid (GA) at 5 levels (0, 250, 500, 750, and 1000 mg/L). All treatments were replicated 4 times with 100 seeds per replicate.

Germinated seeds were counted every 5 days for 65 days and discarded after each weekly count. Removed seedlings were transplanted to observe and check for abnormal growth or morphological variation. Germinated seedlings were transferred to a greenhouse maintained at 15.5°C and transplanted into a soilless media mix (Sunshine Mix #1 ™). Morphological abnormalities were identified by comparing normal shoot and root development in each seedling. At the end of the final germination experiment, a TZ test was completed and non-viable seeds were not considered when calculating the final germination %. Following the TZ analysis, the final germination percentage, the number of days until 50% final germination (T50), and the number of days between 10% and 90% germination (T90) were calculated to examine the rate and uniformity of germination. As germination data was collected every 5 days but not in between, the closest date was used to calculate T50 and T90.

Data was analyzed using a generalized linear model (PROC GLM, SAS University Edition 9.3) with a repeated measures statement to account for the correlation between germination measurements over time. Means were examined utilizing the least-square means test and all pair-wise combinations of all factors and levels were then

examined with Tukey-Kramer HSD at $\alpha=0.05$ significance level (LSMEANS, TUKEY ADJUST SAS University Edition 9.3).

Results

Based on the initial TZ testing, the % viability of 4 replicates with 25 seeds per replicate was at a mean pure live seed (PLS) % of 87%.

Initial Seed Tests to Classify Dormancy

Seed Structure Identification. Utilizing the Martin 1946 seed classification system, the structure of the seed and the shape of the embryo was identified as spatulate, fully developed (Forbis et al. 2002; Finch-Savage and Leubner-Metzger, 2006, Martins 1946).

Experiment 1: Imbibition. The fresh mass (g) of all seeds displayed a mean percent increase in mass at all time periods (5, 10, 15, 30, 45, 60, 90, and 1080 minutes) compared to the unimbibed control (Figure 2.1). This trend shows seeds a steady percent increase in seed mass, however between 90 and 1080, a leveling off in percent increase in seed mass was exhibited, displaying a trend of decreased water uptake after 90 minutes of imbibition

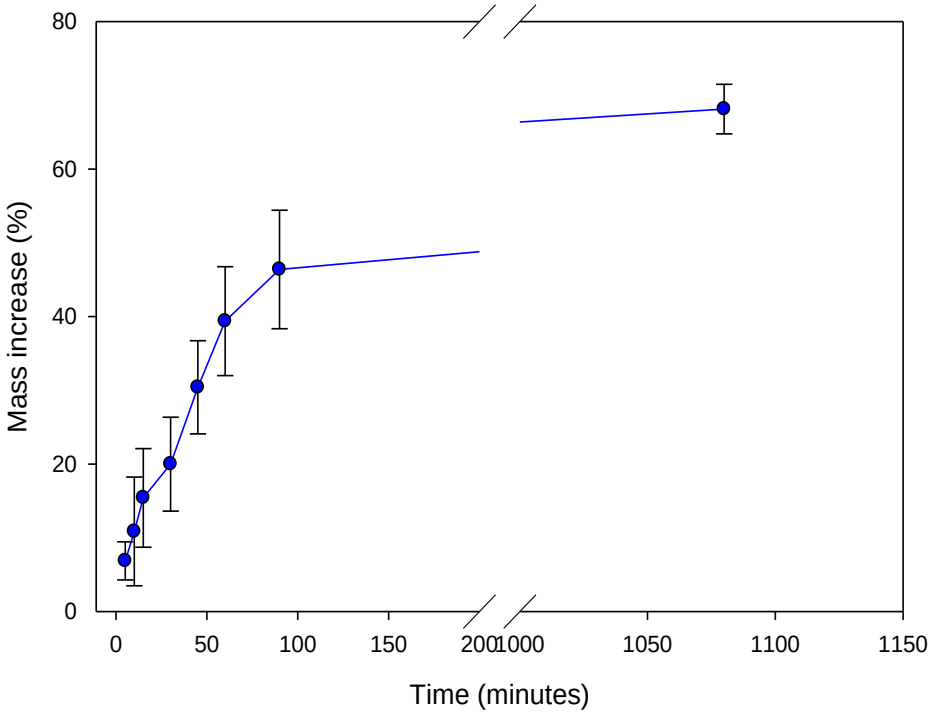

Figure 2.1. Effect of seed imbibition time (minutes) on increase (%) in mass (g) of seeds. Blue circles indicate means and error bars indicate standard error.

Experiment 2.1: Cold: Moist Stratification. There was no significant main effect of cold: moist stratification (CS at 4± 1°C) on % final germination compared to the control (WS at 15± 1°C) (Table 2.1 APPENDIX C). After-ripening temperature (CDS, WDS) did not significantly affect final germination percentage (Table 2.2 APPENDIX C). There was no significant interaction between stratification temperature (CS and WS) and after-ripening temperature on final germination %. The grand mean final germination percentage of all stratification temperatures (CS and WS) and all after-ripening temperatures (CDS and WDS) was 2.5%.

Experiment 2.2: Cold: Moist followed by Warm: Moist Stratification. Warm followed by cold: moist stratification (21 ± 1°C for 8 weeks (WS) followed by 4± 1°C (CS) for 2 weeks) did not significantly impact % final germination compared to the control (CS at 4 ± 1°C for 15 weeks). There was no significant effect of after-ripening temperature (CDS, WDS) on final germination percentage (Table 2.2 APPENDIX C). There was no significant interaction between stratification temperatures (WS followed by CS and control) and after-ripening temperature (CDS and WDS). The grand mean final germination percentage of CS followed by WS, the control, and all levels of after-ripening temperature (CDS and WDS) was 7.1%.

Experiment 3: After-ripening Temperature & Time. There was no significant effect of after-ripening time periods (20, 40, 80, 120, and 240 days) on final germination percentage (Table 2.3 APPENDIX C). After-ripening temperature (CDS, WDS) did not significantly affect the % final germination (Table 2.3 APPENDIX C). There was no significant interaction between after-ripening time periods and temperatures (Table 2.3 APPENDIX C). The grand mean final germination percentage of all after-ripening temperatures (CDS and WDS) in combination all after-ripening time periods (20, 40, 80, 120, and 240 days) resulted in a 6.4%.

Experiment 4: Gibberellic Acid (GA). Seed treatment involving gibberellic acid at 200, 400, and 600 mg/L of GA provided significant improvement in % final germination compared to the control (0 mg/L GA) (Table 2.4 APPENDIX C). After-ripening temperature (CDS, WDS) did not significantly impact final germination percentage

(Table 2.4 APPENDIX C). There was also no significant interaction between GA and after-ripening temperatures (CDS and WDS) (Table 2.4 APPENDIX C). The grand mean final germination percentage of all GA levels in combination all after-ripening temperatures (CS and WS) resulted in a 29.8%.

Experiment 5: Excised Embryos. All excised embryos failed to germinate by the end of the experimental period. Embryos displayed abnormal conditions as described by Nickelova (1967) such as, cotyledons briefly budding but the embryo blackened exhibiting cellular necrosis, radicles not emerging, or emerged seedling did not survive the experimental period.

Final Germination Test to Classify Dormancy

Experiment 6: Gibberellic Acid (GA). Harvest date did not significantly affect final germination percentage (Table 2.5 APPENDIX C). Warm and cold after-ripening (CDS, WDS) in combination with GA significantly affected final germination percentage (Figure 2.2) (Table 2.5 APPENDIX C). In a two-way interaction, higher levels of GA with after-ripening temperature (CDS at 5± 1°C and WDS at 21± 1°C) significantly increased final germination percentage (Figure 3) (Table 2.5 APPENDIX C).

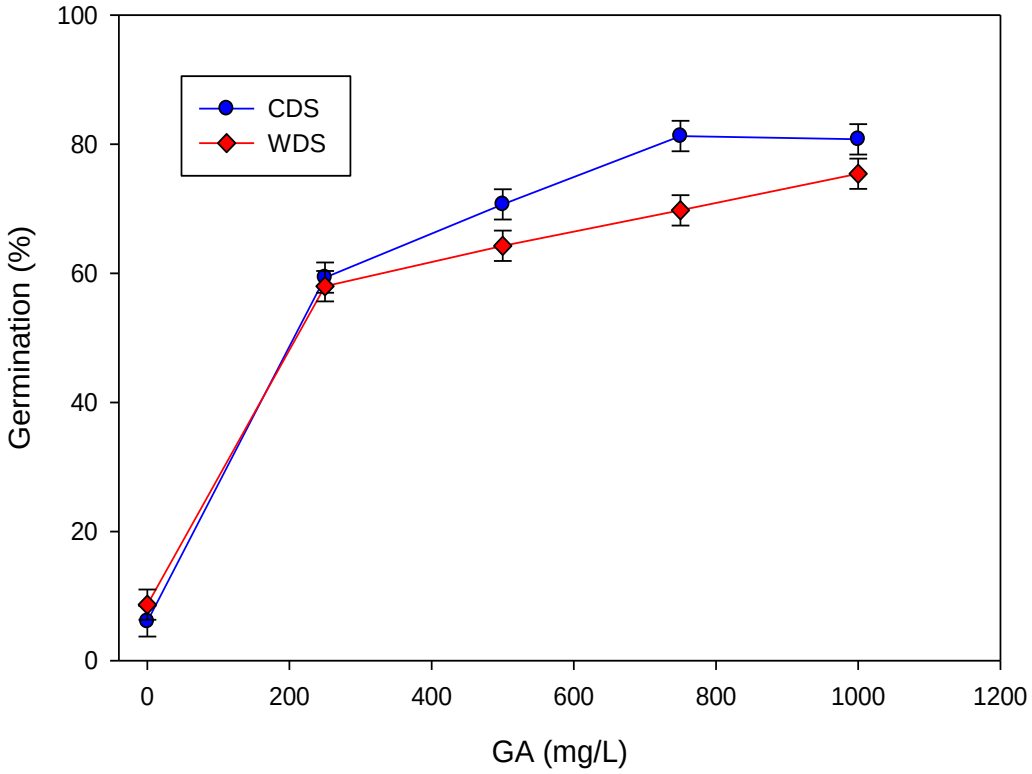

Figure 2.2. Effect of interaction of gibberellic acid (GA mg/L) and after-ripening temperature (CDS at 5± 1°C and WDS at 21± 1°C) on final germination percentage. ANOVA analysis with F=342.38, P=<.0001. Blue circles represent least square means for cold after-ripening temperature (CDS). Red diamonds represent least square means for warm after-ripening temperature (WDS). Error bars indicate 95% confidence at the 0.05 level.

A significant two-way interaction between GA and harvest date, significantly affected the rate of germination with the greatest rate of germination demonstrated by decreased T50 values in H8 (July 8th) at 1000 mg/L GA and H14 (July 14th) at 1000 mg/L GA and H27 (July 27th) at 750 mg/L GA (Figure 2.3) (Table 2.6 APPENDIX C).

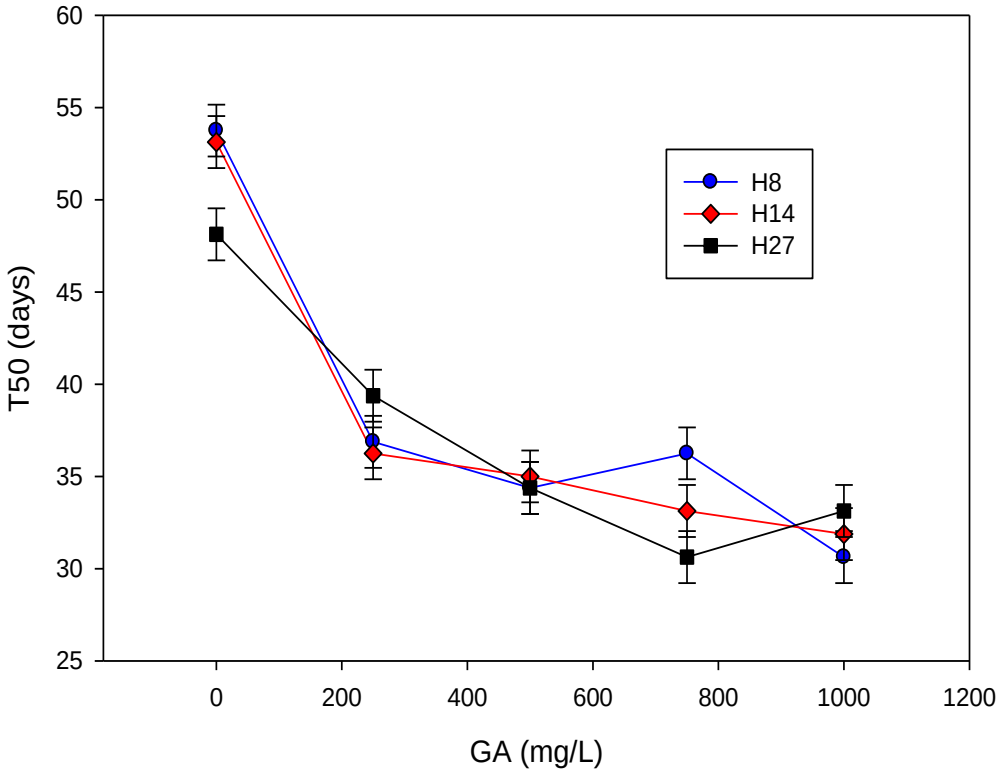

Figure 2.3. Effect of gibberellic acid (GA mg/L) and harvest date (July 8th, 14th, 27th in 2013) on time (days) it takes to achieve 50% final germination (T50). ANOVA analysis with F=2.50, P=0.0168. Blue circles represent July 8th (H8). Red diamonds represent least square means for July 14th (H14). Black squares represent least square means for July 27th (H27). Error bars indicate 95% confidence at the 0.05 level.

In a significant two-way interaction , GA and after-ripening temperature significantly affected rate of germination (T50), with the greatest increase in rate of germination at cold after-ripening 750 and 1000 mg/L GA, and warm after-ripening at 1000 and 500 mg/L GA (Figure 2.4) (Table 2.6 APPENDIX C).

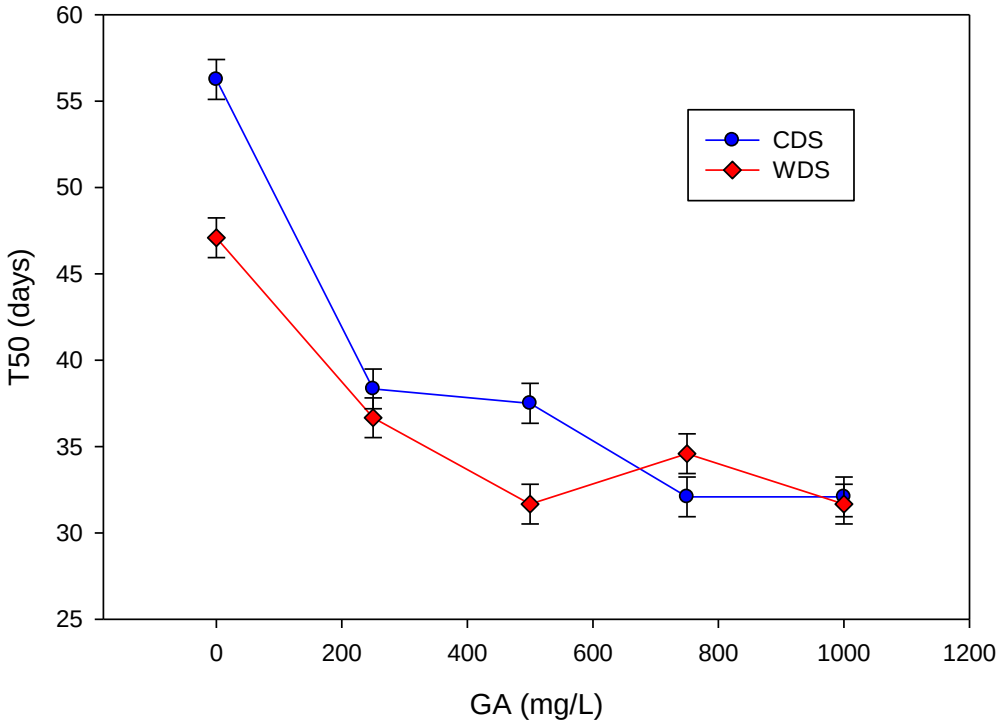

Figure 2.4. Effect of gibberellic acid (GA mg/L) and after-ripening temperature (CDS at 5± 1°C and WDS at 21± 1°C) on time (days) it takes to achieve 50% final germination (T50). ANOVA analysis with F=7.99, P=0.0001. Blue circles represent least square means for cold after-ripening (CDS). Red diamonds represent least square means for warm after-ripening (WDS). Error bars represent 95% confidence at the 0.05 level.

In a significant two-way interaction, after-ripening temperature (CDS and WDS) and harvest date significantly affected the rate of germination (T50) with warm after-ripening (WDS) significantly increasing germination rate for H8 (July 8th) and H14 (July 14th), compared to cold after-ripening (CDS) (Figure 2.5). There was no significant interaction in germination rate (T50) for after-ripening, harvest date, and GA (Table 2.6 APPENDIX C).

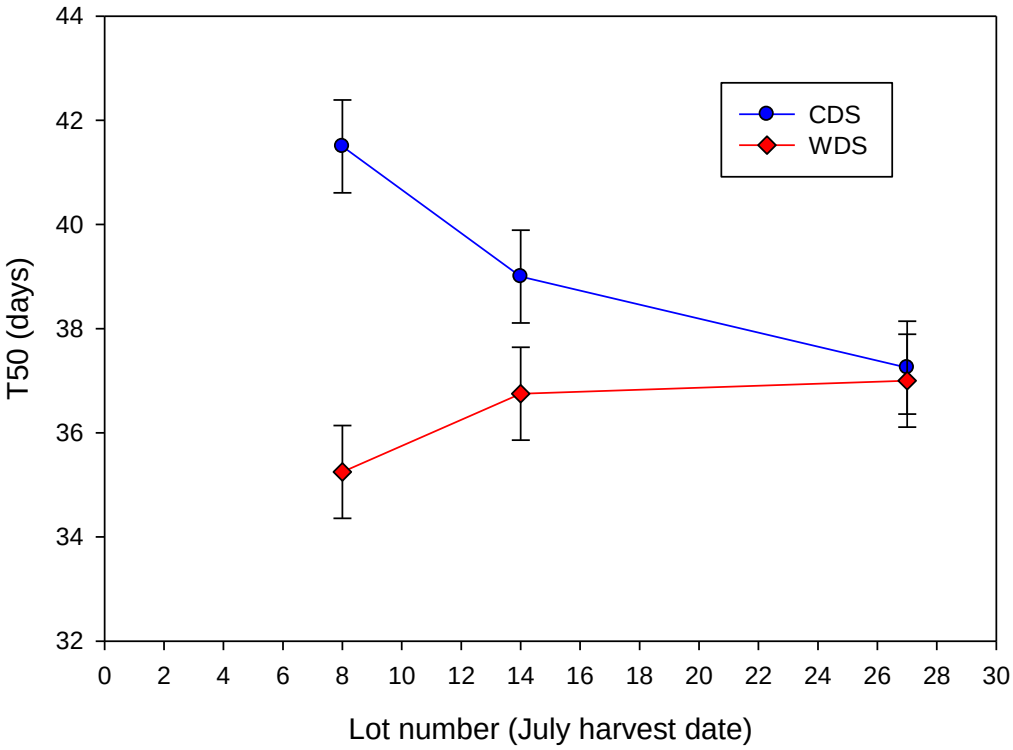

Figure 2.5. Effect of harvest date (July 8, 14, and 27th) and after-ripening temperature (CDS at 5± 1°C and WDS at 21± 1°C) on time (days) it takes to achieve 50% final germination (T50). ANOVA analysis with F=2.50, P=0.0168. Blue circles represent cold after-ripening (CDS). Red diamonds represent warm after-ripening (WDS). Symbols represent least square means. Error bars indicate 95% confidence at the 0.05 level.

There was a significant three-way interaction in germination rate (T90) for after-ripening, harvest date, and GA (Table 2.7 APPENDIX C) (Figure 2.6). However, the T90 values do not accurately depict the values of the control (cold after-ripening at 0 GA and warm after-ripening at 0 GA), as this measurement does not account for the increased delay in germination for the control seeds, compared to all other treatments. The seeds in the control began to germinate 2 to 5 weeks after seeds exposed to GA had already started germinating. The delay in the initiation of germination and the low % final

germination for the control values caused T50 and T90 values to depict a fast germination rate, when in actuality; they represent a delay and decreased final germination percentage when compared to other treatments.

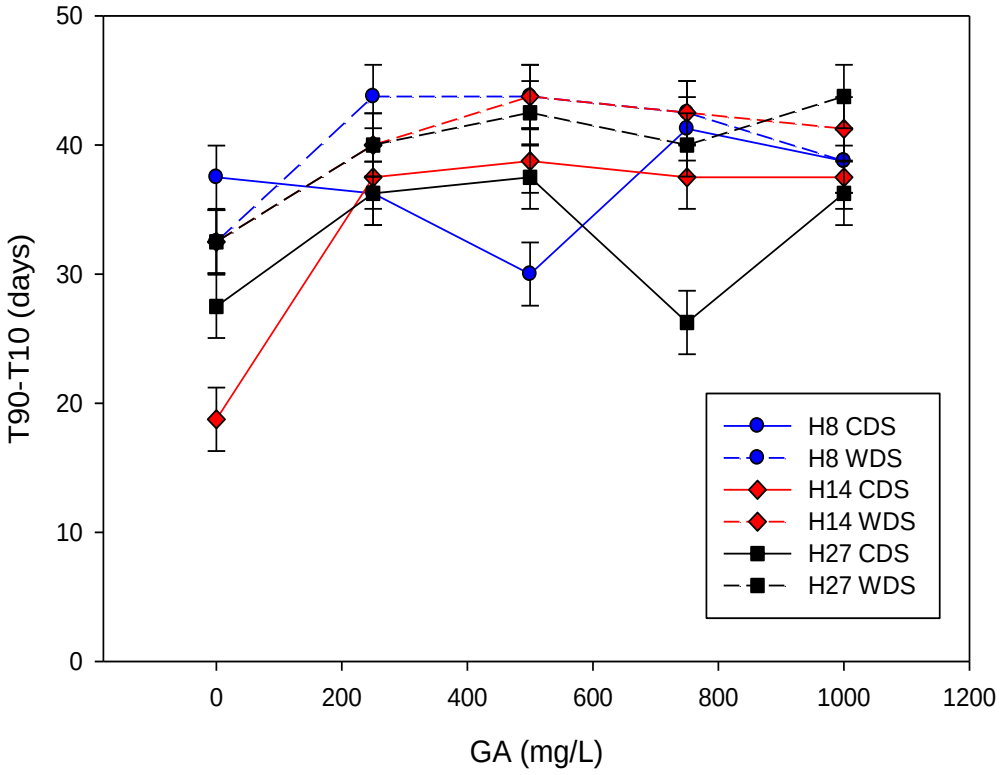

Figure 2.6. Effect of gibberellic acid (GA mg/L), harvest date (July 8th, 14th, and 27th), and after-ripening temperature (CDS at 5± 1°C and WDS at 21± 1°C) on time (days) it takes to achieve between 10% and 90% of final germination (T90). ANOVA analysis with F=3.31, P=0.0023. Solid lines represent cold after-ripening (CDS) and short-dashed lines represent warm after-ripening (WDS). Blue circles represent harvest date of July 8th (H8). Red diamonds represent harvest date of July 14th (H14). Black squares represent harvest date of July 27th (H27). Symbols represent least square means. Error bars indicate 95% confidence at the 0.05 level.

Discussion

Seed Dormancy Classification Process

Classifying seed dormancy to specific level and type often requires the examination of multiple physiological mechanisms or dormancy-breaking requirements, all which may occur simultaneously. Multiple dormancy mechanisms present in different proportions present challenges to the classification of dormancy (Schwienbacher et al. 2011). This is evident especially when attempting to distinguish between different levels and types of physiological dormancy. As physiological dormancy is defined by a number of factors such as; 1) developmental state of embryo when removed from mother plant 2) physical attributes of the seed, and 3) response of the mother plant and seed to environmental stimuli, it becomes difficult to confirm a clearly defined level and type to the class of physiological dormancy (Finch-Savage and Leubner-Metzger 2006).

A lack of a clear level and type classification of physiological dormancy was apparent in the classification process for arrowleaf balsamroot seeds. In this study, multiple types within more than one level of physiological dormancy were supported. In order to clarify the level and type of seed dormancy, the classification scheme by Baskin and Baskin (1998, 2004) and reclassification scheme by Schwienbacher (2011) was utilized. Under the Baskin and Baskin (1998, 2004) classification scheme, the following levels and types of physiological dormancy were characteristic of arrowleaf balsamroot seeds: nondeep physiological dormancy type 2, intermediate physiological dormancy type 2, and deep physiological dormancy type 1 and 3. Under the reclassification scheme

by Schewienbacher et al. 2011 seeds of arrowleaf balsamroot suggest deep physiological dormancy, type 3.

Deep Physiological Dormancy. Arrowleaf balsamroot displayed deep physiological dormancy, type 1 and type 3.

Deep physiological dormancy, types 1 and 3, was supported as the following type requirements: for type 1: excised embryos produced abnormal seedlings, and for type 3: 3 to 4 months of cold: moist stratification was required to break dormancy.

However, type 2, which occurs when GA does not promote germination, was not exhibited as the treatment of GA resulted in % final germination > 80%.

Under the Schwienbacher (2011) reclassification scheme, deep physiological dormancy type 3, which occurs when seed exposed to an after-ripening time period of a minimum of 133 days, to a maximum of 148 days, achieve a % final germination of less than 50%. This was supported as the final germination experiment (experiment 6), with an after-ripening period of 378 days at 5±1°C, achieved a final germination of 4.1% ± 2.4% for cold after-ripening and 6.7 ± 2.4% for warm after-ripening.

Intermediate Physiological Dormancy. Arrowleaf balsamroot displayed intermediate physiological dormancy type 2.

Intermediate physiological dormancy type 2, which occurs when GA promotes germination, was supported by the result of greater than 80% germination with GA treatment.

However, intermediate physiological dormancy type 1, 3, and 4, were not supported, as the following type requirements did not occur: type 1: which occurs when excised embryos produce normal seedlings, was not supported as excised embryos exhibited abnormal qualities, type 3: which occurs when 2 to 3 months of cold scarification breaks dormancy, was not supported as low germination percentages < 20% were exhibited, and type 4: which occurs when dry storage (after-ripening) shortens the cold: moist stratification period, was not supported as 40 and 80 days of after-ripening resulted in a low final germination percentage of < 2.5%.

Nondeep Physiological Dormancy. Arrowleaf balsamroot seeds displayed nondeep physiological dormancy type 2.

Nondeep physiological dormancy type 2, which occurs when GA promotes germination, was supported as treatment of GA resulted in greater than 80% germination.

However nondeep physiological dormancy types 1, 3, and 4 were no supported, as the following type requirements did not occur: type 1: which occurs when excised embryos produce normal seedlings, was not supported as excised embryos exhibited abnormal qualities, type 3: which occurs when cold: moist stratification (0 to 10°C) or warm: moist stratification (> 15°C) breaks dormancy, did not occur as stratification temperatures obtained a mean final germination percentage of 2.5%.

Nondeep physiological dormancy type 4, which occurs when seeds after-ripen in dry storage, was not supported as after-ripening (after-ripening periods of 20, 40, 80, 120, and 240 days) did not significantly promote germination with a % final germination of < 2.5%.

Physical, Morphological and Morphophysiological Dormancy. The ability of arrowleaf balsamroot to uptake water with an intact seed coat suggests that arrowleaf balsamroot seeds do not have physical dormancy. Arrowleaf balsamroot seeds exhibited a fully developed and differentiated embryo, with little to no endosperm, which is not indicative of morphological and morphophysiological seed dormancy.

Reclassification. Utilizing the Baskin and Baskin (1998) classification scheme, arrowleaf balsamroot seed dormancy is indicative of nondeep physiological dormancy type 1, intermediate physiological dormancy type 2, and deep physiological dormancy types 1 and 3. This may be due to the difficulties in clearly defining a level and type to the class of physiological dormancy. The challenges with clearly defining a level and type to the class of physiological dormancy may be evident by the conflicting guidelines which have been utilized to classify the levels and types of physiological dormancy. In the Baskin and Baskin (1998, 2004) classification scheme, GA does not promote germination of seeds exhibiting deep physiological dormancy.

However, in Schwienbacher (2011), after-ripening is identified as a precondition, seeds which are exposed to a range of 133 to 448 days of after-ripening at 4°C, and do not achieve greater than 50% germination as a result of after-ripening, are classified under deep physiological dormancy. After 378 days of warm and cold after-ripening, arrowleaf balsamroot seeds exposed to the warm temperature achieved a final germination percentage of 4.1% ± 2.4% and seeds exposed to the cold temperature achieved a final germination percentage of 6.7% ± 2.4%.

Based on less than 10% final germination after 378 days of after-ripening, the Schwienbacher (2011) reclassification scheme suggests arrowleaf balsamroot seeds exhibit deep physiological dormancy. Schwienbacher (2011) does not consider GA and excised embryos a part of the classification scheme.

From an ecological perspective, the classification of arrowleaf balsamroot seed dormancy as deep physiological dormancy type 3 may be supported. Baskin and Baskin (2004) has documented the requirement of a longer period of cold: moist stratification as an adaptive strategy for survival in specific environmental conditions, such as a lengthy, cold winter, which is characteristic of the Intermountain West. The ability of arrowleaf balsamroot to germinate at lower temperatures is also characteristic of deep physiological dormancy, as species displaying this type often germinate in colder spring temperatures (Baskin and Baskin 2004).

Conclusion

With treatment of GA final germination percentage of arrowleaf balsamroot seeds was successfully doubled compared to previous studies. In the current study, treatment with GA, in combination with 378 days of after-ripening, increased germination to greater than 80%, and germination rate (T50) was highest with the combination of 750 to 1000 mg/L GA treatment under the influence of harvest date, and after-ripening temperature.

Upon examination of the classification of arrowleaf balsamroot seed dormancy to the specific level and type of physiological dormancy, nondeep physiological dormancy

type 2, intermediate physiological dormancy type 2, and deep physiological dormancy types 1 and 3 was indicated, utilizing the Baskin and Baskin classification scheme (1998, 2004). The Schwienbacher (2011) scheme suggests that arrowleaf balsamroot seeds are most closely aligned with the class and level, physiological deep dormancy.

CHAPTER 3

GREENHOUSE PROPAGATION OF ARROWLEAF BALSAMROOT

Abstract

The objective of this study was to evaluate greenhouse growth conditions for the propagation of arrowleaf balsamroot (*Balsamorhiza sagittata*). Upon plug establishment, seedlings were transplanted into 4-inch pots square pots or Cone-tainer cells and fertilized with 20-10-20 NPK synthetic, water-soluble fertilizer (Peat-Lite Special, Jack's Professional ™) at 5 rates (0, 50, 100, 200, 400 mg nitrogen /L). Plants were fertilized 1 time per week for a total of 98 days in the greenhouse. Based on shoot dry weight (SDW), leaf width, and length measurements, rates of 200 to 400 mg nitrogen /L resulted in greatest positive shoot growth. Supplemental nitrogen beyond 100 mg nitrogen/L may have decreased plant health by decreasing root mass. Based on the root morphology, root dry weight, and a positive growth response in leaf width, the Cone-tainer is an effective container type for propagating arrowleaf balsamroot in the greenhouse.

Introduction

Arrowleaf balsamroot is an Intermountain West native perennial plant species with a showy, sunflower-like flowerhead, stabilizing taproot, high drought tolerance, and high ecological value as a year-around food source for domestic livestock and undomesticated wildlife (Wasser 1982; McWilliams 2002; Chambers 2006). This species

has been identified as a valuable restoration species and also as a native plant with a high horticultural potential (McWilliams 2002; Chambers 2006).

Utilization of native plant material may enhance native pollinator habitat, ecological stability, reduce the need for chemical pest, and reduce fertilizer use, thus limiting resource consumption and pollution (Meyer et al. 2005; Erikson 2008). Furthermore, use of native plant material increases the sustainability of small-scale and large-scale agricultural operations, reduces consumption of non-renewable resources such as gas used to transport plant materials and ultimately may promote the long-term safety and health of both humans and the environment (Potts et al. 2002; Meyer et al. 2005; Erikson 2008; Peppin et al. 2010).

Due, in part, to growing consumer awareness of sustainability issues, there is a growing demand for native plants (Potts et al. 2002). A number of surveys have identified a clear market demand for native plants from both growers and homeowners (Meyer et al. 2005; Helfand et al. 2006; Larsen and Harlan 2006). In the last 30 years, national policy has increasingly focused on encouraging federal land management agencies to use native plant material in large-scale restoration projects (Peppin et al. 2010). Increasing popularity of native plants has helped create greater demand for native plant material (NPM) (Potts et al. 2002). Yue (2011) found that native plants have an average premium of $0.35 per plant compared to non-native plants. In the same survey 92% of the respondents stated that they would be willing to pay the $0.35 premium for native ornamental plant species vs. non-native or invasive species (Yue et al. 2011).

Rapid production of native plants in a commercially viable setting is often lacking due to limited knowledge regarding the propagation of these plants. This paucity of knowledge has been identified as one of the most significant limitations to producing native plant material (Potts et al. 2002; Lynn et al. 2008; Brzuszek and Harkess 2009; Perez et al. 2010; Peppin et al. 2010). Greenhouse production protocols for optimizing growth of many native plant species remain unknown. Further research is needed to establish greenhouse production protocols for one such species, arrowleaf balsamroot. The purpose of this project was to identify basic production requirements and greenhouse growth conditions for arrowleaf balsamroot, specifically, to examine the effect of fertilizer rate and container-type on arrowleaf balsamroot in the greenhouse.

Materials and Methods

Arrowleaf balsamroot seeds were germinated in a germination box (cut comb honey container - 4-5/16” x 4-5/16” x 1-3/8” © Pioneer Plastics Inc.) containing sterile blue blotter paper. After successful germination seeds were transplanted into 150 cell plug trays (4.5 cm x 4.5 cm cells) in Sunshine Mix #1 ™ (SunGro Horticulture ™) and grown to the cotyledon stage. Twenty eight days after sowing, cotyledons which exhibited their first true primary leaf were then transplanted into two types of growth containers; a standard 4-inch square pot (670 ml) or a Ray Leach Cone-tainer (164 ml), filled with Sunshine Mix #1 ™.

Plants in both container types were manually fertigated one time per week with 20-10-20 NPK with water-soluble fertilizer (Peat-Lite Special, Jack’s Professional) at

rates of 0, 50, 100, 200 and 400 mg nitrogen/L. Fertilizer rates (mg nitrogen/L) were chosen to mimic commercial bedding plants. Although highly dependent on species, growth stage, and additional production protocols, Bailey (1996) recommends utilization of 20-10-20 NPK at 50 to 250 mg/L of nitrogen and potassium fertilizer rates for bedding plants which may need less nutrients and those species which may require a higher concentration would require 20-10-20 NPK at 200 to 400 mg/L of nitrogen and potassium. Every fourth irrigation cycle plants were irrigated with water and included a leaching percentage (the percentage of irrigation solution that drains from the container after irrigation) of 10 to 15% per container.

Fifteen replicates were completely randomized on a greenhouse bench. Mean ambient air temperature of $30^{\circ}/21^{\circ}$C day/night. The experiment was initiated in July 2013 and continued for 98 days, at which point plants health reached a critical point and seemed likely to decline with increased leaf senescence with greater than the 98 day time period in the greenhouse. Average day length from July 2014 to October 2014 ranged from 13 hours and 30 min to 11 hours 55 minutes. Length (mm) and leaf width (mm) were measured once per week using a digital caliper. After 98 days, plant shoots were separated from the roots, roots were washed, and shoots and roots were immediately dried in paper bags at 54^{0}C for 48 hrs. The shoot dry weight (SDW) and root dry weight (RDW) in grams was determined using a Mettler Toledo AG104 balance. The root-to-shoot ratio was calculated as a plant quality measurement assessing root health.

The experiment was designed as a randomized complete factorial experiment with 2 factors (container type and fertilizer) and multiple levels per factor, with container type

at two levels (4-inch square pot and Cone-tainer) and fertilizer at 5 rates (0, 50, 100, 200, and 400 mg nitrogen/L). Leaf width and height measurements was analyzed between and within 10 time periods (weeks) through the REPEATED statement in PROC GLM (SAS University Ed. 9.11). Biomass measurements were analyzed in PROC GLM (SAS University Ed. 9.11). All pairwise combinations were examined for utilizing Tukey's honestly significant difference test with a significant level of α=0.05.

Results and Discussion

Container Type:

In examination of the effect of container type on leaf measurements for potted arrow-leaf balsamroot, leaf width measurements exhibited increased growth in the Cone-tainer compared to the 4-inch square pot (Figure 3.1) (Table 3.2 APPENDIX D).

Container type did not have a statistical significant effect on SDW (Table 3.3 APPENDIX D). The RDW and SDW graphically depicted as a root-to-shoot ratio exhibited significantly greater decreased root-to-shoot ratio in the 4-inch square pot compared to the Cone-tainer (Figure 3.2) (Table 3.5 APPENDIX D).

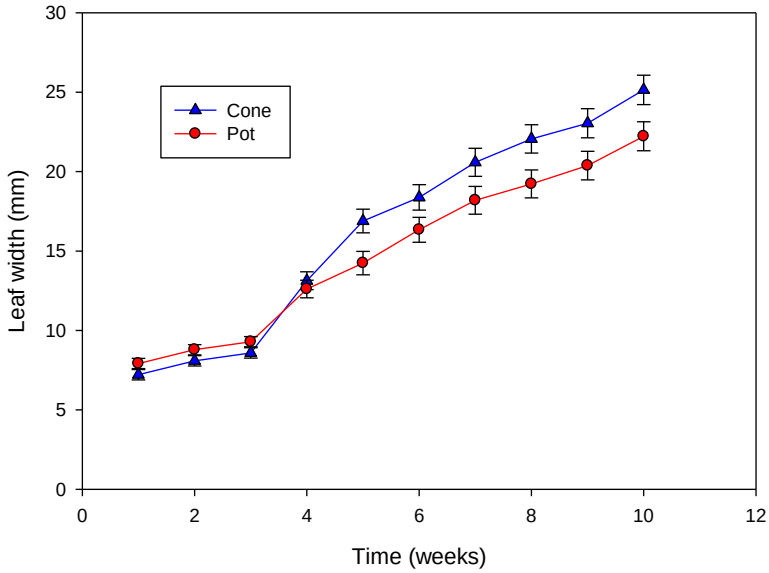

Figure 3.1. Effect of container type on leaf width. ANOVA analysis with F=5.08, P=0.0259. Blue upward facing triangles indicate least-square means for the Cone-tainer (cone). Red circles indicate least square means for the 4-inch square pot (pot). Error bars indicate 95% confidence at the 0.05 significance level.

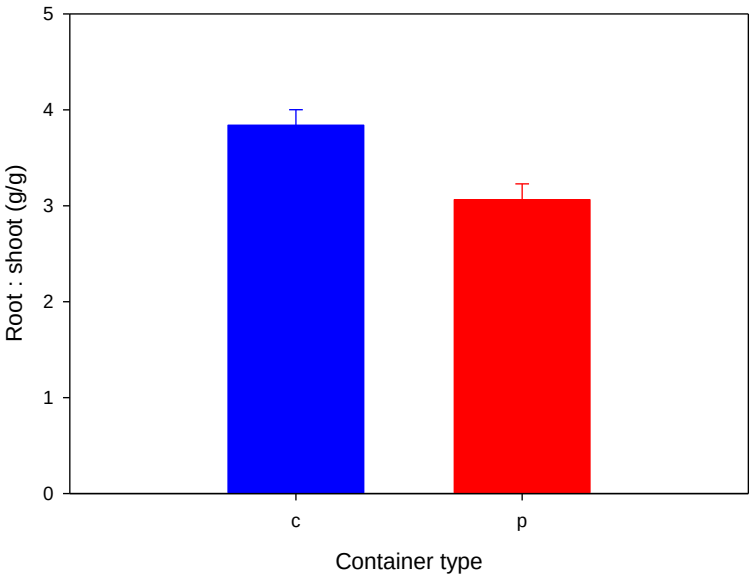

Figure 3.2. Effect of container type on root: shoot ratio. ANOVA analysis with F=12.53, P=0.0006. Blue bar indicates least-square means for Cone-tainer (c). Red bar indicates least square means for 4-inch square pot (p). Error bars indicate 95% confidence at the 0.05 significance level.

Container type appears to be an important variable to consider when growing native plants in the greenhouse as it may influence growth, production efficiency, and also out-planting success (Landis and Simonich 1984). Many native plants may require a longer production period than their domesticated counterparts so any advantage that decreases production time is valuable in a native plant nursery.

Size, shape, root control features, cost, support, and ease of handling are important characteristics to consider when selecting containers for plant growth and production. For example, a 4-inch pot is shallow and wide, without root control features, and may be better for plants with a small, more fibrous root system. The Cone-tainer type is deeper, with anti-spiraling ridges that encourage taproot growth while inhibiting the spiraling of roots. Container type should be carefully considered and should meet the growth characteristics and requirements of the species (USFS 2014).

Arrowleaf balsamroot has a taproot which may reach up to 2.44 meters long (Wasser 1982; McWilliams 2002). When considering container characteristics such as; shape and size, root control features, cost, and support and handling, Cone-tainer type has an advantage over 4-inch pots by providing depth for the taproot development of arrowleaf balsamroot, reduce cost through saving space, and are easier to handle due to the cells being culled and consolidated (USFS 2014).

Fertility: The effect of fertility on leaf measurements for potted arrowleaf balsamroot, leaf length did not show a statistical significant growth response to fertility (Table 3.1 APPENDIX D). At all levels of fertility (50, 100, 200, and 400 mg nitrogen/L) compared to the control, leaf width showed a significant positive growth

response (Figure 3.3) (Table 3.2 APPENDIX D). Between nitrogen levels, there was only a slight significant increase in leaf width growth response (Figure 3.3) (Table 3.2 APPENDIX D).

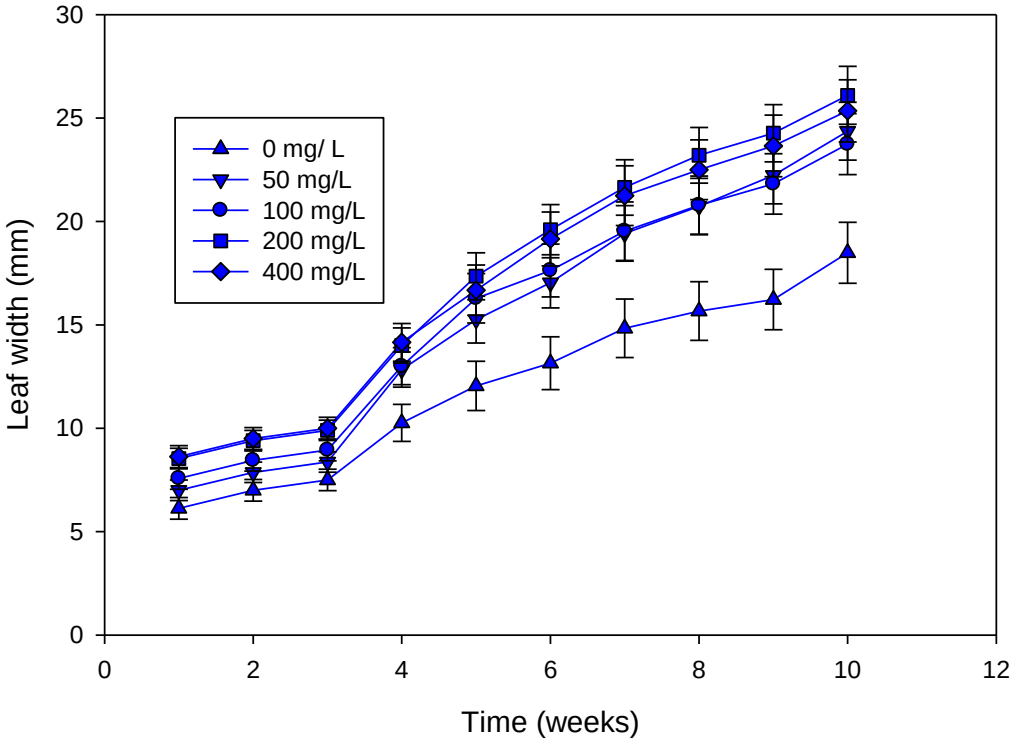

Figure 3.3. Effect of fertilizer on leaf width. ANOVA analysis with F=4.2, P=0.0031. Blue symbols indicate least-square means for container type (Cone-tainer and 4-inch square pot). Upward triangles indicate 0 mg nitrogen/L. Downward triangles indicate 50 mg nitrogen/L. Circles indicate 100 mg nitrogen/L. Squares indicate 200 mg nitrogen/L. Diamonds and error bars indicate 400 mg nitrogen/L. Error bars indicate 95% confidence at the 0.05 significance level.

Increasing nitrogen levels at 50, 100, 200, and 400 mg nitrogen/L showed increased shoot dry weight (Figure 3.4) (Table 3.3 APPENDIX D). However, shoot dry weight between rates of 50 and 100 mg nitrogen/L, and 200 and 400 mg nitrogen/L did

not demonstrate a statistically significant difference. Supplemental fertilizer ranging from 200 to 400 mg nitrogen/L showed the greatest positive shoot dry weight effect. Greater than 100 mg nitrogen/L resulted in a statistically significantly negative response in the root-to-shoot ratio (Figure 3.5) (Table 3.5 APPENDIX D).

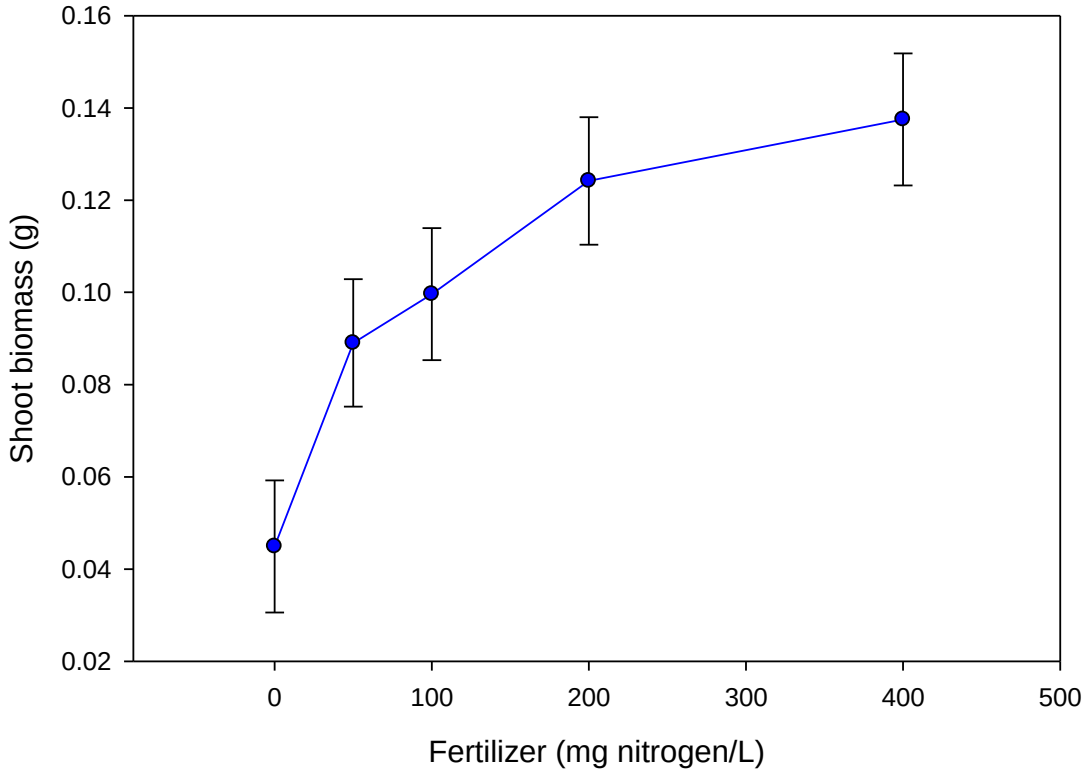

Figure 3.4. Effect of fertilizer on shoot dry weight. ANOVA analysis with F=6.36, P=0.0001. Blue circles indicate least-square means for fertilizer at 0, 50, 100, 200, and 400 (mg nitrogen/L). Error bars indicate 95% confidence at the 0.05 significance level.

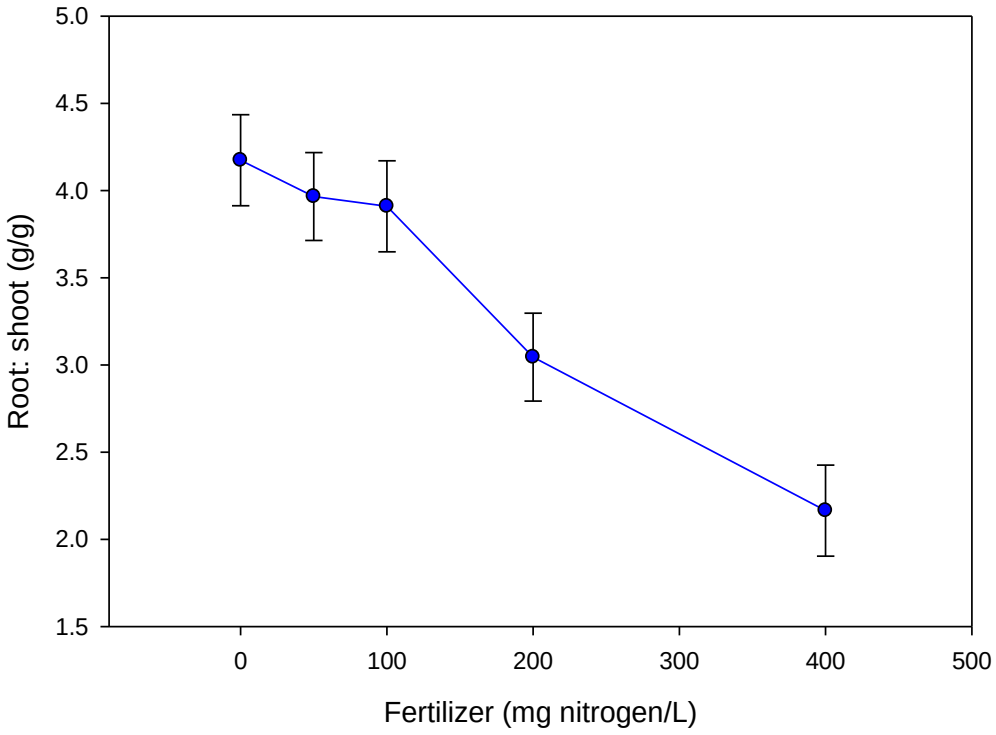

Figure 3.5. Effect of fertilizer on root: shoot ratio. ANOVA analysis with F=10.56, P=<0.0001. Blue circles indicate least-square means for fertilizer at 0, 50, 100, 200, and 400 mg nitrogen/L. Error bars indicate 95% confidence at the 0.05 significance level.

In general, increasing nitrogen levels encouraged root growth of arrowleaf balsamroot and increased caudex mass and depth of rooting. However, the growth response at higher fertility was closely connected to container type, highlighting the importance of the interaction between container type and fertility levels.

Container Type by Fertilizer Interaction: The effect of the significant interaction between container type and fertility on leaf measurements, leaf length measurements showed a significant negative growth interaction in the 4-inch square pot at high fertility levels (200 and 400 mg nitrogen/L) (Figure 3.6) (Table 3.1 APPENDIX D).

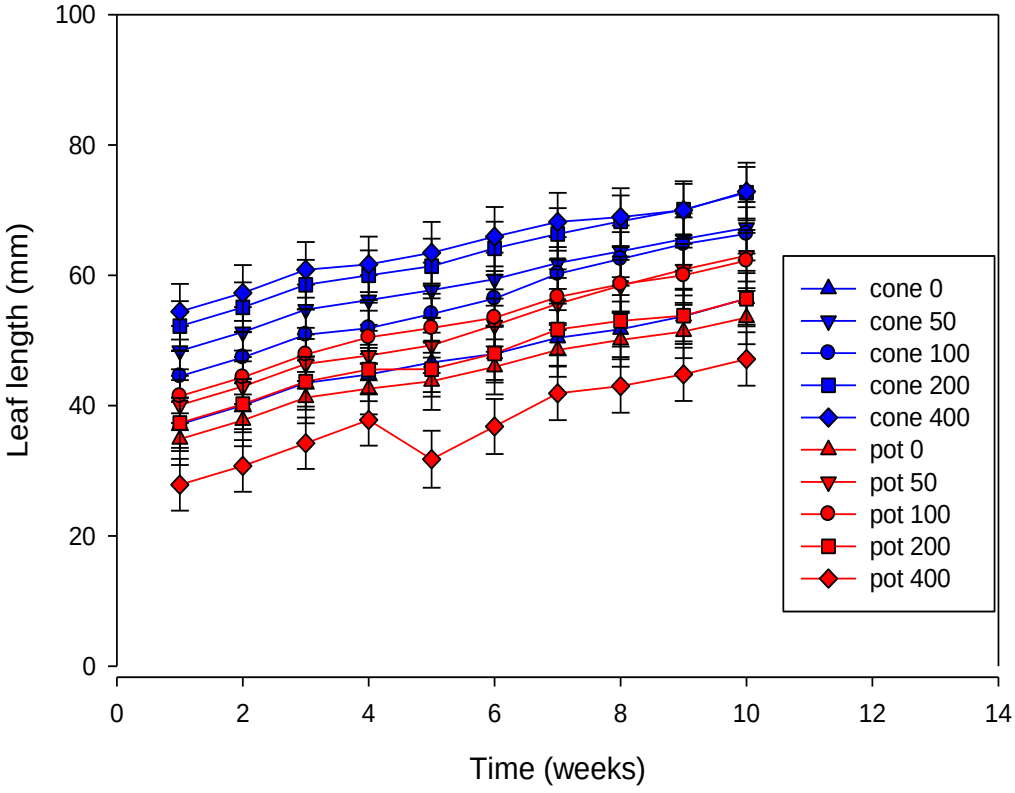

Figure 3.6. Effect of container type (cone, pot) and fertilizer (0, 50, 100, 200, and 400 mg nitrogen/L) on leaf length. ANOVA analysis with F=2.87, P=0.0256. Blue symbols represent the Cone-tainer (cone). Red symbols represent the 4-inch square pot (pot). Upward triangles indicate 0 mg nitrogen/L. Downward triangles indicate 50 mg nitrogen/L. Circles indicate 100 mg nitrogen/L. Squares indicate 200 mg nitrogen/L. Diamonds indicate 400 mg nitrogen/L. Symbols represent least square means. Error bars indicate 95% confidence at the 0.05 significance level.

There was no statistically significant container type-by-fertilizer interaction for leaf width measurements (Table 3.2 APPENDIX D). There was no significant container type-by-fertilizer interaction for SDW (Table 3.3 APPENDIX D). Root dry weight showed a significant positive growth interaction in the Cone-tainer and a significant negative growth interaction in the 4-inch square pot at high fertility levels (200 and 400

mg nitrogen /L) (Figure 3.7) (Table 3.4 APPENDIX D). There was no significant container type-by-fertilizer interaction for the root-to-shoot ratio (Table 3.5 APPENDIX D).

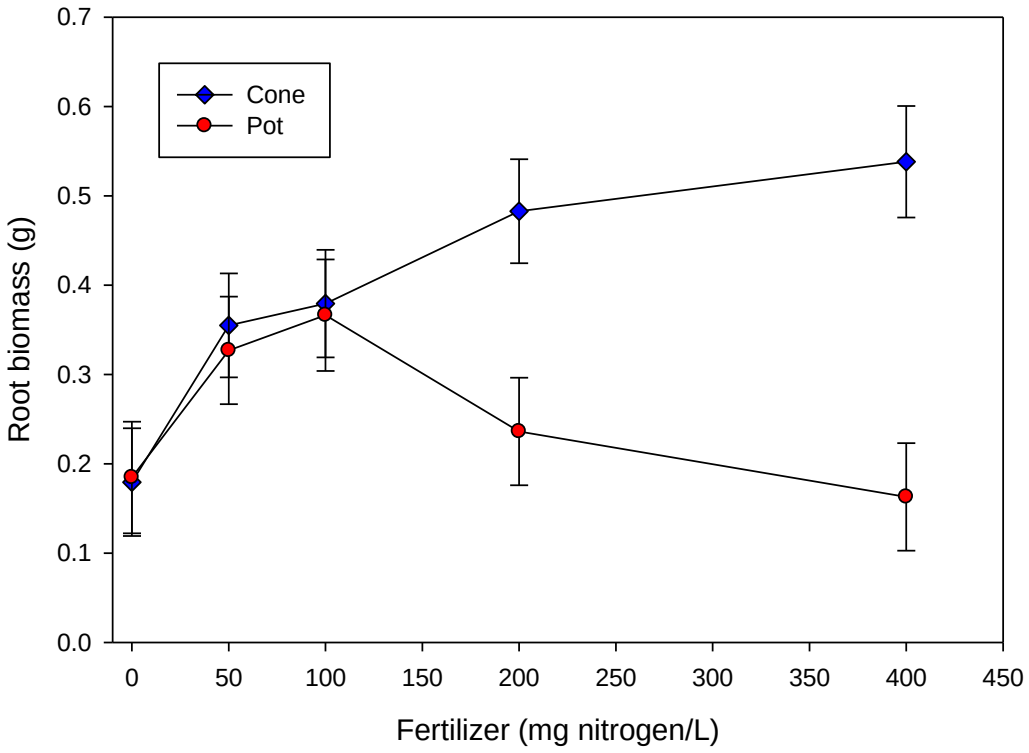

Figure 3.7. Effect of container type and fertilizer rate (mg nitrogen /L) on root dry weight (g). ANOVA analysis with F=3.91, P=0.005. Blue diamonds indicate least square means for the Cone-tainer (cone). Red circles indicate least square means for the 4-inch pot (pot). Error bars indicate 5% confidence at the 0.05 significance level.

Arrowleaf balsamroot requires a long establishment period (6 to 8 years) with slow shoot growth and winter senescence (McWilliams 2002). Although shoot growth is slow, the taproot has been found to grow more quickly (McWilliams 2002). An important vegetative structure of arrowleaf balsamroot is the caudex, which consists of stem tissue

that stores carbohydrates and supports rapid regrowth under other favorable conditions (Wischnofske et al. 1983; McWilliams 2002; Flann 2013).

In the greenhouse, the statistical significant container type-by-fertilizer interaction for leaf measurements may be partly due to the senescence and regeneration which was observed with higher nitrogen levels in only the 4-inch square pots. At 400 mg nitrogen/L a total of four plants senesced and then regrew from the caudex six weeks after the development of the first primary leaf. Additionally, at 200 mg nitrogen/L one plant senesced and regrew. Senescence and regeneration at the higher nitrogen levels decreased the mean growth response of leaf measurements to fertilizer, lowering the significance of the effect of 400 mg nitrogen/L on leaf measurements and SDW (Figure 5). The significant interaction shown in the almost doubled negative reduction in root dry weight in the 4-inch square pot compared to the Cone-tainer at 200 and 400 mg nitrogen/L highlights how arrowleaf balsamroot performs better in the Cone-tainer at higher fertility. Decreased RDW in the 4-inch pots at higher nitrogen levels could be indicative of root spiraling and root rot caused by the decreased drainage in 4-inch pots and the lack of anti-spiraling features.

The statistically significant negative interaction in the root-to-shoot ratio at higher fertility rates suggest that rates above 100 mg nitrogen/L may result in decreased root mass incapable of supporting an increased shoot mass. An increased SDW and decreased RDW with higher supplemental nitrogen levels may lead to a less transplantable product as the smaller root system may not as effectively support a larger root system once transplanted. This decrease in root-to-shoot ratio with increasing nitrogen levels was

expected in a containerized seedling exposed to higher rates of nitrogen. Landis and Simonich (1984) reported that although synthetic fertilizers allow growers to quickly produce plants for nursery production, excess fertilization may be toxic and reduce plant growth.

Conclusion

Although arrowleaf balsamroot is categorized as a slow-growing native plant species, a significant positive increase in shoot mass may be achieved with increasing fertility applied through manual fertigation at rates of 50, 100, 200, and 400 mg nitrogen/L. A significant negative interaction in shoot mass may occur in a 4-inch square pot at higher fertility of 200 to 400 mg nitrogen/L. The significant negative effect of higher fertility levels on root-to-shoot ratio is exhibited in a negative shoot-to-root ratio with increasing fertility. This displays how fertility beyond 100 mg nitrogen/L may lead to a less transplantable product. The significant negative effect of the 4-inch square pot is shown in a reduced root-to-shoot ratio in the 4-inch square pot. The increased positive response of the root-to-shoot ratio and the shoot mass supports how the Cone-tainer appears to better than the 4-inch square pot for propagating arrowleaf in the greenhouse.

CHAPTER 4

SEED DORMANCY OF SILVERLEAF PHACELIA

Abstract

Silverleaf phacelia (*Phacelia hastata* var. *hastata*) is a versatile and hardy native plant species, with an aesthetically pleasing flower, silver-tinged leaves, and the ability to respond positively to nursery propagation protocols. However, attempts to germinate this species without suitable pretreatment have resulted in low and erratic germination due to seed dormancy. Although previous studies have identified the seed dormancy class of silverleaf phacelia to be physiological, further research is needed. In this study a minimum mechanical scarification increased germination rate and final germination percentage. Ninety seconds of scarification using new 40 grit sandpaper produced a maximum final germination of 87% ± 5%. Utilizing the Baskin and Baskin (1998, 2004) seed dormancy scheme to classify silverleaf phacelia seed dormancy, seeds exhibit nondeep physiological dormancy type 1, 2, and 5, and intermediate dormancy type 1 and 2. Based on the results of this study, addressing nondeep physiological dormancy type 5 would be the most effective method to break seed dormancy and allow more efficient propagation of silverleaf phacelia.

Introduction

Dormancy is the temporary failure of a seed to germinate (the emergence of the radicle from the embryo) even when the seed is exposed to favorable conditions for

germination (Baskin and Baskin 2001). Seed dormancy is caused by characteristics of the seed (Baskin and Baskin 2001; Finch-Savage and Leubner-Metzger 2006; Bewley et al. 2013). According to which characteristics may be causing dormancy, seed dormancy has been classified into five main classes: 1) physiological dormancy (PD), 2) morphological dormancy (MD), morphophysiological dormancy (MPD), physical dormancy (PY) and a combinational dormancy (PY + PD) (Nikolavea 1969; Baskin and Baskin 2004; Schwienbacher et al. 2011). These five classes of dormancy have been expanded upon so each class may have sublevels and subtypes to each level (Baskin and Baskin 2004).

Besides being a fascinating biological process for plant scientists and ecologists, the study of seed dormancy has many practical implications (Stoehr and El-Kassaby 2011). For example, the identification of seed dormancy through the classification process may be important for predictive plant establishment and survival analyses, especially in unique and changing climate conditions (Baskin and Baskin 2004; Bewley et al., 2013). However, seed dormancy may be a barrier to the propagation of many native wildflowers with potential for horticultural marketing, as many native wildflower species exhibit complex and difficult-to-break dormancy patterns (Cseresnyes 1979; Baskin and Baskin 1998).

Silverleaf phacelia (silverleaf scorpionweed) (*Phacelia hastata* var. *hastata*) is an Intermountain West native perennial forb in the waterleaf (Hydrophyllaceae) family which occurs at a broad elevation range of 899.77 to 2279.29 meters with a distribution from Southern British Columbia to the Sierras of California, and east to the Rocky Mountains (Hitchcock and Cronquist 1990). Silverleaf phacelia has numerous, tightly

coiled, small 3 to 7 mm calyx lobed lavender to white flowers. The inflorescence uncoils loosely from the center as the flowers mature (USDA 2015).

The silver tinge to the leaves and the loosely coiled inflorescence accounts for one of this species' common name, silverleaf scorpion weed. *Phacelia* is the largest genus in the waterleaf family and has 4 varieties: *Phacelia hastata* var. *charlestonensis*, *Phacelia hastata* var. *compacta*, *Phacelia hastata* var. *dasyphylla*, *Phacelia hastata* var. *hastata* (Glass and Levey 2011; Gilbert et al. 2005). Silverleaf phacelia and its varieties are found within valley grasslands, as well as mountainous foothills to rocky ledges in alpine regions (Mitchell et al. 1966; Bamberg and Major 1968; Morefield 1992; Bates et al. 2000; Glass and Ley 2011).

With growing environmental instability and disturbance, the need to restore dysfunctional ecosystems has become increasingly important (Clewell and Aronson 2013). Rehabilitation of disturbed areas due to waste and spoils from abandoned mine sites presents a challenge for national and state land management agencies. Native plant species which establish in areas of increased environmental degradation are economically valuable for rehabilitation purposes (Munshower 1994; Brown 1997). Wildfire is another form of disturbance which is projected to increase in arid regions (Beyers 2004). With increased fire occurrence, native plant material recognized as early colonizers may be economically valuable for revegetation of large burn areas. Recent changes in national policy have dictated that federal land management agencies give priority to use of native plant materials in revegetation activities of disturbed areas (Peppin et al. 2010). Many state land management regulations also require utilization of locally sourced native

species in site restoration (Roundy et al. 1997). This increased focus on using native plant material in revegetation activities has created a rising demand for locally-adapted native plant material (Peppin et al. 2010).

Silverleaf phacelia has been noted as an early colonizer after fire occurrence (Bates et al. 2000; Wayman and North 2006). Scianna (2004) has also listed silverleaf phacelia as a species which is tolerant of heavy metals. Similarly, Schladweiler (2005) noted that silverleaf phacelia represented 22% of the abundance of forbs growing on an abandoned coal mine in Wyoming. An additional study looking at restoration using native plant species on coal mine spoils in the Rocky Mountains of Canada, found that within four years following restoration, silverleaf phacelia was one of the dominant native species (Smyth 1997). Evidence suggests silverleaf phacelia is a valuable species for restoration due to its ability to colonize after fires and establish in disturbed areas and arid regions.

Silverleaf phacelia may have high potential to be sold in nurseries and landscape businesses due to its high establishment success, aesthetic value, and significance for pollinators. Floral species which provide pollinator habitat are economically valuable as they facilitate healthy pollinator populations for pollination of agricultural crops. The flower of silverleaf phacelia have been shown to host three pollinator specialists, Mason Bees (*Chelostoma phaceliae*), Sweat Bee (*Dufourea trochantera*), and Pollen Wasps (*Pseudomasaris zonalis*) (Yu 2014). Silverleaf phacelia may have a high potential to be sold in nurseries and landscape businesses due to its high establishment rates, aesthetic

value, and significance for pollinators. Nursery propagation of silverleaf phacelia in the Intermountain West may help fill a niche in the regional native plant market.

Attempts to propagate this species often result in initial low and erratic germination, and a longer delay than desirable for marketability. Dormancy may is one of the likely causes of production delays. Previous research has classified the dormancy of this species as physiological, stating that 60 days of cold, moist stratification prior to germination may overcome dormancy (USDA 2014). However, the level and type of physiological dormancy of silverleaf phacelia remains largely unknown. Issues related to unknown seed dormancy mechanisms may, however, limit initial acceptance of silverleaf phacelia in the horticultural market.

The aim of this study was to gain a better understanding of the seed dormancy release requirements and to increase the cumulative germination percentage of silverleaf phacelia. This was achieved through identification of the seed dormancy class, level, and type, along with identification of methods for seed dormancy release.

Materials and Methods

Seeds of silverleaf phacelia were collected in late August of 2013 at the U.S. Department of Agriculture Natural Resources Conservation Service, Bridger Plant Material Center ($45^0$17'11.42" N, $108^0$53'11.13" W) at an elevation of 1118 meters. The collection site was a study site used by Keating (2014). Seeds varied in maturity at collection date due to indeterminate flowering and seed shattering upon maturity. Capsules of a light to dark brown color were harvested by removing filled flower heads

from peduncles before the flower heads dehisced and shattered. Seeds were cleaned through a series of different sized screens and chaff was separated from seed using a South Dakota Seed Blower (Keating 2014). Seeds were exposed to different storage conditions over a period of four months from August to January 2014, after which they were stored at 5 ± 1^{0}C in paper envelopes for a period of one year (Keating personal comm. 2014).

Initial tetrazolium chloride (TZ) tests for seed viability were conducted, following the TZ Seed Testing Handbook protocols from the Association of Official Seed Analysts (ASOCA). Tests were conducted in January, 2014 after 12 months of storage and included 4 replicates of 100 seeds that were imbibed overnight in dH2O, and bisected longitudinally but left intact. Intact seeds were placed in a glass dish in 5 mL of 1% 2, 3, 5-triphenyltetrazolium chloride solution and incubated in the dark at 35°C for 24 hours. Dissected seed embryos were then examined under magnification at 10x. Missing, unstained, or all or partially black or yellow stained embryos were considered unviable. Embryos stained red to dark orange were considered viable (ASOCA, 1993).

A total of six seed germination experiments and one seed structure identification experiment were completed to classify level and type of seed dormancy and identify successful treatments to increase the final germination percentage of silverleaf phacelia. Unless otherwise noted all experiments followed general methods for seed germination with steps 1 through 7: 1) random selection of seeds, 2) surface sterilization of seeds utilizing a soak in a 15% bleach and deionized water solution for 10 minutes, 3) sterilization of germination container (cut comb honey container - 4-5/16” x 4-5/16” x 1-

3/8” © Pioneer Plastics Inc.) via bleach wash, 4) blue blotter paper sterilized for 15 minutes in an autoclave 5) surface sterilized seeds placed on blue blotter paper, 6) blue blotter paper moistened with deionized water or a growth regulator solution, and, 7) after the initial treatment, germination containers were moistened with deionized water as needed .

Experiments were designed as a complete factorial experiment with 4 replicates. Germinated seeds (radicle ≥ 1mm) were counted daily for 19 days (unless otherwise noted) which is when final germination percentage reached 80% or greater in the majority of treatments. After each count, seedlings were planted and growth was examined separately until normal primary leaf development occurred. At the end of the experiment, a TZ test was completed and non-viable seeds were not considered when calculating the final germination percentage. Data was analyzed using a generalized linear model (PROC GLM, SAS University Edition 9.3) with a repeated measures statement to account for the correlation between germination measurements over time. Where only one factor was examined, data was analyzed using a one-way analysis of variance (PROC ANOVA, SAS University Edition 9.3). Means were examined utilizing the least-square means, and all pair-wise combinations of all factors and levels were then examined with Tukey Kramer HSD at $\alpha=0.05$ significance level (LSMEANS, TUKEY ADJUST SAS University Edition 9.3).

Experiments to Classify Dormancy

Seed Structure Identification. The seed classification system of Martins (1964), revised by Baskin and Baskin (2007), was used to classify the size and structure of the seed (Forbis et al., 2002; Finch-Savage and Leubner-Metzger, 2006; Baskin and Baskin 2007).

Experiment 1: Imbibition. To examine water uptake by intact seeds, imbibition was measured. Four replicates of 25 randomly selected seeds were nicked with a scalpel. The seed coats of an additional four replicates of 25 randomly selected seeds remained intact. The initial dry mass (Wn) was measured in grams using a Mettler Toledo AG104 balance. After determining the average for initial mass (g), seeds were imbibed. At 5, 10, 15, 30, 45, 60, 90 and 1080 minutes, samples were taken out and the imbibed tissue was weighed (Wi); increase in fresh weight after imbibition was calculated using the formula [(Wi- Wn)/Wn] x 100.

Experiment 2: Excised Embryos. To determine whether excised embryos could germinate, four replicates of 25 seeds each were randomly selected, and the embryo was excised from the seed coat under a dissecting microscope using tweezers and a scalpel. Excised embryos were then placed in germination containers at 21 ± 2^{0}C, under ambient laboratory lighting. Germinated embryos were counted daily for 6 days.

Experiment 3: Gibberellic Acid (GA). To examine the effect of GA on germination, four replicates of 200 seeds each were randomly selected, and separately

exposed to four concentrations of GA at 0 (control), 250, 500, 750, or 1000 mg/L. Seeds were then placed in germination containers at 21± 2^0C, under ambient laboratory lighting. Germinated seeds were counted daily for 19 days.

Experiment 4: Cold: Moist & Warm: Moist Stratification. To examine the effect of cold: moist or warm: moist stratification, four replicates of 100 seeds were randomly selected, separately exposed to warm: moist stratification (WS) of 34± 1^0C for 8 days or cold: moist stratification (CS) of 4± 1^0C for 8 days, and control was incubated under an identical moist treatment, with a different temperature of 21± 2^0C, for a period of 8 days (control). Germinated seeds were counted daily for 19 days. Although a longer period of cold: moist stratification was not tested, a longer period of colder stratification was predicted to decrease seed viability and final germination percentage through the increased likelihood of entering conditional dormancy and increased mold growth.

Experiment 5: Mechanical Scarification & Stratification. Utilizing a mechanical seed scarifier (Seedburo Electric Seed Scarifier) with 40 grit sandpaper, four replicates of 200 seeds were randomly selected and scarified for 0, 90, 180, 270, or 360 seconds. Seeds were then exposed to cold: moist stratification at 4±1^0C for 0, 3, 5, and 8 days. Germinated seeds were counted daily for 19 days.

Experiment 6: Mechanical Scarification & Sandpaper Age. Utilizing a mechanical seed scarifier (Seedburo Electric Seed Scarifier) four replicates of 200 seeds were randomly selected and scarified at times of 0, 90, 180, 270 and 360 seconds, utilizing either older, worn 40 grit sandpaper, or new, unused 40 grit sandpaper. Seeds were then

germinated at 21± 2^0C, under ambient laboratory lighting. Germinated seeds were counted daily for 19 days. Final germination percentage, the number of days till 50% of the final germination percentage (T50), and the number of days between 10% and 90% of the final germination percentage (T90) were calculated for this experiment as this treatment yielded the most successful results.

Results

Based on the initial TZ testing, the % viability from 4 replicates of 25 seeds per replicate was at a mean pure live seed (PLS) % of 81%.

Experiments to Classify Dormancy

Seed Structure ID. The revised seed classification system of Martins (1946) identified the structure of the seed and the shape of the embryo as linear axile, fully developed, with endosperm present (Forbis et al. 2002).

Experiment 1: Imbibition. Fresh weight of all seeds after imbibition significantly increased in mass at all time periods (5, 10, 15, 30, 45, 60, 90 and 1080 minutes) compared to the unimbibed control for both scarified and unscarified seeds (Figure 4.1). Scarified seeds had a significantly greater mass than unscarified seeds at time periods of 15, 30, and 45 minutes. However, at 5 minutes, 60 minutes, and 1080 minutes the two treatments were very similar.

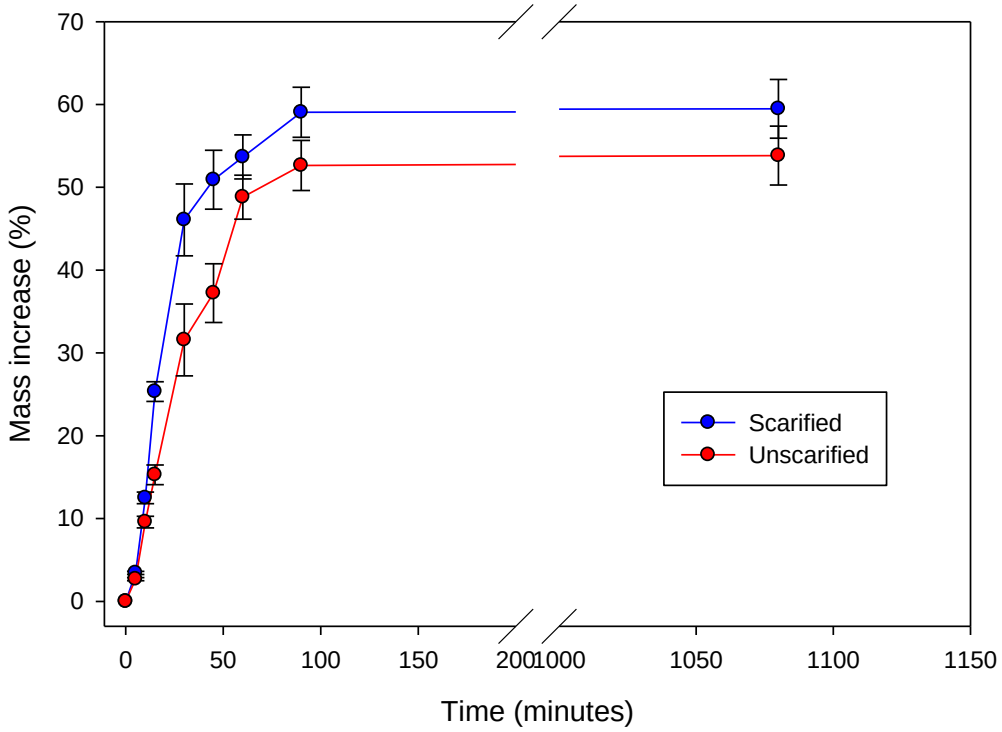

Figure 4.1. Effect of seed imbibition time (minutes) and scarification on increase (%) in mass (g) of seeds. Blue symbols represent means for mass (g) of scarified seeds. Red symbols represent means for mass (g) of unscarified seeds. Error bars indicate 95% confidence at the 0.05 significance level.

Experiment 2: Excised Embryos. Excised embryos had a final germination percentage of 100% by six days after TZ testing (Figure 4.2). Embryos exhibited normal development of radicle and cotyledons. Normal development of the first primary leaf was also exhibited by all surviving embryos.

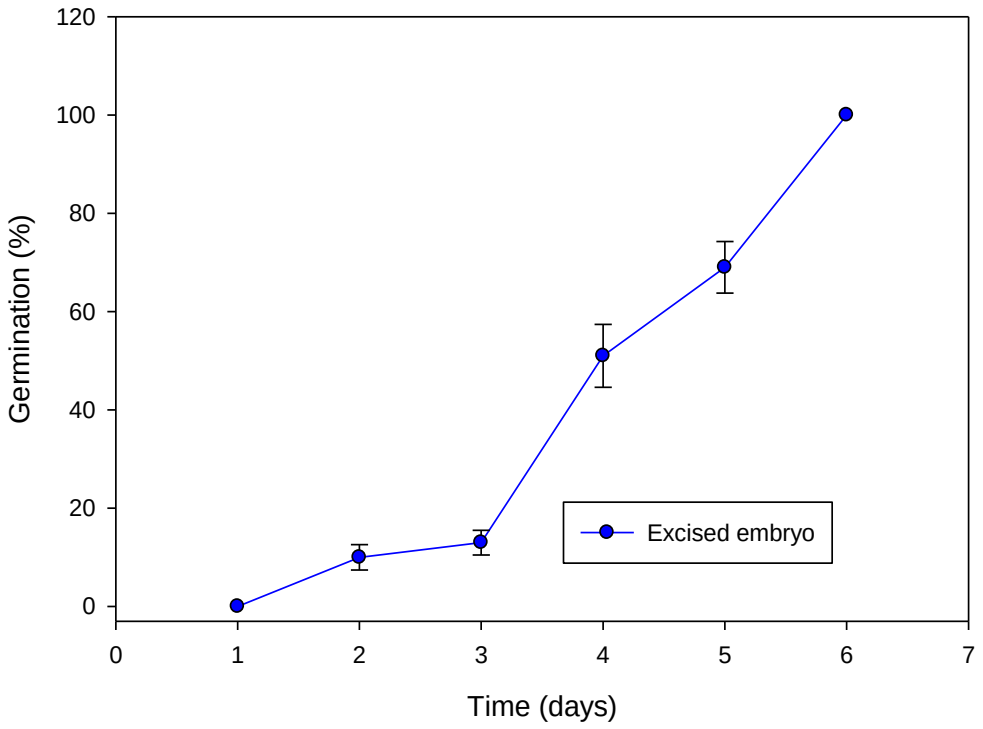

Figure 4.2. Final germination percentage of excised embryos after six days of incubation in ambient laboratory conditions at 21 ± 1^0C. Circles indicate means and error bars indicate the standard error.

Experiment 3: Gibberellic Acid (GA). Treatment with GA (mg/L) produced a significant increase in final germination percentage at all application levels of GA (250, 500, 750, 1000 mg/L) compared to the control level of 0 mg/L (Figure 4.3) (Table 4.1 APPENDIX E). Although not tested in this experiment, additional tests should explore whether the response in final germination percentage would continue to increase, reach a peak and decrease, with exposure to progressively higher levels of GA.

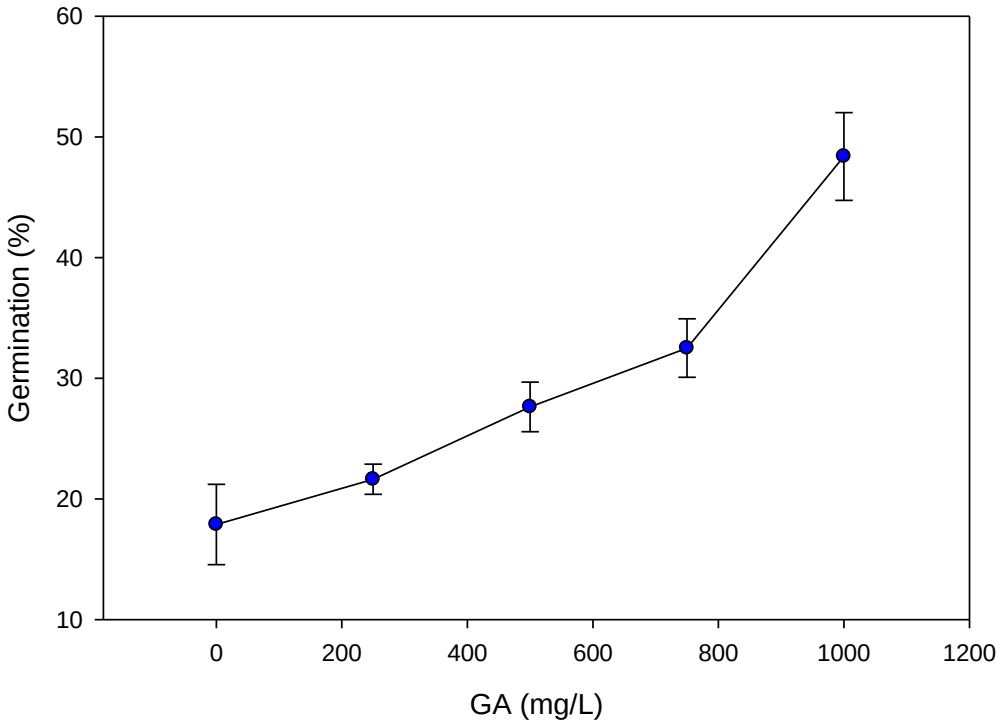

Figure 4.3. Effect of gibberellic acid (GA mg/L) on final germination percentage. ANOVA analysis with F=78.78, P=<.0001. Circles indicate means and error bars indicate 95% confidence at the 0.05 level.

Experiment 4: Cold: Moist & Warm: Moist Stratification. Cold: moist stratification (CS) resulted in the highest final germination percentage (11% ± 1.4%) which was significantly higher than final germination percentage at warm: moist stratification (WS) (2.3% ± 1%) (Figure 4.4) (Table 4.2 APPENDIX E). However, there was no significant difference in final germination percentage between the control (8.7% ± 1.7%) and the CS stratification treatment (Table 4.2 APPENDIX E). Cold: moist stratification delayed germination compared to no stratification treatment. Although a longer period of cold: moist stratification was not tested, results from 3, 5, and 8 days of

cold: moist stratification indicated that a longer period of cold: moist stratification may decrease seed viability with no significant positive effect on final germination percentage.

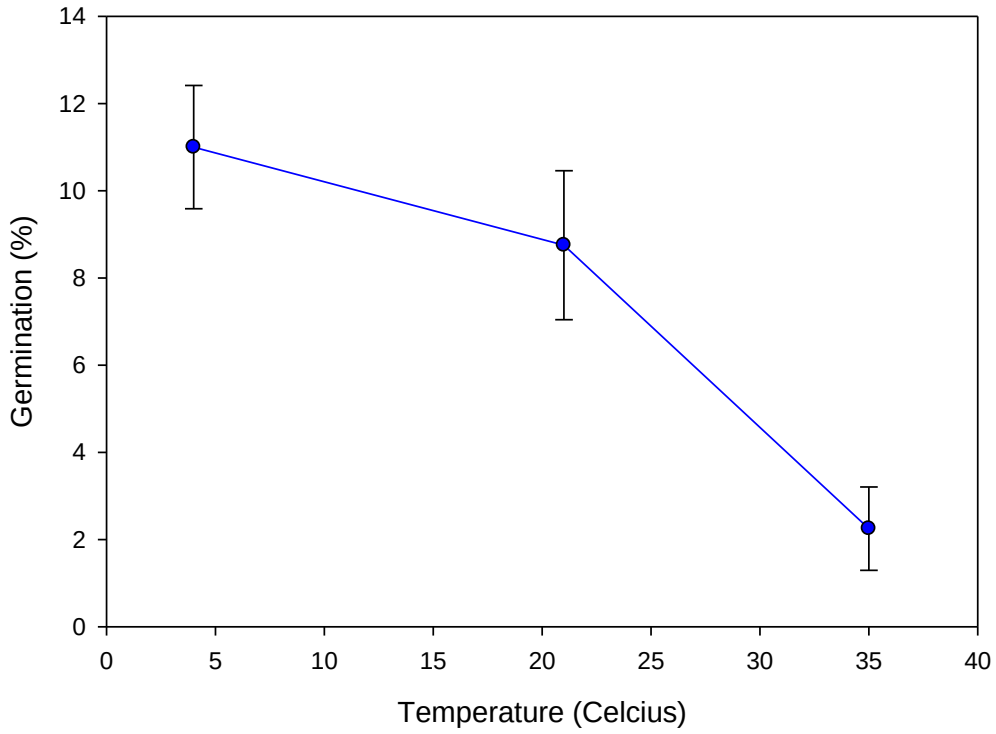

Figure 4.4. Effect of stratification temperature on final germination percentage. ANOVA analysis with F=42.47, P=<.0001. Circles indicate means and error bars indicate 95% confidence at the 0.05 level.

Experiment 5: Mechanical Scarification & Stratification. All levels of scarification (90, 180, 270, 360 seconds) significantly increased final germination percentage compared to the control (0 seconds) Figure 4.5) (Table 4.3 APPENDIX E). Scarification times beyond 90 seconds did not indicate a strongly significant improvement in final germination percentage. The addition of post-scarification cold: moist stratification at 4 ± 1^{0}C for 3, 5, and 8 days did not significantly increase % final

germination compared to the scarified, non-stratified control at 0 days (Table 4.3 APPENDIX E).

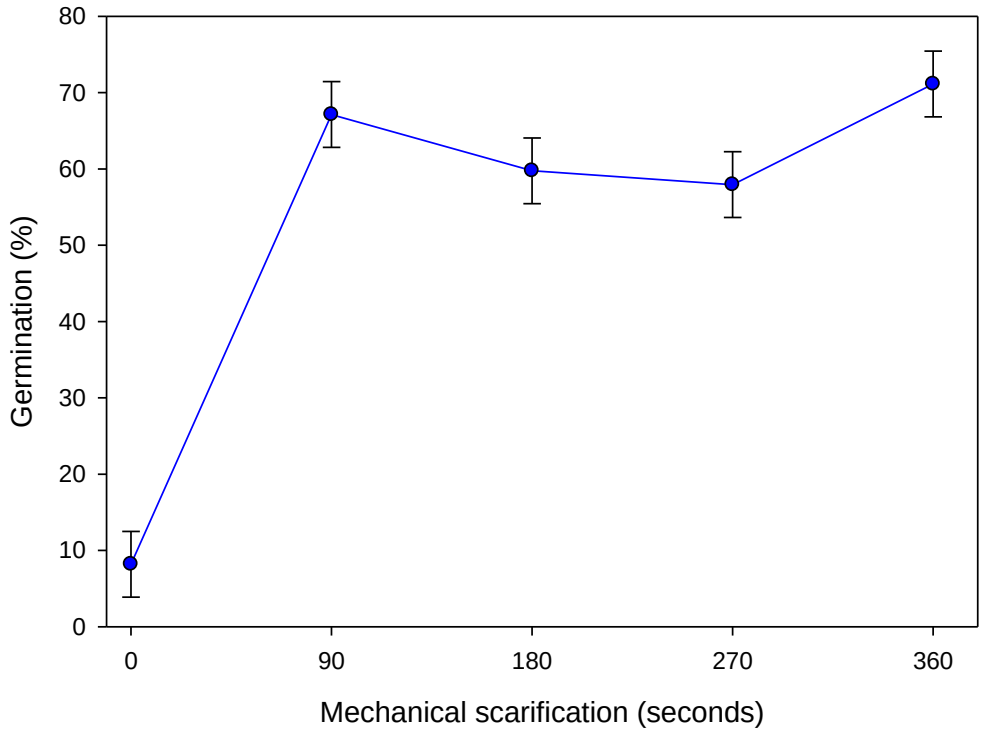

Figure 4.5. Effect of mechanical scarification on final germination percentage. ANOVA analysis for scarification with F=35.07, P=<.0001. Circles indicate means and error bars indicate 95% confidence at the 0.05 significance level.

Experiment 6: Mechanical Scarification & Sandpaper Age. Scarification time at 90, 180, 270, and 360 seconds significantly increased % final germination compared to the control (0 seconds of scarification) (Figure 4.6). Scarification times beyond 90 seconds did not result in a strongly significant improvement in final germination percentage. The new 40 grit sandpaper significantly increased final germination

percentage compared to the worn 40 grit sandpaper (Figure 4.7) (Table 4.4 APPENDIX E).

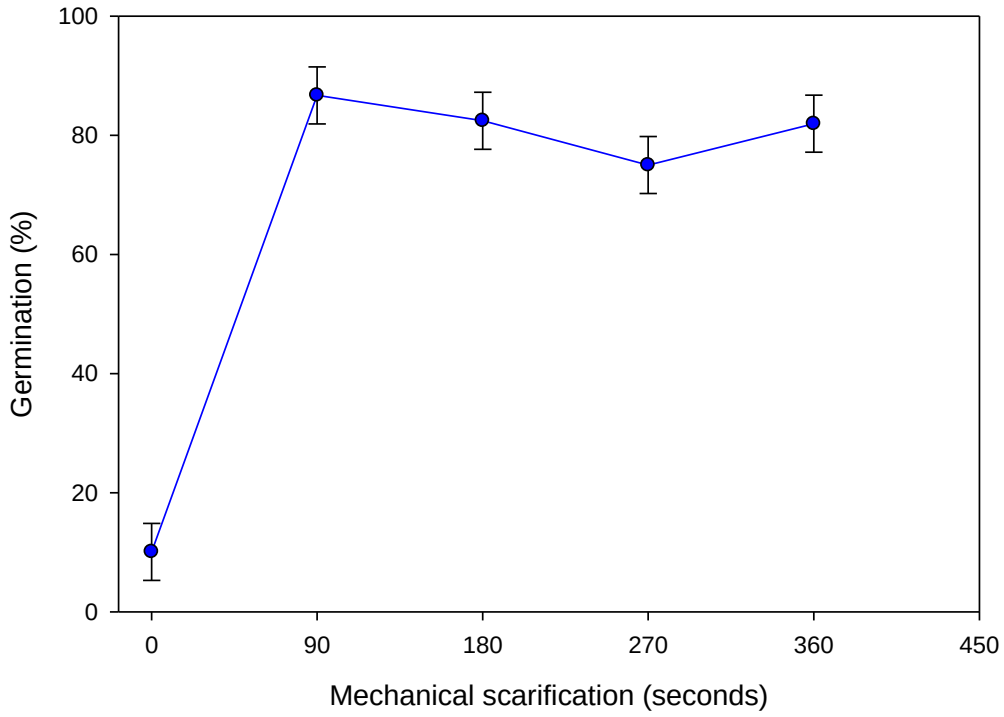

Figure 4.6. Effect of mechanical scarification with cold: moist stratification on final germination percentage. ANOVA analysis with F=45.31, P=<.0001. Circles indicate least-square means and error bars indicate 95% confidence at the 0.05 significance level.

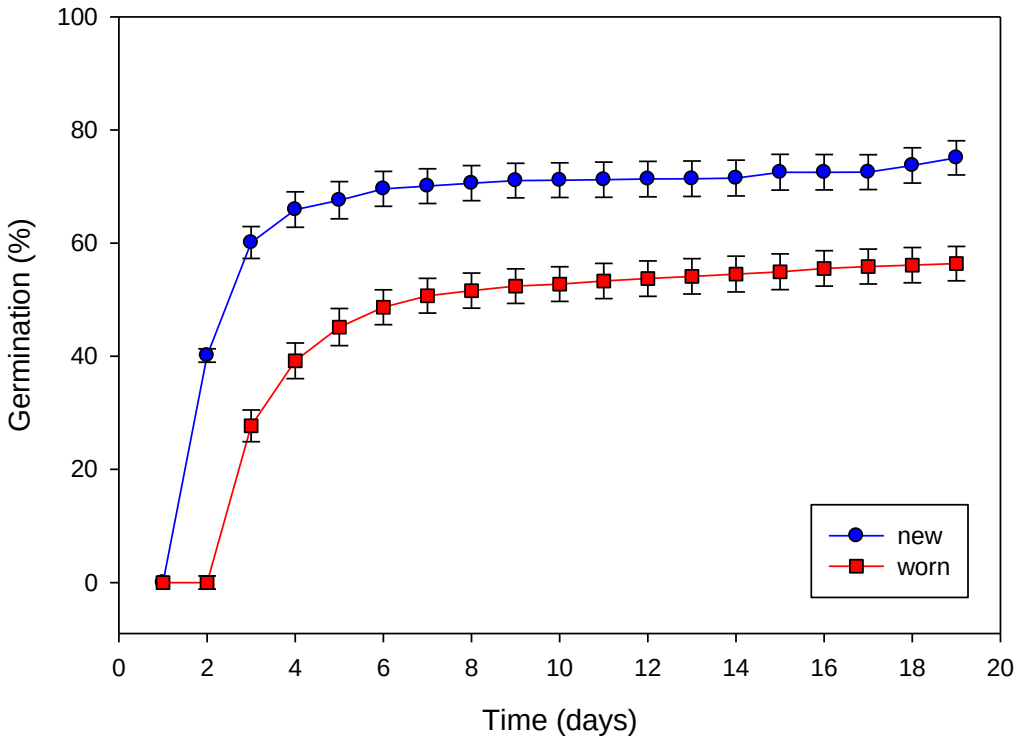

Figure 4.7. Effect of sandpaper age with mechanical scarification on final germination percentage. ANOVA analysis with F=25.68, P=<.0001. Blue circles indicate means for new 40 grit sandpaper. Red squares indicate means for old 40 grit sandpaper. Error bars indicate 95% confidence at the 0.05 significance level.

Upon examination of the time to 50% final germination (T50) with the data combined across sandpaper treatments, rate of germination increased with scarification time with at least 90 seconds of scarification, as exhibited by decreased T50 values. The lack of a significant effect between the two sandpaper treatments on T50 shows that although new sandpaper significantly increases final germination percentage compared to old sandpaper, there is no difference in the rate of germination between new and worn sandpaper. There was no significant difference in rate between seeds scarified by old versus new sandpaper (Figure 4.8) (Table 4.5 APPENDIX E). Also, longer scarification

times of 180, 270, and 360 seconds did not significantly improve the rate of germination compared to 90 seconds.

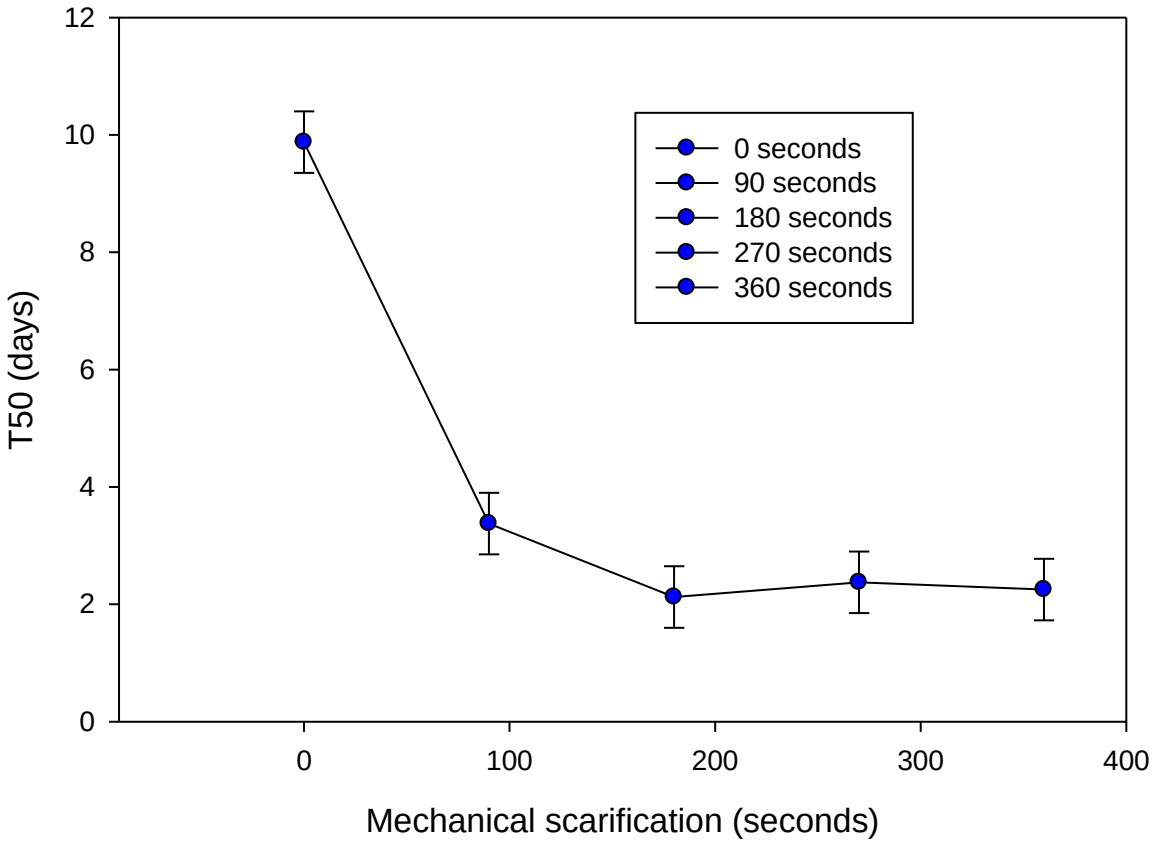

Figure 4.8. Effect of scarification with new and old sandpaper on time (days) to 50% germination (T50). ANOVA analysis with F=40.11, P=<.0001, Circles indicate means at scarification times (seconds) and error bars indicate 95% confidence at the 0.05 level.

Upon examination of the time interval between 10% and 90% final germination (T90) with results from the new and worn 40 grit sandpaper, rate of germination increased with at least 90 seconds of scarification. Longer scarification times of 180, 270 and 360 seconds slightly increased rate of germination compared to 90 seconds. (Figure

4.9)(Table 4.6 APPENDIX E). At 90 seconds of scarification new sandpaper significantly increased rate of germination (T90) compared to worn sandpaper.

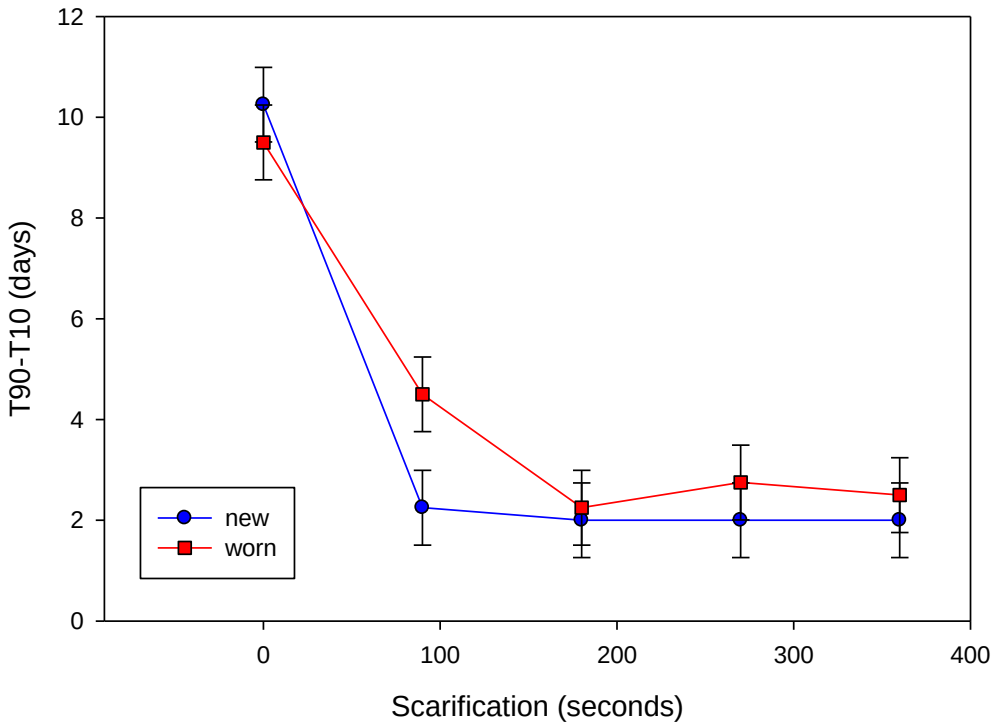

Figure 4.9. Effect of scarification with new and worn sandpaper on time (days) between 10% and 90% final germination (T90). ANOVA analysis for sandpaper age (old and new) with F=14.31, P=0.0007. ANOVA analysis for scarification with F=6.24, P=0.0009. Blue circles indicate means for new sandpaper. Red squares indicate means for worn sandpaper. Error bars indicate 95% confidence at the 0.05 significance level.

These results suggest that germination of silverleaf phacelia may be increased by mechanical scarification for at least 90 seconds. Warm: moist stratification inhibits germination and cold: moist stratification does not significantly increase final germination percentage. Newer sandpaper utilized for the scarification process may

increase rate of germination. Additional studies examining the effect of scarification and moist stratification on freshly collected and harvested seeds could prove valuable.

Discussion

Seed Dormancy Classification Process

The classification of seed dormancy is difficult, as dormancy status is continuously changing with time. This is of particular importance to this study because the seeds utilized in this study were exposed to a pre-treatment of 1 year of after-ripening (dry storage) at 5 ± 1^{0}C. After-ripening is considered a dormancy-release mechanism for seeds exhibiting non-deep physiological dormancy. As such, it should be noted that the dormancy-release treatment of scarification utilized within this study was preceded by a pretreatment of after-ripening at 5 ± 1^{0}C for one year. The effect of after-ripening on germination in this study is not known.

Nondeep Physiological Dormancy. Silverleaf phacelia seeds displayed nondeep physiological dormancy type 1, 2, and type 5.

The occurrence of type 1 nondeep physiological dormancy, which occurs when excised embryos result in normal seedlings, was supported as all embryos germinated and exhibited normal growth and development.

Upon examination of type 2, which occurs when GA promotes germination, evidence was provided that GA promoted germination, however final germination percentage was < 20%, thus GA does not appear to be a strong dormancy release mechanism.

In exploration of type 3, which is present when cold or warm stratification breaks dormancy, the occurrence of low germination percentages of < 12% with cold: moist stratification and < 2% with warm: moist stratification did not provide evidence that stratification is a dormancy-breaking treatment. Although a longer time period of cold: moist stratification was not tested, results indicate a longer time period may result in decreased seed viability and % final germination through the increased likelihood of entering conditional dormancy and increased mold growth.

The occurrence of type 4, which occurs when after-ripening promotes germination, may be an important part of the seed physiology of silverleaf phacelia; however, this was not examined in this project. Research suggests that this may be an important part of the dormancy-release process in other seeds exhibiting characteristics similar to silverleaf phacelia (Baskin and Baskin 2006).

The occurrence of type 5, which occurs when a treatment of scarification promotes germination, was supported as a % final germination of > 50% was achieved by day 4 and > 80% by day 19.

Intermediate Physiological Dormancy. Silverleaf phacelia seeds displayed intermediate physiological dormancy type 1 and type 2, however type 4 could not be examined as all seeds experienced after-ripening.

The occurrence of intermediate physiological dormancy type 1, present when excised embryos germinate and produce normal seedlings, was met, as 100% of embryos of silverleaf phacelia resulted in normal seedlings.

The occurrence of type 2, which is present when GA promotes germination, was weakly met, as GA promoted germination by about 10% compared to no GA.

The occurrence of type 3, which is present when seeds require 2 to 3 months of cold: moist stratification to break dormancy was not met. Cold: moist stratification resulted in a delay in germination and thus did not significantly increase % final germination. Although a longer period of cold: moist stratification was not tested, results from 3, 5, and 8 days of cold: moist stratification indicated that a longer period of cold: moist stratification may decrease seed viability and have no significant positive effect on final germination percentage.

The occurrence of type 4, when dry storage shortens the cold: moist stratification period required to release dormancy, appears unlikely, as cold: moist stratification delayed germination. However, as all seeds were in dry storage (after-ripening) for 12 months with no control for comparison, the results are inconclusive.

Deep Physiological Dormancy. Seeds of silverleaf phacelia did not display any indications of deep physiological dormancy.

The occurrence of type 1, which is present when excised embryos grow abnormally, was not met, as all excised embryos germinated.

The occurrence of type 2, which is present when GA does not promote germination, was not met, as GA promoted germination by about 10% compared to no GA.

The occurrence of type 3, which is present when seeds require 3 to 4 months of cold: moist stratification to break dormancy was not met, as results from a shorter period

of cold: moist did not significantly increase final germination percentage Although a longer period of cold: moist stratification was not tested, results from 3, 5, and 8 days of cold: moist stratification indicated that a longer period of cold: moist stratification may decrease seed viability and have no significant positive effect on final germination percentage.

Physical, Morphological and Morphophysiological Dormancy. The ability of silverleaf phacelia to uptake water with an intact seed coat is not indicative of a seed with physical dormancy. As silverleaf phacelia has a non-dwarf size embryo with cotyledons and endosperm present, its seed structure does not fit the characteristics of seeds which exhibit morphological and or morphophysiological dormancy.

Morphological and morphophysiological seed dormancy classes are characterized by a small embryo to endosperm ratio and an underdeveloped embryo. Based on the seed size, structure, the fully developed stage of the embryo, and the permeability of the seed coat to water, silverleaf phacelia falls explicitly under the class of physiological seed dormancy.

Conclusion

Silverleaf phacelia displays nondeep physiological dormancy, with dormancy breaking occurring after scarification (Finch-Savage and Leubner-Metzger 2006). The purpose of addressing nondeep physiological dormancy of silverleaf phacelia seeds with scarification is to address the resistance of the seed coat, which provides enough resistance to prevent the protrusion of the radicle. When mechanical scarification was

provided for at least 90 seconds with new sandpaper, a final germination of 87% ± 5% was achieved in 4 days. Upon examination of the classification of silverleaf phacelia seed dormancy to the specific level and type of dormancy, nondeep physiological dormancy, types 1, 2, and 5, and intermediate dormancy types 1 and 2, were indicated. For propagation purposes, addressing seed dormancy-release requirements of nondeep physiological dormancy type 5 would be the most effective method to break seed dormancy. The effect of after-ripening (nondeep physiological dormancy type 4) could not be studied as seeds received a pretreatment of 1 year of after-ripening before seed experiments began. Further studies should be completed to test the effect of after-ripening and scarification on newly harvested seeds.

CHAPTER 5

GREENHOUSE PROPAGATION OF SILVERLEAF PHACELIA

Abstract

The objective of this study was to evaluate greenhouse cultural treatments on the production of silverleaf phacelia (*Phacelia hastata* var. *hastata*). Upon plug establishment, seedlings were transplanted into 4-inch square pots or Cone-tainer cells and fertilized with 0, 50, 100, 200, and 400 mg of nitrogen / L of 20N-10P-20 K synthetic, water-soluble fertilizer. Fertilizer rates of 50 to 200 mg nitrogen/L resulted in increased shoot growth based on shoot dry weight (SDW), plant height, and plant spread measurements. Overwhelmingly, based on SDW, RDW, plant height and plant spread measurements; the 4-inch square pot was superior to the Cone-tainer for production of silverleaf phacelia in the greenhouse.

Introduction

Silverleaf phacelia, also known as silverleaf scorpionweed (*Phacelia hastata* var. *hastata*) is an Intermountain West native perennial plant species in the waterleaf family (Hydrophyllaceae) which may be a valuable restoration species and also a valuable native perennial wildflower for the Montana floriculture industry.

Utilizing native plant material for restoration purposes has become increasingly important as environmental degradation and instability has been rapidly occurring (Clewell and Aronson 2013). Rehabilitation of disturbed areas due to spoils from

abandoned mine sites is a challenge for land management agencies. Increased drought and fire occurrence in arid and semi-arid regions may increase the need for native plant material for revegetation and restoration purposes (Westerling et al. 2006). In a survey of the Colorado green industry, respondents agreed that they believe the native plant market will continue to grow with increasing concerns over water conservation issues (Potts et al. 2002).

With the passage of the National Environmental Policy Act (NEPA) in 1969, the Native Plant Conservation Initiative National Strategy in 1995, and the establishment of the Federal Interagency Native Plant Material (NPM) Development Committee in 2000 (USDA and USDI 2002), revegetation policies have increasingly focused on utilizing and producing locally adapted and genetically compatible native plant material for restoration purposes (Richards et al. 1998; Potts et al. 2002; Beyers 2004).

Silverleaf phacelia is an early colonizer species after fire disturbances and is able to establish and thrive in extremely arid regions (Major and Rejmanek 1982; Winslow 2005). Additionally, Smyth (1997) and Schladweiler (2005) noted silverleaf phacelia established on sites contaminated by mining wastes, such as abandoned coal mine spoils. Silverleaf phacelia is a valuable species for restoration and revegetation activities due to the following characteristics: it colonizes after fire disturbance, has a mid to high establishment success, is drought tolerant, and survives on sub-par soils (Smyth 1997; Schladweiler 2005; USDA 2014).

In addition to utilization for restoration, silverleaf phacelia may be of horticultural value due to its aesthetically pleasing inflorescence, which is also an

important source of pollen. Although demand for native plants exists, production is often costly. Yue (2011) found that on average, a non-native or invasive plant had an average discount per plant of $0.66 to $1.33 compared to a native plant. Despite the extra cost, 92% of survey respondents were willing to pay a $0.35 premium per plant, for plants labelled as native species vs. those labelled as non-native or invasive (Yue et al. 2011).

However, a lack of availability of locally adapted plant material is still reported as a topmost concern in the development of the native plant market (Potts et al. 2002; Beyers 2004; Peppins et al. 2010). Lack of availability may be partly due to the difficulty of producing some native species (Potts et al. 2002; Beyers 2004). Propagation requirements of many native plant species including silverleaf phacelia are unknown (Potts et al. 2002). Further research is needed to establish greenhouse growth protocols for propagation of silverleaf phacelia. The objective of this project was to determine suitable greenhouse propagation fertilizer rate (mg nitrogen/L) and container type requirements for silverleaf phacelia.

Materials and Methods

Silverleaf phacelia seeds were germinated in a germination box (4-5/16”x 4-5/16” x 1-3/8” © Pioneer Plastics Inc.) containing sterile blue blotter paper and after successful germination seeds were transplanted into 150 cell plug trays (4.5 cm x 4.5 cm cells) in Sunshine Mix 1 (Sun Gro Horticulture ™) and grown to the cotyledon stage. Twenty nine days after sowing, cotyledons which exhibited their first true primary leafs were then transplanted into one of two types of growth containers; a 4-inch pot (670 ml) and a Ray

Leach Cone-tainer ™ (164 ml), filled with Sunshine Mix #1. Plants were irrigated by hand one time per week with 20-10-20 NPK water-soluble fertilizer (Peat-Lite Special, Jack's Professional ™) at rates of 0, 50, 100, 200 and 400 mg of nitrogen/L. Nitrogen test rates reflect common application rates for bedding plants in the commercial industry. Although highly dependent on species, growth stage, and other production protocols, common fertilizer rates for bedding plants such as impatiens and pansy may be utilizing 20-10-20 NPK at 50 to 250 mg/L of nitrogen and potassium (Bailey 1996). However bedding plants which may require heavy fertilization, such as begonia and verbena, may require use of higher amounts of fertilizer, such as 20-10-20 NPK at 200 to 400 mg/L of nitrogen and potassium (Bailey et al. 1996). Every fourth irrigation cycle, plants were irrigated with water with a leaching percentage (the percentage of irrigation solution that drains from the container after irrigation) of 10 to 15% per container. Plant height and plant spread was measured one time per week (mm) using a digital caliper. Flowering incidence was measured throughout the experiment once per week at the same time plant measurements were taken. The characteristic of flowering was determined by the existence of a flowering bud which has broken and opened, displaying flower petals. The incidence of flowering was measured per treatment level from start to end of the experiment.

Fourteen replicates of each combined treatment of container type and nitrogen rate was completely randomized on a greenhouse bench, with mean ambient air temperature at 30/21°C day/night and no supplemental lighting. The experiment was initiated in June 2014 and continued for a total of 71 days, at which point in time plants

were flowering and large enough to be marketable. Average day length from June 2014 to August 2014 ranged from 14 hr. 24 min to 17 hr. 9 min. After 71 days in the greenhouse, plant shoots were separated from roots, roots were washed, and shoots and roots were immediately dried in paper bags at 54^0C for 48 hrs. The shoot dry weight (SDW) and root dry weight (RDW) were determined.

The experiment was designed as a randomized two way factorial experiment with 2 container types and 5 fertilizer rates. The repetition of the measurements was analyzed between and within 6 time periods (weeks) through the REPEATED statement in PROC GLM (SAS University Ed. 9.11). Pairwise combinations were examined utilizing Tukey-Kramer HSD test with a significant level of α=0.05.

Results

Results from plant height measurements (mm) indicate that silverleaf phacelia increases in growth with increasing rates of fertilizer in both containers (Figure 1)(Table 5.1 APPENDIX F). In both container types, plant height measurements doubled with just 50 mg nitrogen/L, compared to the control (0 mg nitrogen/L). At higher rates of 200 to 400 mg nitrogen/L, plant height in the 4-inch square pots almost doubled compared to the Cone-tainer (Figure 5.1). These results showed a significant two-way interaction between container type and fertilizer, with the greatest positive growth exhibited by plant height measurements occurred in the 4-inch square pot at 200 to 400 mg nitrogen/L.

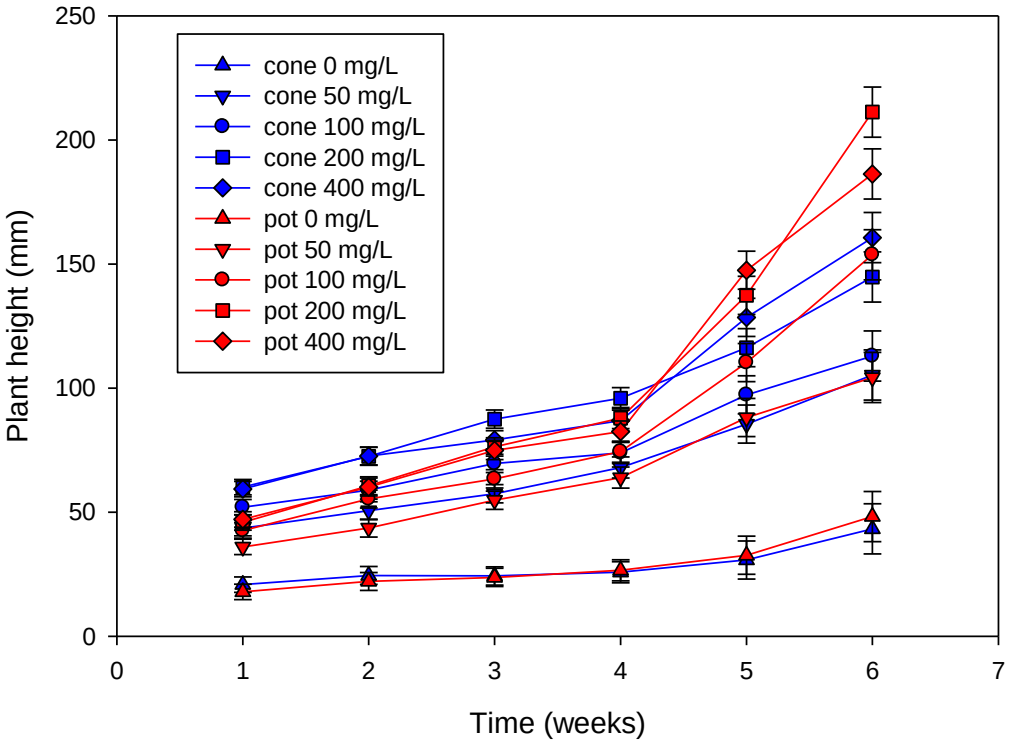

Figure 5.1. Effect of container type and fertilizer levels on plant height. ANOVA analysis F=3.69, P=0.007. Blue symbols indicate means for Cone-tainer (cone). Red symbols indicate means for the 4-inch square pot (pot). Upward triangles and error bars indicate 0 mg nitrogen/L. Downward triangles indicate 50 mg nitrogen/L. Circles indicate 100 mg nitrogen/L. Squares indicate 200 mg nitrogen/L. Diamonds indicate 400 mg nitrogen/L. Error bars indicate 95% confidence at the 0.05 significance level.

This significant difference in plant height between container types occurred by the fourth week, at which time flowering, which was included in the plant height measurements, was occurring. Flowering incidence increased with greater nitrogen rates and was greatest at 400 mg nitrogen/L in both containers. Flowering incidence was greatest at 400 mg nitrogen/L in the 4-inch square pot compared to the Cone-tainer (Figure 5.2) (Table 5.2 APPENDIX F). These results show a significant two-way

interaction between container type and fertilizer on flowering incidence, displaying that plant maturity based on flowering occurrence and shoot growth, when utilizing 200 to 400 mg nitrogen/L, was reached more quickly in the 4-inch square pot versus the Cone-tainer.

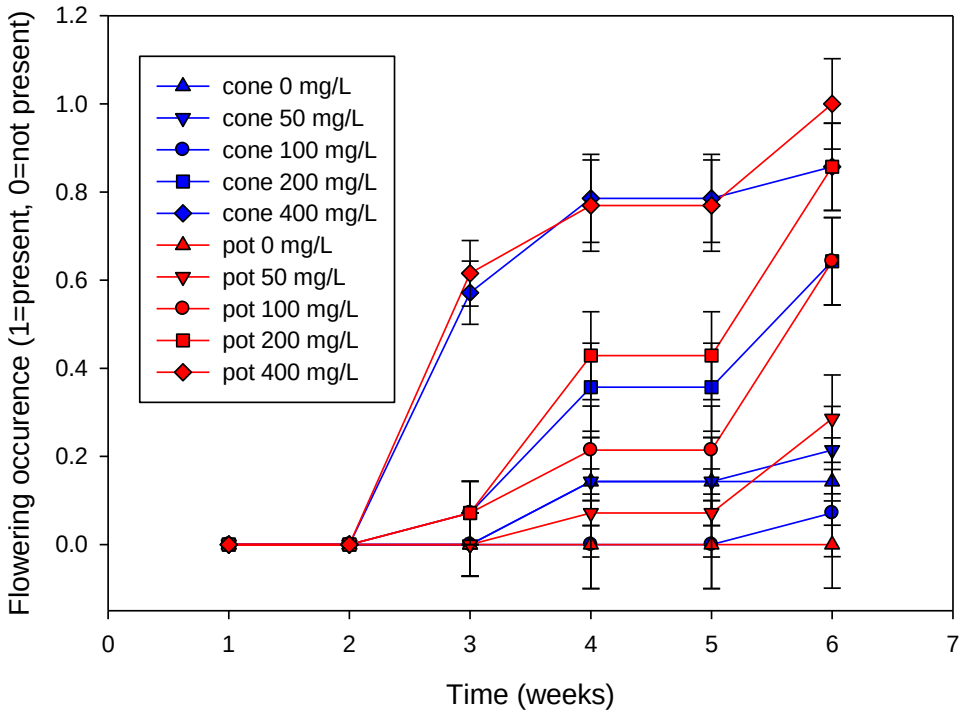

Figure 5.2. Effect of container type and fertilizer levels on flowering incidence. ANOVA analysis with F=3.46, P=0.0101. Blue symbols indicate mean occurrence in the Cone-tainer (cone). Red symbols indicate mean occurrence in the 4-inch square pot (pot). Upward triangles indicate 0 mg nitrogen/L. Downward triangles indicate 50 mg nitrogen/L. Circles indicate 100 mg nitrogen/L. Squares indicate 200 mg nitrogen/L. Diamonds indicate fertilizer level of 400 mg nitrogen/L. Error bars indicate 95% confidence at the 0.05 significance level.

Results from plant spread measurements (mm) indicates that silverleaf phacelia increases in growth with increasing rates of nitrogen in both the 4-inch square pot and the

Cone-tainer (Figure 5.3) (Table 5.3 APPENDIX F). In both container types, plant spread measurements almost tripled with just 50 mg nitrogen/L of fertilizer, compared to the control. Plant spread increased successively with increasing nitrogen concentration in the 4-inch square pot, however in the Cone-tainer plant spread measurements successively increased between 100 and 200 mg nitrogen/L, but there was no significant difference in plant spread between 50 and 100 mg nitrogen/L and 200 and 400 mg nitrogen/L. Results display a significant interaction in plant spread between container type and fertilizer as plant spread in the 4-inch square pot significantly exceeded spread in the Cone-tainer at higher nitrogen levels.

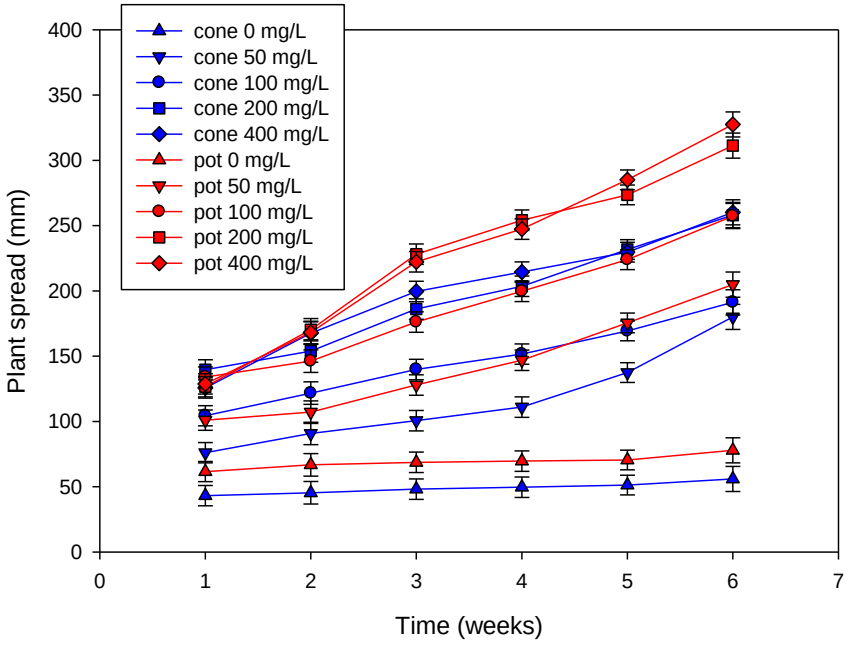

Figure 5.3. Effect of container type and fertilizer on plant spread. ANOVA analysis F=2.61, P=0.0387. Blue symbols indicate means for Cone-tainer (cone). Red symbols indicate means for the 4-inch square pot (pot). Upward triangles indicate 0 mg nitrogen/L. Downward triangles indicate 50 mg nitrogen/L. Circles indicate 100 mg nitrogen/L. Squares indicate 200 mg nitrogen/L. Diamonds indicate 400 mg nitrogen/L. Error bars indicate 95% confidence at the 0.05 significance level.

In both container types shoot dry weight (SDW) significantly increased with increasing levels of nitrogen. In the 4-inch square pot there was no significant difference between 200 and 400 mg nitrogen/L (Figure 5.4)(Table 5.4 APPENDIX F). In the Cone-tainer, SDW increased with increasing levels of nitrogen. There was no significant difference between 50 and 100 mg nitrogen/L (Figure 5.4)(Table 5.4 APPENDIX F). Also SDW in the Cone-tainer significantly decreased at 400 mg nitrogen/L compared to 200 mg nitrogen/L. Results exhibit increased SDW with increasing levels of nitrogen in both container types; however, a significant two-way interaction between container type and fertilizer as in the 4-inch square pot there was a greater significant increase in SDW between increasing levels of nitrogen, compared to the Cone-tainer.

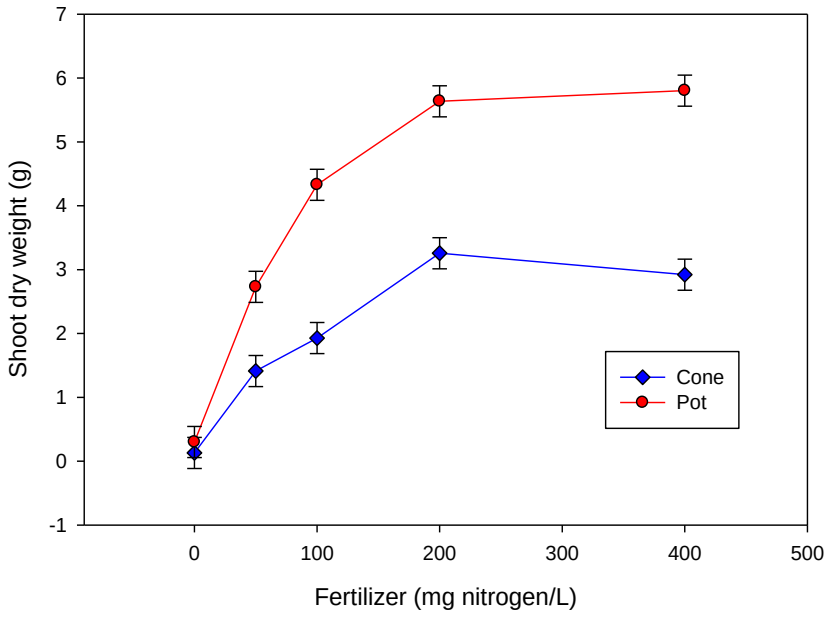

Figure 5.4. Effect of container type and fertilizer on shoot dry weight (SDW). ANOVA analysis with F=10, P=<0.0001. Blue diamonds indicate means for Cone-tainer (cone). Red circles indicate means for the 4-inch square pot (pot). Error bars indicate 95% confidence at the 0.05 significance level.

Similarly, root dry weight (RDW) in both container types significantly increased with increasing levels of nitrogen. In the 4-inch square pot there was no significant difference between 100 and 200 mg nitrogen/L and between 200 and 400 mg nitrogen/L (Figure 5.5)(Table 5.5 APPENDIX F). In the Cone-tainer, RDW increased there was no significant difference in increased RDW between 50, 100, 200 and 400 mg nitrogen/L (Figure 5.5)(Table 5.5 APPENDIX F). Also RDW in both container types, especially in the Cone-tainer, slightly decreased at 400 mg nitrogen/L. Results demonstrate a significant two-way interaction between container type and fertilizer for RDW, as RDW significantly doubled in the 4-inch square pot compared to the Cone-tainer.

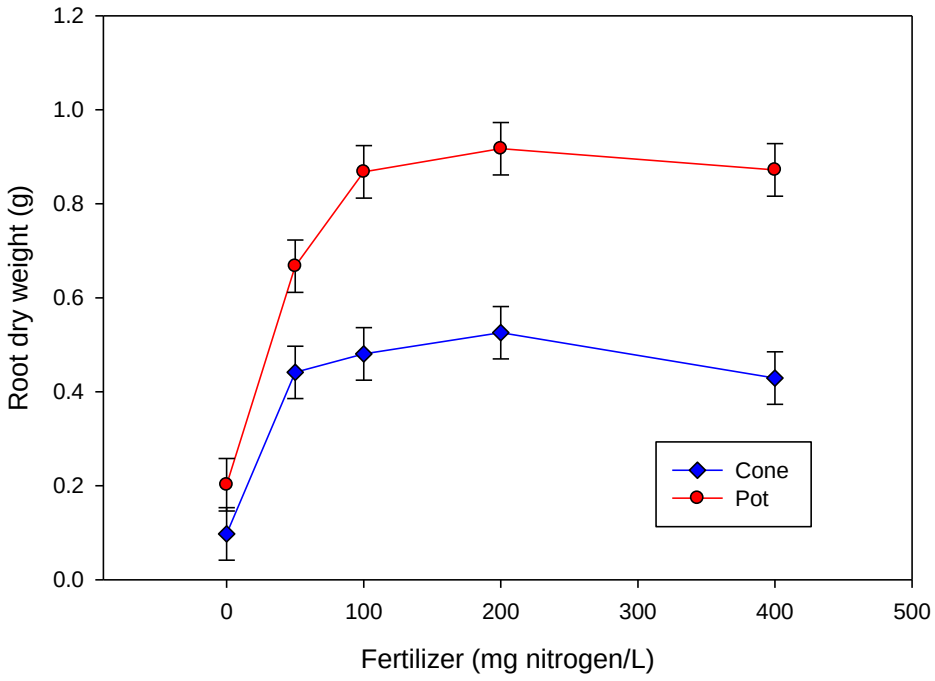

Figure 5.5. Effect of container type and fertilizer on root dry weight (RDW). ANOVA analysis with F=3.19, P=0.0154. Blue diamonds indicate means for Cone-tainer (cone). Red circles indicate means for the 4-inch square pot (pot). Error bars indicate 95% confidence at the 0.05 significance level.

The RDW and SDW graphically depicted as a root-to-shoot ratio showed declined root mass with higher levels of nitrogen, resulting in decreased root-to-shoot values, especially at fertilizer rates greater than 100 mg nitrogen/L (Figure 5.6)(Table 5.6 APPENDIX F). The root-to-shoot ratio was not significantly different between container types (Figure 5.6)(Table 5.6 APPENDIX F). Results for the root-to-shoot ratio exhibit a shared response for both container types of a reduction in root-to-shoot ratio with increasing nitrogen rates.

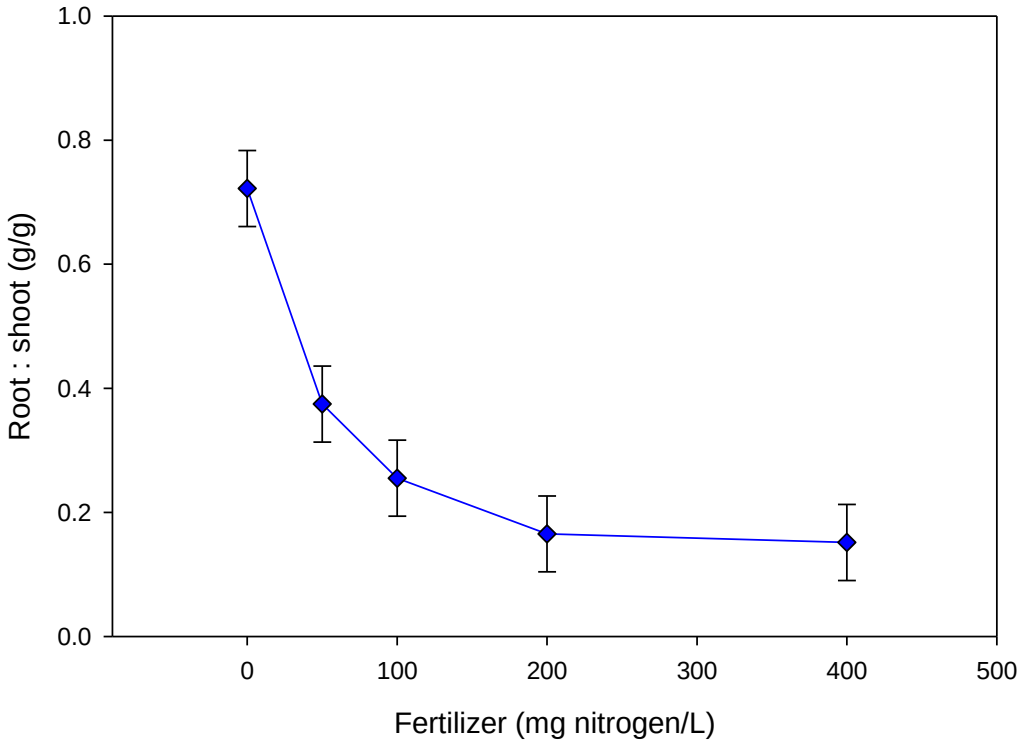

Figure 5.6. Effect of fertilizer on root-to-shoot ratio (g/g). ANOVA analysis with F=14.68, P=<0.0001. Blue diamonds indicate means for both container types (4-inch square pot & Cone-tainer). Error bars indicate 95% confidence at the 0.05 significance level.

Discussion

In discussion on the effect of fertilize on silverleaf phacelia, increasing the concentration of fertilizer showed a significant increase in plant height, plant spread, flowering incidence, shoot dry weight, and root dry weight, compared to the control. This positive growth response to increased nitrogen rates is expected as fertilizer is utilized to push growth rates in plant nurseries (Wilkinson et al. 2014). Silverleaf phacelia, although small-seeded and more likely to expend stored nutrients soon after germination, grew quickly, compared to other native wildflowers which may have production schedules as long as 2 to 3 years (Landis and Simonich 1984).

Increased nitrogen rates also displayed a slightly reduced RDW and a significant reduction in the root-to-shoot ratio in silverleaf phacelia for both container types. The reduction in RDW and significant decline in root-to-shoot values with increasing nitrogen levels is an expected response in greenhouse production of containerized plants. Landis and Simonich (1984) reported that although synthetic fertilizers allow growers to quickly produce plants to a desired growth rate for the nursery culture, beyond a certain point too much fertilizer causes plant growth to decrease and may become detrimental.

Fertilizer levels above 50 mg nitrogen/L may result in reduced root mass for silverleaf phacelia as with increased nutrient availability; less energy is invested into increasing root size. Fertilizer levels above 50 mg nitrogen/L may also result in a reduced root-to-shoot ratio with increased nutrient availability as instead of investing nutrients in increased shoot and root mass, more nutrients are utilized for increased shoot mass rather

than root mass. Reduced root mass in conjunction with a larger shoot may be detrimental for transplantation (Wilkinson et al. 2014).

In discussion of the effect of container type on silverleaf phacelia, a greater positive growth response for plant height, spread, flowering incidence, shoot dry weight, and root dry weight was shown in the 4-inch square pot compared to the Cone-tainer. Container volume, height, diameter, shape, drainage, and root-pruning characteristics all help to predict plant quality. Volume dictates how large the plant may be grown, height helps to determine root plug depth, diameter is important for the specific species being grown, and shape is important for root features of species being grown (Wilkinson et al. 2014). The 4-inch square pot has a broader shape and about triple the amount of soil volume as the Cone-tainer. However, the Cone-tainer is deeper with root pruning features and increased drainage. Silverleaf phacelia displayed almost a tripled value of shoot growth in the 4-inch square pot compared to the Cone-tainer; this may be attributed to the almost tripled amount of soil volume in the 4-inch square pot compared to the Cone-tainer.

In discussion of the effect of a significant interaction between container type and fertilizer for silverleaf phacelia, plant height, spread, flowering incidence, shoot dry weight, and root dry weight showed an increased positive response at higher nitrogen rates in the 4-inch square pot compared to the Cone-tainer. This may be due to the more spreading, fiberous root system of silverleaf phacelia fitting better into the broader shape of the 4-inch square pot compared to the narrower and deeper shape Cone-tainer. The 4-inch square pot also had a greater size and soil volume, with increased growth correlated

with increased nitrogen levels, the greater soil volume in the 4-inch square pot resulted in an increased positive growth response compared to the Cone-tainer.

Conclusion

Silver-leaf phacelia is a versatile and hardy native plant species, with the ability to respond positively to less costly greenhouse production protocols such as; low application rates of nitrogen, standardized pot configuration, and a relatively quick greenhouse production schedule of about 32 days to produce flowering plants in typical greenhouse propagation conditions. In summary, silver-leaf phacelia has a high ornamental and conservational value, and with limited additional research, may be ready for commercial production. Suggested propagation experiments include may examination of different media types and vernalization periods to improve outplanting success or bloom period.

CHAPTER 6

MONTANA NATIVE PLANT MARKET SURVEY

Abstract

The need for native plant products has increased within the last thirty years. Native plants may provide increased ecological stability, control pests and pathogens on agricultural crop blocks, reduce consumption of non-renewable resources, and reduce herbicide use. Commercial availability of many native plants is limited and the reasons for this limitation differ from region to region. The native plant material being grown and sold locally could significantly increase in-state revenue and potentially increase the development of a new niche market within the Montana horticultural industry. To assess market potential, it is imperative that we determine the role native plant material may have in Montana's horticultural industry. The objective of this study was to survey 30 Montana native plant nurseries or growers to better understand how Montana green industry professionals view the challenges, successes, and future projection of the Montana native plant market.

Introduction

The demand for native plants has grown as a result of policy changes within the federal government, among state agencies, and within private organizations, as well as increased consumer awareness of the importance of locally adapted, native plant material, (Richards et al. 1998; Tamimi 1999; National Wildlife Federation 2007; Peppin et al.

2010; Kauth and Perez 2011). The federal government has completed major expenditures on native plant material (Peppin et al. 2010). In 2006 alone, the Bureau of Land Management (BLM) nationally spent a total of $270,000 and the BLM nationally spent a total of $151,000 on native plant material (Beyers 2004; Peppin et al. 2010). The majority of this native plant material was used for ecological restoration and burn area rehabilitation (Peppin et al. 2010).

Federal land management agencies, such as the United States Forest Service (USFS) and BLM, are obligated to provide restoration and rehabilitation measures, utilizing native plant material if practical, after large-impact ecosystem disturbances such as forest fires (Richards et al. 1998; Erikson 2008). At the small business/homeowner level, a survey in the Colorado Plateau region suggested that customers are interested in native plants for a variety of reasons, including that they provide a new array of garden plants compared to traditional non-native offerings (Potts et al. 2002). Further McMahan (2005) noted rising interest from the individual private consumer in utilizing native plant material in residential landscapes. In the Southwestern United States increased drought conditions and fires, may have partly contributed to the surge in demand for native plant products.

Utilization of native plants facilitates the following: ecological stability, reduced ecosystem degradation, increased soil stabilization, biodiversity, species richness, and increased preservation of local genotype (Yue et al. 2011). Utilizing non-native or invasive plant species may cause environmental and economic harm through ecosystem degradation. Purple loosestrife, (*Lythrum salicaria*) an invasive species initially

introduced as a horticultural species, sold in nurseries as early as 1829 (Mack 1991; Delisle et al. 2003), and still widely sold in markets today (Anderson 2004; Barbier and Knowler 2006; Yue et al. 2010), has cost an estimated $45 million in damages annually, through land restoration expenses (National Sustainable Agriculture Information Service 1997; Pimentel et al. 2000; Yue et al. 2011). Incorporating non-local genotypes may reduce potential in-state revenue for the floriculture industry and also create problems through the introduction of non-local genetic materials, potentially threatening the sustainability of adapted local genotypes (Huenneke 1991; Lynch 1991; Schmid 1994; Huford & Mazer 2003; Rogers & Montalyo 2004)).

The horticultural industry, one of the most prolifically growing industries in the United States, increased its revenue by $9.3 million from 1997 to 2005. In 2004, 13% of the total horticultural sales in the United States were from native plant sales (Brooker et al. 2005; Hodges and Haydu 2006; Kauth and Perez 2011). Native plants are considered to be a developing niche for the green industry (Potts et al. 2002). According to a survey, landscape architects in Utah reported that compared to 5 years ago, 41% of practicing architects utilize a higher frequency of native plants (Brzuszek and Harkess 2008). In the year 2000 with the development of the Federal Interagency Native Plant Material (NPM) Development Committee (USDA and USDI 2002), interagency projects have been created to increase availability and production of native plant material (Pellant et al. 2004; Shaw et al. 2005; Peppin et al. 2010). In regions across the United States, such as in Florida, where native plants accounted for 19% of total annual plant sales in 2008, the

demand for native plants exceeds the supply (Smith 2007; Brzuszek and Harkess 2008; Hodges 2011).

The lack of availability of native plants seems to result from a variety of limitations and challenges indicative of the native plant market (Potts et al. 2002; Norcini 2006; Peppin et al. 2010; Kauth and Perez 2011). In large-scale, federally-managed, restoration projects, a large supply of local genotypes are needed quickly (Peppin et al. 2010; Rogers & Montalvo 2004). The regional lack of availability of native plant material in the United States has been examined and a number of causes have been proposed: 1) the non-native plant material market is more localized, 2) native plant growers are smaller and lack necessary monetary resources, 3) funding to increase availability of native plant material is limited, 4) native plants may be more expensive for customers, 5) native seed stock is limited, difficult to obtain, and may be more expensive than non-native seed, and 6) customer demand may not be as great for natives as for non-natives (Norcini 2006; Brzuszek and Harkess 2008; Potts et al. 2002; Peppin et al. 2010).

Potts (2002) suggests limited knowledge and education regarding the utilization and identification of native plants as a challenge for consumers and industry professionals. Kauth and Perez (2011) stated that there is: 1) a lack of shared knowledge on how to propagative native plants among growers, 2) questions regarding seed origin, and 3) a lack of efficient and cost-effective methods for collecting and processing seed (Kauth and Perez 2011). However, numerous sources have stated that cost may not play a major role in the limitation of the native plant market, as despite extra cost, it appears that consumers are not less willing to purchase native plants (Gagliardia and Brand 2007; Yue

et al. 2010; Kauth and Perez 2011; Ricordi et al. 2014). In St. Paul, Minnesota, researchers found that on average, customers were willing to pay a $0.35 premium for plants which were identified as noninvasive and native, compared to a $1.01 to $1.66 discount for plants labelled as nonnative or invasive (Yue et al. 2010).

In summary, the limitations of native plants are dictated by many challenges and may differ regionally. Native plant material being grown and sold locally could significantly increase in-state revenue for the horticultural industry through the development of a new niche market. As of yet, no feasibility studies or even surveys have been implemented in Montana to determine the status of the native plant market.

Materials and Methods

The objective of this study was addressed through the questions with following concerns: 1) gather demographic information on current Montana native plant growers, 2) assess limitations to the native plant market identified through current Montana native plant growers, and 3) assess potential for the promotion of information sharing and networking among current Montana native plant material growers.

A survey approved by the Institutional Review Board (IRB) by Montana State University of 14 questions (Table 6.1 APPENDIX G) in a mixed closed and open-ended format was delivered to a total of 30 Montana native plant growers involved in propagating herbaceous native plants that were identified through a list compiled by the Montana Native Plant Society (MNPS 2011). To ensure the largest survey population possible, this list was verified using additional lists located on the WERA 1013 website

(http://www.uwyo.edu/wera1013/default.as) and the Montana Department of Environmental Quality website (http://deq.mt.gov/wqInfo/wetlands/NurseriesSeed Sources.mcpx).

Due to the small population size, the survey was delivered to the entire population instead of a randomized subset of the population. To ensure an adequate response, the survey was administrated through both interview-based and self-completed modes. The surveys were delivered through three methods as recommended by the Survey Design and Analysis handbook (NOAA 2007); 1) online via email software company (Survey Monkey ©); 2) orally via the telephone; 3) written forms were delivered via US Postal Service. Reminders were sent to enhance survey return rate (Dillman 2000).

Of the total target population of 30 Montana native plant growers, 19 received an email or call on January 7, 2015, February 3, 2015, and March 20, 2015. On March 31st, the remaining 11 native plant growers received letters through the postal service with a printed questionnaire and self-addressed and stamped envelope. The response data was then analyzed using PROC FREQ for frequencies (version 9.2; SAS University Edition, SAS Institute, Cary, NC). Answer frequencies were calculated for all applicable questions and as all questions were answered, sample size was equal for all questions.

Results

Demographics

From the list of nurseries involved in propagating or selling native plant material, a total of 77.4% of growers were confirmed to be actively growing and or selling

herbaceous native plant material. A total of 70.8% were reached via email with a response rate of 57.1%, 29.1% via the telephone with a response rate of 57.1% for surveys delivered via telephone, and 7% sent via United States Postal Service with a response rate of 2.6%.

The Montana Office of tourism organizes the state into 6 regions: Glacier County, Southwest Montana, Central Montana, Yellowstone Country, Missouri River Country, and Southeast Montana. The majority of nurseries were located in the Glacier region (52.94%), secondly in the Yellowstone region (29.41%) and lastly in the Southwest region (17.65%) (Figure 6.1). There was no native plant nurseries located in Central Montana, Southeast Montana or in the Missouri River Country.

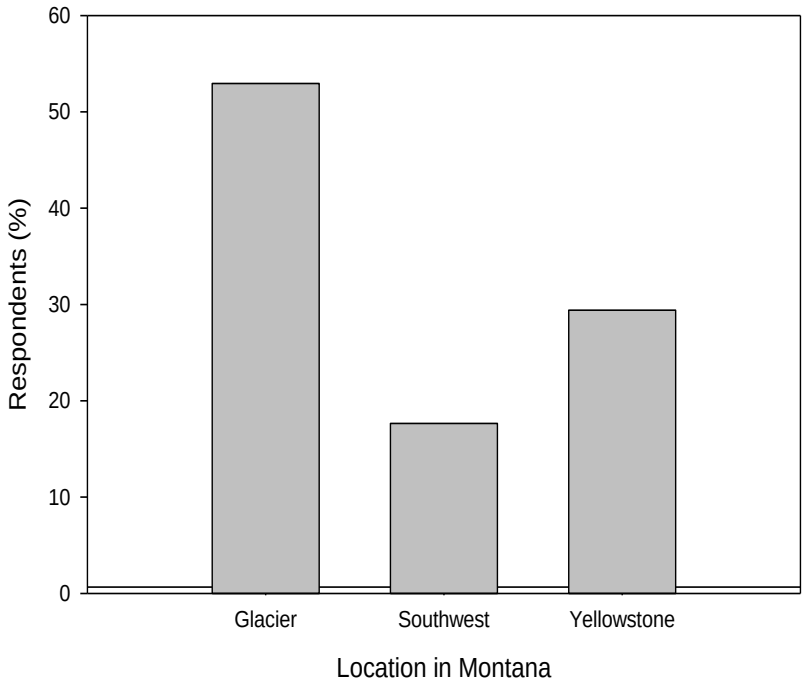

Figure 6.1. Frequency percentage of respondent's answers for question assessing location of Montana native plant nurseries surveyed. PROC FREQ analysis.

When examining whether wholesale and retail growers propagate native plants on-site or buy and then sell native plants propagated by other growers, the majority of Montana native plant nurseries who responded to the survey grew native plants on-site (76.92%). The minority purchased native plants from other growers and then sold them to consumers on-site (23.08%)(Figure 6.2).

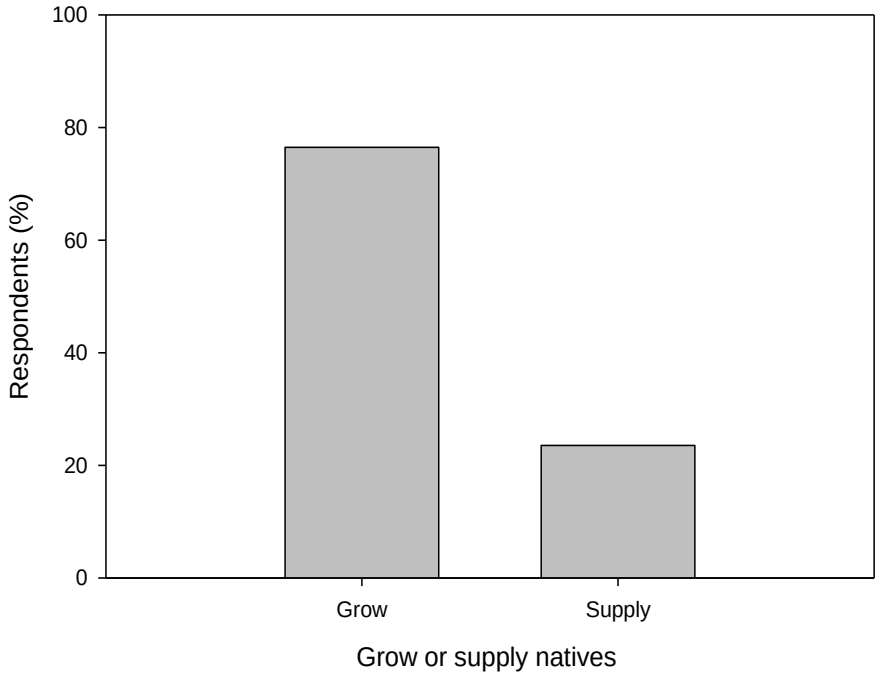

Figure 6.2. Frequency percentage of respondent's answers for question assessing whether the Montana native plant grower grew or supplied native plants. PROC FREQ analysis.

The majority of respondents (47.1%) said that on average, 0 to 20% of their customers come in to browse or purchase native plants. With the next most popular response tied (17.6%) at an average of 61 to 80% and 21 to 40% of customers coming in to browse or purchase native plant species. A minority of respondents (11.6%) said 81%

to 100% of customers asked for native plant material, with the least amount of growers (5.8%) responding that 41% to 60% requested native plants (Figure 6.3).

It is important to note that we have an inherent bias in our data as only growers already involved in native plant material production were surveyed. Additionally, these results may be a byproduct of a different interpretation of what consists as a positive response, as some growers may have counted a verbal customer request for native plant material as a positive response, whereas other growers may have counted a customer's interest as a positive response.

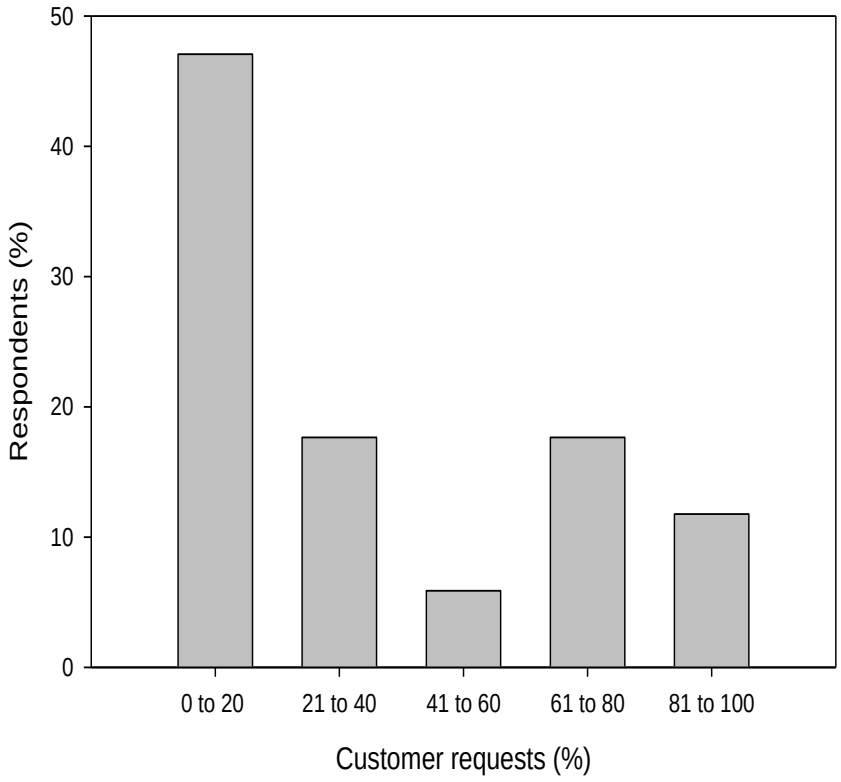

Figure 6.3.Frequency percentage of respondent's answers to question assessing percentage of customers who request native plants. PROC FREQ analysis.

In total, the majority of respondents considered their business a combination of wholesale and retail (52.94%), whereas 41.18% considered their business solely retail, and only 5.88% as solely wholesale (Figure 6.4). Most respondents held the role of owner (82.35%) with the minority of respondents as staff (11.76%) or manager (5.88%)(Figure 6.5).

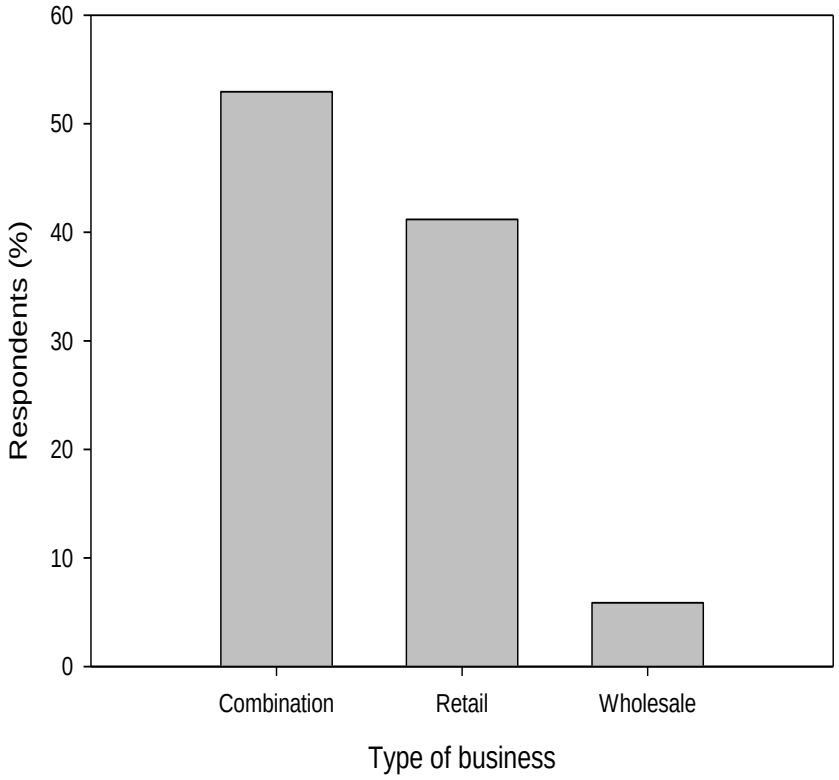

Figure 6.4. Frequency percentage of respondent's answers for question assessing type of business category of Montana native plant growers. PROC FREQ analysis.

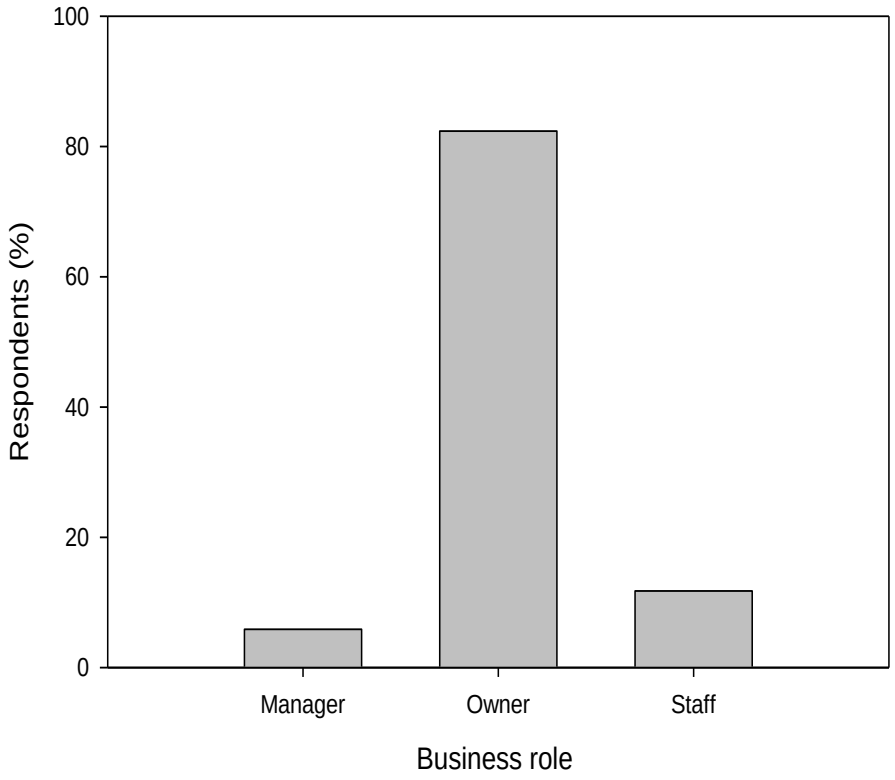

Figure 6.5. Frequency percentage of respondent's answers for question assessing role within business for person surveyed representing Montana native plant grower. PROC FREQ analysis.

Market Trends

The results from our survey suggest that customer demand exists for native plant material. When asked why they sell native plants, the majority of respondents stated it was due to customer demand (58.8%), with ecological reasons being the second most important reason respondents chose to grow native species (17.6%). Additional responses for why growers sell native plants;1) grower's personal interest, and 2) variety of plant material sold were both at a percent response (11.7%)(Figure 6.6).

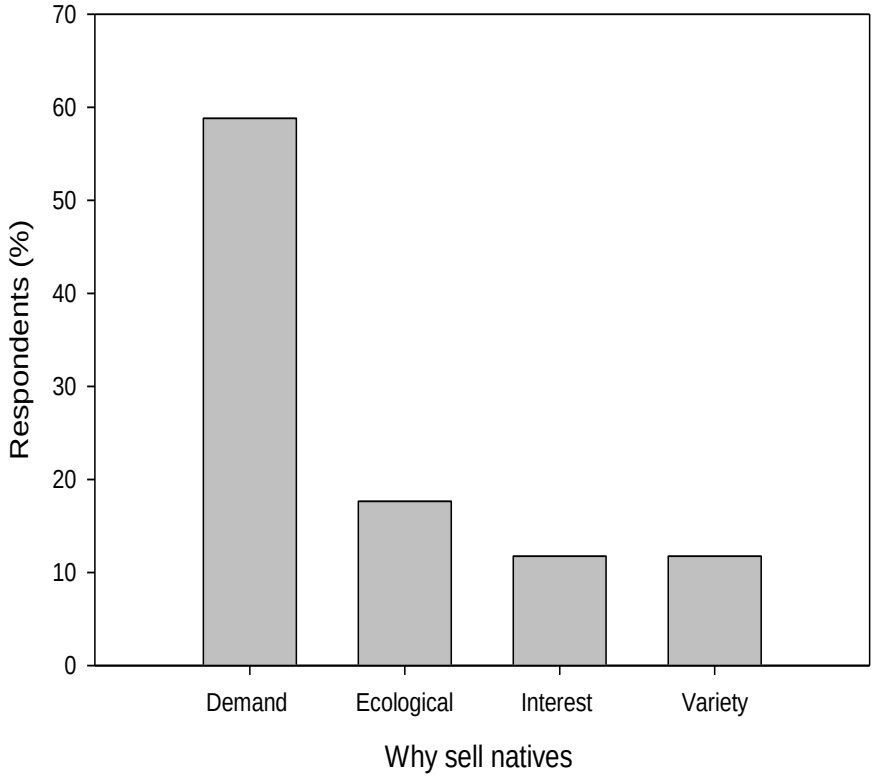

Figure 6.6. Frequency percentage of respondent's answers for question assessing why grower sells native plants. PROC FREQ analysis.

As an indication of currently increasing customer demand for native plants, the greatest number of native plant nurseries surveyed was recently established businesses. The majority of respondents stated that they have been growing and or supplying native plants for no more than 10 years (35.2%). Additionally, 29.4% of growers stated that they have been in the industry for 11 to 20 years. The remaining respondents stated that they had been growing native species for: 1) 21 to 30 years (11.7%), 2) 31 to 40 years (11.7%), and 3) 41 or a greater number of years (11.7%) (Figure 6.7).

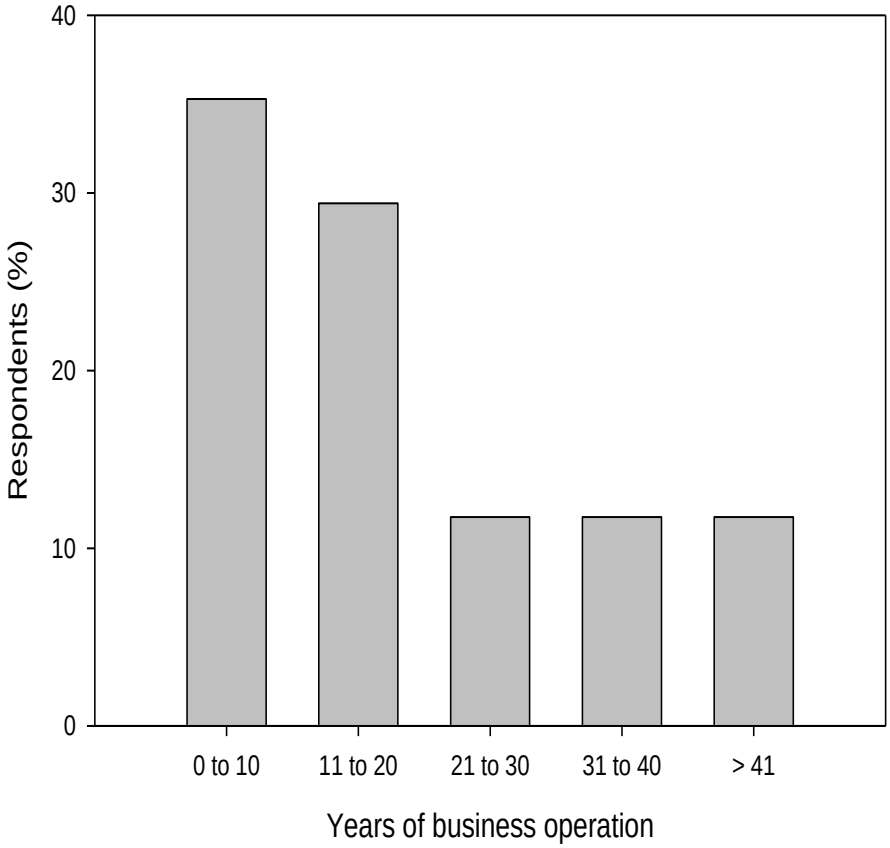

Figure 6.7. Frequency percentage of respondent's answers for question assessing the length of time the business has been established. PROC FREQ analysis.

Respondents listed over 25 different species when asked to list the top five most common species they sell. Quaking aspen (*Populus tremuloides*), red-osier dogwood (*Corunus sericea*), blanketflower (*Gaillardia aristata*), big sagebrush (*Artemesia tridentata*), and chokecherry (*Prunus virginiana*).

Market Challenges

When participants were asked what they believe may be hindering the native plant market, with the request to choose only one selection, the top concern was the lack of

seed supply (41.1%), the second ranked concern was propagation difficulties (23.5%), and thirdly respondents were concerned with a lack of consumer demand for natives (17.6%). Other respondents mentioned increased labor associated with natives (11.6%) and expense (5.8%) (Figure 6.8).

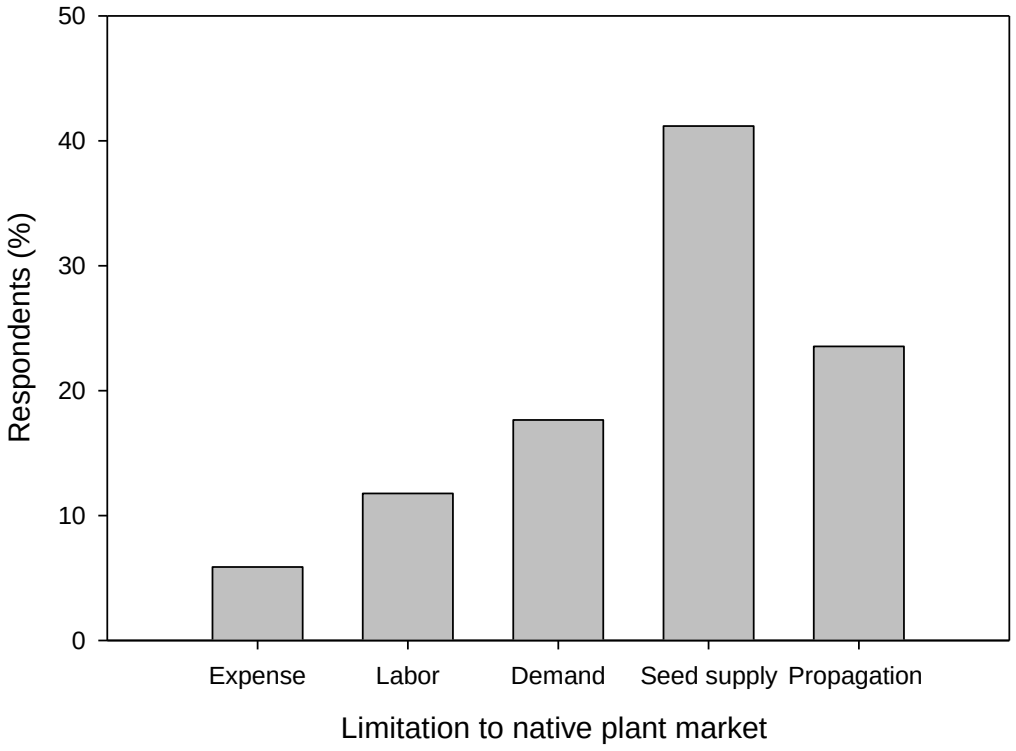

Figure 6.8. Frequency percentage of respondent's answers for question assessing limitations to the Montana native plant market. Dollars required is indicated as expense. Labor required is indicated as labor. Lack of demand is indicated as demand. Lack of seed supply is indicated as seed supply. A propagation difficulty is indicated as propagation. PROC FREQ analysis.

When participants were asked if they had trouble propagating natives, the majority said yes (76.4%) with a minority responding no (23.53%). For participants who said yes, the following species were listed as desirable but present propagation

challenges; ocean spray (*Holodiscus discolor*), mountain hollyhock (*Iliamna rivularis*), fuzzytongue penstemon (*Penstemon eriantherus*), Bitterroot (*Lewisia rediviva*), skyrocket (*Ipomopsis aggregata*), beargrass (*Xerophyllum tenax*), curl-leaf mountain-mahogany (*Cercocarpus ledifolius*), and shrubby penstemon (*Dasiphora fruticose*).

Market Projection

Addressing industry concerns is important for the future development and stabilization of the Montana Native Plant Market. Successfully creating and sustaining a collaborative framework or network to support the native plant market may require increased communication and collaboration of Montana native plant growers. Based on our survey results, Montana native plant growers are willing, even eager, to increase lines of communication and avenues for potential collaboration. When asked if they would be interested in networking and other professional development opportunities, the majority said yes (82.35%)(Figure 6.9).

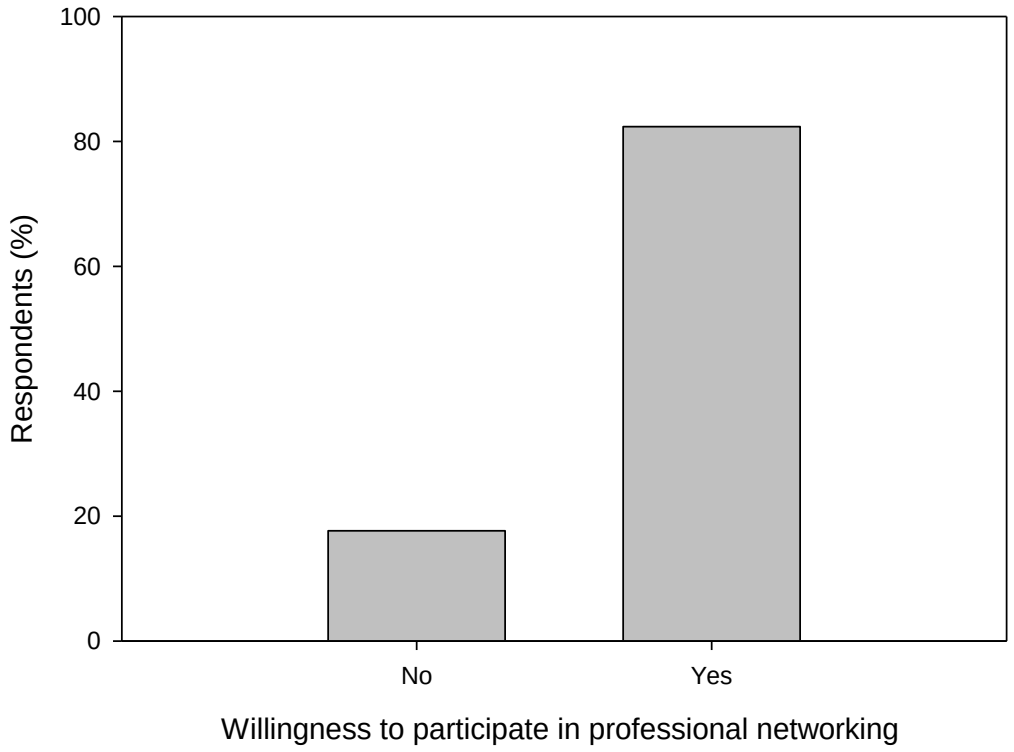

Figure 6.9. Frequency percentage of respondent's answers for question assessing willingness to network (yes or no) with other Montana native plant growers. PROC FREQ analysis.

When asked how they keep up to date with the native plant species which they may grow or sell, the majority indicated that they chose species based off previous year's sales and plant requests (46.1%), followed by personal experimentation and research (23.1%), through a society or social group, such as the Montana Native Plant Society (MNPS) (15.3%) and through online information (15.3%). When growers were asked if they would be interested in displaying educational information (brochures, fliers, etc.) on the growth and maintenance of native plant material, the majority said yes (70.59%),

suggesting growers were willing to help increase educational and information opportunities for native plant consumers (Figure 6.10).

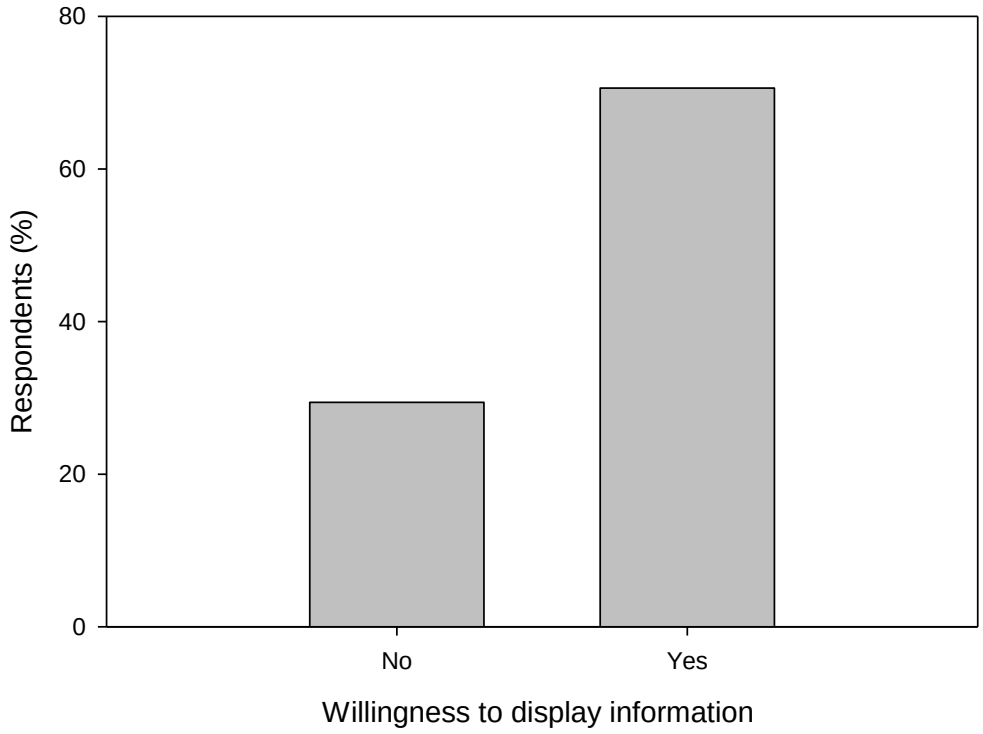

Figure 6.10. Frequency percentage of respondent's answers for question assessing willingness to display information (yes or no) regarding native plant material. PROC FREQ analysis.

Discussion

Demographics & Market Trends

Of the responses, the greatest frequency of respondents grew native plants on-site and also considered their business as a combination of wholesale and retail. The higher frequency of growers who grow their own plant material may indicate that Montana native plant nurseries may be smaller sized businesses and also may be categorized as

businesses participating in a specialized niche market. Another indication of the size of the native plant market may be the increased frequency of respondents whom considered their business either as a combination of wholesale or retail, or solely retail. Retail businesses, compared to wholesale businesses, may be smaller sized and less influential on the market. These results are similar to a survey of the Colorado plateau, in which the majority of nurseries surveyed also were retail or a combination of wholesale and retail (Potts et al. 2002).

As an additional likely refection of the small business nature of most Montana native plant nurseries, the majority of respondents held the role of owner of the business. This is similar to the results of Brzuszek and Harkess (2009) who also had a majority of the surveys completed by either owners or managers. This may reflect that the management of the nursery is principally involved in their business and has a level of experience or expertise in the propagation of native plants. However, it may also be a potential indication of a lack of resources and or supporting staff. Further questions regarding the number of staff and their roles within the business would help to clarify the size and availability of supporting resources, such as staff, for each nursery.

Our survey results indicating that the greatest number of native plant nurseries surveyed were recently established businesses may reflect a recent increase in demand for native plant material. Other surveys have reported similar trends from native plant market surveys. In a survey of nurseries in the Colorado plateau region, participants stated that native plant material is increasing in popularity (Potts et al. 2002). Brzuszek and Harkass

(2009) in a survey of the green industry in the Southeastern United States found that growers believe customers have become increasingly more interested in native plants.

Respondents listed over 25 different species when asked to list the top five most common species they sell with the majority of those species as woody vs. herbaceous native plant species. The majority of these species are woody (80%) vs. herbaceous (20%). These results differ from Brzuszek (2008) in which tree, shrub, and herbaceous plant material was roughly equal in popularity. However in Brzuszek (2011) tree species were in the highest demand (53.9%), followed by herbaceous plants (8.7%), and grasses were the least popular type of native plant material sold (5.5%).

Market Challenges

In our results growers indicated that a lack of seed supply was the top most limiting factor to the growth of the Montana native plant market. A smaller percentage of respondents believe that the limiting factor to the native plant market is customer demand. In our survey, when growers elaborated, a lack of information about native plant species for producers and consumers was thought to contribute to the lack of customer demand. Related factors including, a lack of professional networking and educational opportunities for native pant growers, and reduced communication and information sharing among growers, hinders the promotion of information among growers on how to propagate unfamiliar or new native plant species (Peppin et al. 2010).

Ultimately, all other limitations identified in the question, such as propagation difficulties, increased labor and cost, lack of demand, and lack of information for native plant producers and consumers, may all play a role in limiting the supply of native plants.

Limited supply and reduced availability of native plants as a major limiting factor in native plant market is also evident in other studies. In a survey of the Colorado plateau, 27% of respondents stated that the availability of seed was the primary limitation to the native plant market and 8% said that lack of seed supply was a top limitation to supplying native plant material (Peppin et al. 2010).

Other studies have reported that the lack of educational opportunities and information on the growth and maintenance of native plants is a significant challenge for the native plant market. Kauth and Perez (2011) stated that the education of customers was among the top concerns of the green industry in Florida (Kauth and Perez 2011). In In a survey of the Colorado plateau, 10% of respondents said lack of consumer knowledge of native plant material production was limiting the purchasing of native material and 21% said the lack of the grower's knowledge was limiting the supply of native plant material (Potts et al. 2002). There is also a lack of information for customers on how to maintain and establish native plants (Potts et al. 2002).

Market Projection

In summary, all of the challenges to the native plant market identified in our results, including: 1) lack of seed supply, 2) propagation difficulties, 3) lack of customer demand, 4) increased labor, and 5) expense, may contribute to creating a destabilizing effect on the Montana native plant market. In the Colorado Plateau, inconsistency and unreliability of the native plant market was the most significant limitation to supply of native plants (Peppin et al. 2010). Similarly, Kauth and Perez (2011) in a survey of

industries involved in the Florida native wildflower market stated that demand for native plants may be highly dependable upon region.

This inconsistency may be caused, in part, by regional market characteristics. Demand may be increased in western states where federal land management agencies may purchase up to 50% of the native plant material offered in one year (Peppin et al. 2010). Likewise, in Utah during 2003, the USFS and BLM were the two highest purchasers of native plant material in the western United States (Lynn et al. 2008; Peppin et al. 2010; Kauth and Perez 2011). Alternatively, Harkess (2009) stated that purchase of native plant material by government agencies consisted of about 4% of total sales in the Southeastern US. This inconsistency of demand may significantly contribute to the challenge of initiating and developing a native plant market (Beyers 2004; Peppin et al. 2010; Kauth and Perez 2011, Potts et al. 2002).

Conclusion

In summary, results from this survey indicate there is a demand for native plants in the state of Montana but additional work is needed to stabilize and increase the reliability of the Montana native plant market. Montana native plant growers who responded have stated the following top challenges to the native plant market; lack of seed supply, propagation difficulties, lack of customer demand, increased labor, and expense. However, the majority of respondents displayed a willingness to increase collaboration, perhaps helping to address one part of the challenge to the production of native plant species.

Results from this survey provide baseline information on the status of the Montana native plant market, which may be helpful in directing a future native plant market feasibility study. A number of studies have proposed market plans which may act as a model for the state of Montana. In Utah, the Utah Intermountain Native Plant Growers Association created a specialized identity for native plants and labelled them as "Utah Choice" (Meyer 2005). Another example, the Great Basin project, is entirely federally funded with objectives to conduct research on producing native plant species and provide growers with initial quantities of seed in the hopes greater seed quantities will be returned to be utilized in restoration projects (GBRI 2001; Pellant 2006; Peppin et al. 2010).

Most functioning market models rely on a combination of federal and non-federal funding through collaboration, partnerships, and state cooperatives. Although the greatest demand for native plant material often comes from federal agencies, availability of funding is largely affected by policy changes (Mortlock 1998; Peppin et al. 2010). It has been suggested that an entirely privately-funded market is unable to sustain itself due to the large amount of initial start-up money necessary to sustain the specialized type of production and propagation requirements of native plant material (Peppin et al. 2010).

Ultimately an important future challenge of the Montana native plant market may be to increase collaboration with multiple funding opportunities, such as through state, national, and private entities, in order to invest in the creation of a sustainable and economically profitable Montana native plant market.

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APPENDICES

APPENDIX A

CLASSIFICATION SCHEME BY BASKIN AND BASKIN (1998, 2004)

Baskin and Baskin (1998, 2004) Key

Table 1. A classification system for seed dormancy (modified from Nikolaeva, 1977; Baskin and Baskin, 1998). Does not include seeds with undifferentiated embryos

A. Class: Physiological dormancy (PD)

Level deep: type(s) 1,2,3,4

Level intermediate: type(s) 1,2,3,4

Level non-deep: type(s) 1, 2,3,4,5

B. Class: Morphological dormancy (MD) (does not include seeds with undifferentiated embryos)

C. Class: Morphophysiological dormancy (MPD)

Levels non-deep simple

Level intermediate simple

Level deep simple

Level deep simple epicotyl

Level deep simple double

Level non-deep complex

Level intermediate complex

Level deep complex (also see Table 3)

D. Class: Physical dormancy (PY)

E. Class: Combinational dormancy (PY + PD)

Level non-deep PD: type(s) 1,2

Table 2. Characteristics of dormancy in seeds with deep, intermediate and non-deep physiological dormancy (from information in Baskin and Baskin, 1998)

A. Level: Deep

Type 1: Excised embryo produces abnormal seedling

Type 2: GA does not promote germination

Type 3: Seeds require 3–4 months of cold stratification to germinate

B. Level: Intermediate

Type 1: Excised embryo produces normal seedling

Type 2: GA promotes germination in some (but not all) species

Type 3: Seeds require 2–3 months of cold stratification for dormancy break

Type 4: Dry storage can shorten the cold stratification period

C. Level: Non-deep

Type 1: Excised embryo produces normal seedling

Type 2: GA promotes germination

Type 3: Depending on species, cold (c. 0–10°C) or warm (15°C) stratification breaks dormancy

Type 4: Seeds may after-ripen in dry storage

Type 5: Scarification may promote germination

Table 3. Eight levels of morphophysiological dormancy (Baskin and Baskin 1998; Walck et al. 1999) and temperature, or temperature sequence, required to break them

MPD type:	To break dormancy	At time of embryo growth	GA3 overcomes dormancy
Non-deep simple	W or C	W	+c
Intermediate simple	W + C	W	+
Deep simple	W + C	W	+/–
Deep simple epicotyl	W + C	W	+/–
Deep simple double	C + W + C	W	?
Non-deep complex	C	C	+
Intermediate complex	C	C	+
Deep complex	C	C	–

KEY: W= warm stratification; C = cold stratification; MPD = morphophysiological dormancy; + = yes; +/– = yes/no; – = no

APPENDIX B

RECLASSIFICATION SCHEME BY SCHWIENBACHER (2011)

Schwienbacher (2011) Key

1. ND/PD

1.1. FGP (FRESHLD) ≥ FGP(CDSfresh) and FGP (FRESHLD) ≥ FGP (CWSfresh/CWSsubs) and MGT (FRESHLD) ≤ MGT (CDSfresh) and MGT (FRESHLD) ≤ MGT(CWSfresh/CWSsubs) and FGP (FRESHLD) ≥ 50%

Yes ND

No 1.2

1.2. FGP (CDSfresh) ≥ FGP(CWSfresh/CWSsubs) and MGT (CDSfresh) ≤ MGT (CWSfresh/CWSsubs) and FGP (CDSfresh) ≥ 50%

Yes PD, non-deep level

No 1.3

1.3 FGP (FRESHLD) ≥ 50% or FGP (CDSfresh) ≥ 50% or FGP (CWSfresh/CWSsubs) ≥ 50%

Yes PD, intermediate level

No PD, deep level

2. MD/MPD

2.1. FGP (FRESHLD) ≥ 50%

Yes MD

No MPD

3. PY/PY + PD

3.1. FGP (FRESHLD) ≥ FGP(FRESHsc) and MGT (FRESHLD) ≤ MGT (FRESHsc) and FGP (FRESHLD) ≥ 50%

Yes (assumption of impermeable seed coat dismissed!) ND

No 3.2

3.2. FGP (FRESHsc) ≥ 50%

Yes PY

No PY + PD

* For unknown values of FGP or MGT the truth-value of the functional operation was assumed to become TRUE, except for the comparison of FGP with 50%, where missing values resulted in a FALSE.

KEY

FGP = final germination percentage; MGT = mean germination time;

ND = non-dormant; PD = physiological dormancy; MD = morphological dormancy;

MPD = morphophysiological dormancy; PY = physical dormancy;

PY + PD = combinational dormancy; FRESHLD = seeds without storage under long day conditions; CWSfresh = cold-wet storage; CWSsubs = cold-wet storage subsequent to a germination experiment; CDSfresh = cold-dry storage; FRESHsc = scarification of seeds without storage.

APPENDIX C

ANOVA ANALYSIS TABLES FOR CHAPTER 2

Chapter 2 ANOVA Analysis Tables

Table 2.1. ANOVA analysis of stratification (W / C) in combination with after-ripening (CS, WS) on final germination percentage.

Source	DF	Type I SS	F Value	Pr > F
Model	3	7	0.7	0.578
After-ripening	1	5.3333	1.6	0.2415
Stratification	1	0.3333	0.1	0.7599
After-ripening x Stratification	1	1.3333	0.4	0.5447

Table 2.2. ANOVA analysis of combined stratification (W+ C) with after-ripening (CS/ WS) on final germination percentage

Source	DF	Type I SS	F Value	Pr > F
Model	3	13.6666	1.3	0.339
After-ripening	1	3	0.86	0.3816
Combined Stratification	1	5.3333	1.52	0.2521
Combined Stratification x After-ripening	1	5.3333	1.52	0.2521

Table 2.3. ANOVA analysis of after-ripening temperature (CS / WS) with after-ripening time periods (20, 40, 80, 120 & 240 days) on final germination percentage.

Source	DF	Type I SS	F Value	Pr > F
Model	9	184.0333	5.73	0.0006
Temperature	1	2.7	0.76	0.3946
After-ripening	4	169.5333	11.88	<.0001
Temperature x After-ripening	4	11.8	0.83	0.5235

Table 2.4. ANOVA analysis of GA (mg/L) with after-ripening temperature (CS, WS) on final germination percentage.

Source	DF	Type I SS	F Value	Pr > F
Model	7	4077.167	8.63	0.0002
After-ripening	1	192.6666	2.86	0.1104
Gibberellic Acid (GA)	3	3829.833	18.92	<.0001
After-ripening x GA	3	54.6666	0.27	0.846

Table 2.5. ANOVA analysis of harvest date, after-ripening temperature (CS, WS), and GA (mg/L).

Source	DF	SS Type I	F Value	Pr > F
Model	29	83916.67	49.13	<.0001
Harvest date	2	285.2167	2.42	0.0946
After-ripening	1	580.8	9.86	0.0023
Harvest date x After-ripening	2	101.45	0.86	0.4261
Gibberellic Acid (GA)	4	80656.42	342.38	<.0001
GA x Harvest date	8	814.7833	1.73	0.1023
After-ripening x GA	4	681.1167	2.89	0.0266
Harvest date x After-ripening x GA	8	796.8833	1.69	0.1113

Table 2.6. ANOVA analysis of harvest date, after-ripening temperature (CS, WS), and GA (mg.L) on T50 values.

Source	DF	SS Type I	F Value	Pr > F
Model	29	7708.542	16.71	<.0001
Harvest date	2	31.6666	1	0.3735
After-ripening	1	255.2083	16.05	0.0001
Harvest date x After-ripening	2	186.6666	5.87	0.004
Gibberellic Acid (GA)	4	6186.667	97.26	<.0001
GA x Harvest date	8	318.3333	2.5	0.0168
After-ripening x GA	4	508.3333	7.99	<.0001
Harvest date x After-ripening x GA	8	221.6666	1.74	0.0994

Table 2.7. ANOVA analysis of harvest date, after-ripening temperature (CS, WS), and GA (mg/L) on T90 values.

Source	DF	SS Type I	F Value	Pr > F
Model	29	3980	5.71	<.0001
Harvest date	2	105	2.18	0.1184
After-ripening	1	907.5	37.77	<.0001
Harvest date x After-ripening	2	65	1.35	0.2638
Gibberellic Acid (GA)	4	1505	15.66	<.0001
GA x Harvest date	8	688.75	3.58	0.0012
After-ripening x GA	4	71.6666	0.75	0.5634
Harvest date x After-ripening x GA	8	637.0833	3.31	0.0023

APPENDIX D

ANOVA ANALYSIS TABLES FOR CHAPTER 3

Chapter 3 ANOVA Analysis Tables

Table 3.1. ANOVA analysis of container type (Cone-tainer and 4-inch square pot) and fertilizer (mg nitrogen/L) on leaf length (mm).

Source	DF	SS Type I	F Value	Pr > F
Model	9	8787.595	4.16	<.0001
Container	1	4020.319	17.11	<.0001
Fertilizer	4	2070.717	2.2	0.0721
Container x Fertilizer	4	2696.56	2.87	0.0256

Table 3.2. ANOVA analysis of container type (Cone-tainer and 4-inch square pot) and fertilizer (mg nitrogen/L) on leaf width (mm).

Source	DF	SS Type I	F Value	Pr > F
Model	9	1775.803	3.36	0.001
Container	1	298.2763	5.08	0.0259
Fertilizer	4	986.6533	4.2	0.0031
Container x Fertilizer	4	490.8738	2.09	0.0858

Table 3.3. ANOVA analysis of container type (Cone-tainer and 4-inch square pot) and fertilizer level (mg nitrogen/L) on shoot dry weight (g).

Source	DF	SS Type I	F Value	Pr > F
Model	9	0.1939	3.9	0.0002
Container	1	0.0111	2.03	0.157
Fertilizer	4	0.1405	6.36	0.0001
Container x Fertilizer	4	0.0421	1.91	0.1131

Table 3.4. ANOVA analysis of container type (Cone-tainer and 4-inch square pot) and fertilizer level (mg nitrogen/L) on root dry weight (g).

Source	DF	SS Type I	F Value	Pr > F
Model	9	2.0696	4.53	<.0001
Container	1	0.5947	11.72	0.0008
Fertilizer	4	0.6808	3.35	0.012
Container x Fertilizer	4	0.794	3.91	0.005

Table 3.5. ANOVA analysis of container type (Cone-tainer and 4-inch square pot) and fertilizer level (mg nitrogen/L) on root-to-shoot ratio (g).

Source	DF	SS Type I	F Value	Pr > F
Model	9	112.1183	6.77	<.0001
Container	1	23.05314	12.53	0.0006
Fertilizer	4	77.71274	10.56	<.0001
Container x Fertilizer	4	11.35242	1.54	0.1936

APPENDIX E

ANOVA ANALYSIS TABLES FOR CHAPTER 4

Chapter 4 ANOVA Analysis Tables

Table 4.1 ANOVA analysis of GA on final germination percentage.

Source	DF	Sum of Squares	F Value	Pr > F
Model	4	2189.45	172.17	<.0001
Gibberellic acid (GA)	4	2189.45	547.3625	<.0001

Table 4.2. ANOVA analysis of stratification (W / C) on final germination percentage.

Source	DF	Sum of Squares	F Value	Pr > F
Model	2	165.1667	42.47	<.0001
Stratification	2	165.1667	42.47	<.0001

Table 4.3: ANOVA analysis of mechanical scarification (seconds) and stratification (days) on final germination percentage.

Source	DF	SS Type I	F Value	Pr > F
Model	19	47630.55	8.43	<.0001
Scarification	4	41695.68	35.07	<.0001
Stratification	3	689.25	0.77	0.5137
Scarification x Stratification	12	5245.625	1.47	0.1609

Table 4.4: ANOVA analysis of mechanical scarification (seconds) of new and worn 40 grit sandpaper on final germination percentage.

Source	DF	SS Type I	F Value	Pr > F
Model	9	39861.73	24.15	<.0001
Scarification	4	33237.35	45.31	<.0001
Sandpaper	1	4708.9	25.68	<.0001
Scarification x Sandpaper	4	1915.475	2.61	0.0552

Table 4.5. ANOVA analysis of mechanical scarification (seconds) of new and worn 40 grit sandpaper on T50 values.

Source	DF	SS Type I	F Value	Pr > F
Model	9	366	18.48	<.0001
Scarification	4	353	40.11	<.0001
Sandpaper	1	3.6	1.64	0.2106
Scarification x Sandpaper	4	9.4	1.07	0.3895

Table 4.6. ANOVA analysis of mechanical scarification (seconds) of new and worn 40 grit sandpaper on T90 values.

Source	DF	SS Type I	F Value	Pr > F
Model	9	672.1	5.05	0.0004
Scarification	4	369.1	6.24	0.0009
Sandpaper	1	211.6	14.31	0.0007
Scarification x Sandpaper	4	91.4	1.55	0.2144

APPENDIX F

ANOVA ANALYSIS TABLES FOR CHAPTER 5

Chapter 5 ANOVA Analysis Tables

Table 5.1. ANOVA analysis of fertilizer and container type (Cone-tainer and 4-inch square pot) on plant height measurements (mm).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	380370.9	42263.44	29.52	<.0001
Container	1	26194.46	26194.46	18.3	<.0001
Fertilizer	4	333057.4	83264.35	58.16	<.0001
Container x Fertilizer	4	21119.07	5279.768	3.69	0.007

Table 5.2. Effect of fertilizer and container type (Cone-tainer and 4-inch square pot) on flowering occurrence per treatment (n=140 plants total).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	16.89	1.8766	13.67	<.0001
Container	1	0.9461	0.9461	6.89	0.0097
Fertilizer	4	14.0441	3.511	25.57	<.0001
Container x Fertilizer	4	1.8997	0.4749	3.46	0.0101

Table. 5.3. ANOVA analysis of container type (Cone-tainer and 4-inch square pot) and fertilizer (mg nitrogen/L) on plant spread (mm)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	1029385	114376.1	88.01	<.0001
Container	1	76252.71	76252.71	58.68	<.0001
Fertilizer	4	939585.7	234896.4	180.76	<.0001
Container x Fertilizer	4	13546.14	3386.534	2.61	0.0387

Table 5.4. ANOVA analysis container type (Cone-tainer and 4-inch square pot) and fertilizer (mg nitrogen/L) on SDW (g).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	499.4239	55.4915	66.75	<.0001
Container	1	117.2265	117.2265	141.02	<.0001
Fertilizer	4	348.9463	87.2365	104.94	<.0001
Container x Fertilizer	4	33.251	8.3127	10	<.0001

Table 5.5. ANOVA analysis of container type (Cone-tainer and 4-inch square pot) and fertilizer (mg nitrogen/ L) on RDW (g).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	9.9556	1.1061	25.37	<.0001
Container	1	3.3743	3.3743	77.39	<.0001
Fertilizer	4	6.0241	1.506	34.54	<.0001
Container x Fertilizer	4	0.5571	0.1392	3.19	0.0154

Table 5.6. ANOVA analysis of fertilizer (mg nitrogen/ L) on root-to-shoot ratio (g/g)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	6.7286	0.7476	7.12	<.0001
Container	1	0.2791	0.2791	2.66	0.1054
Fertilizer	4	6.1683	1.542	14.68	<.0001
Container x Fertilizer	4	0.2811	0.0702	0.67	0.6145

APPENDIX G

SURVEY QUESTIONS FOR CHAPTER 6

Chapter 6 Survey Questions

Table 5.1. Questions included on the 2015 survey of Montana native plant nurseries.

1. Do you grow or supply native plants in the state?
2. In what region are you located?
3. What position is your role within your business?
4. When was your business created?
5. Are you a retail, wholesale or a combination of both retail and wholesale
6. Why do you supply or grow native plants?
7. What are the most common 5-10 native plant species that you sell?
8. What is an average dollar value of the native plants which you may have sold in 2012-2013?
9. What is an average dollar value of the non-native plants which you may have sold in 2012-2013?
10. What is a challenge to your business prohibiting supply or sale of more native plant species?
11. In your experience, has there been a native plant species which you wish to grow but the growth or germination of this species has been difficult?
12. How do you keep up to date with native plant species which you would like to grow or sell?

13. Would you be interested in participating in a listserv to receive email notifications and updates to free networking and professional development opportunities for growers and nurseries involved in the native plant material market?
14. Would you be interested in displaying educational information (brochures, fliers, etc.) on the growth and maintenance of native plant material?
