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Title: Determining the Influence of Nutrition and Temperature on the Growth and Development of *Camassia* spp.

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As a key historic component of prairies in the Pacific Northwest, *Camassia* spp. should play a role in the restoration of these ecosystems. To do so effectively, further study of *Camassia* spp. propagation is warranted. Thus, the growth, N uptake and allocation, and seasonal thermoperiodicity of *Camassia* spp. was examined. *Camassia leichtlinii* was found to grow and take up N in the spring following a cubic function. Nitrogen was allocated to the leaves and roots prior to the leaves reaching their mature size, and to the daughter bulb thereafter. These results suggest that the most efficient fertilization program may be to deliver N at a rate that follows a Gaussian function. Increasing soil N availability with spring fertilizer applications had only a small impact on the growth of *C. leichtlinii*, however it had a large impact on the N concentration and content of its leaves, roots, and daughter bulb. This suggests that *Camassia* spp. are luxury consumers of N. Increasing the duration of chilling applied to quiescent bulbs resulted in an increased probability of leaf emergence, decreased time between the cessation of chilling and leaf emergence, and faster leaf growth after leaf emergence for the species *C. leichtlinii*, *C. quamash*, and *Toxicoscordion venenosum*. Increasing the duration of the summer rest period (prior to applying chilling) resulted in increased a) daughter bulb and root dry weight at the cessation of chilling, b) growth rate of the leaves, and c) length of the leaves at leaf maturity. These results show that all three species respond to seasonal thermoperiodicity, and thus temperature manipulation may be a useful tool in their propagation.

Determining the Influence of Nutrition and Temperature on the Growth and Development of *Camassia* spp.

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

Matthew Davis, Author

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Chapter 1: Introduction

Prairie Restoration in the Pacific Northwest

Native prairies and oak savannahs dominated the valleys of the Pacific Northwest prior to the 1850s (Fuchs, 2001), and are, as much as old growth forests, a quintessential Pacific Northwest landscape. However, agricultural and urban developments have shattered these ecosystems, and woody encroachment, invasive species, and climate change threaten what remains (Dunwiddie, and Bakker 2011). This is unfortunate because of the ecological, cultural, and aesthetic significance of native prairies. Native prairies are productive ecosystems that provide habitat for endangered wildlife (Taft and Haig, 2003; Ries *et al.*, 2001). They are also biodiverse. For example, Garry oak (*Quercus garryana* Douglas *ex* Hook.) ecosystems are associated with 694 plant taxa, making them home to a greater diversity of plants than any of coastal British Columbia's other ecosystems (Fuchs, 2001). Furthermore, prairies and savannahs have many characteristics that may help to offset the effects of anthropogenic greenhouse gas emissions; they have a relatively high albedo, a strong effect on the vertical distribution of soil organic carbon, and their cool and often saturated soils reduce soil respiration (Chapin III *et al.*, 2011; Jobbágy and Jackson, 2000; McDaniel and Falen, 2014). Culturally, prairies were and continue to be important to many indigenous people in western North America. Aesthetically, prairies are pleasing, with open skies and abundant wildflowers.

For these reasons, the restoration of wet prairie, oak savannah, and lowland meadow ecosystems is emerging as a high priority (Dunwiddie and Bakker, 2011). The science of restoration ecology encompasses a collection of practices, which exist along a continuum from the amelioration of physical and chemical damage to the soil at mining sites to the enhancement of conservation values on already productive landscapes (Hobbs and Norton, 1996). In the context of this paper, successes would be (a) a decrease in invasive species cover and an increase of native species cover in extant prairies, and (b) the conversion of fallow or degraded land back to native prairie. However, only under the

most conservation inclined of future scenarios do stakeholders expect that the amount of native prairie will increase, and this would be at the expense of agricultural land (Baker *et al.*, 2004).

Because of these challenges, efforts to protect remnant prairies and create future prairies will undoubtedly require strong public support, and both restoration successes and a compelling narrative will be key in creating and maintaining such support. Perhaps the conservation of one of the Pacific Northwest's most cherished native plants will help to root prairie restoration in the public's imagination. These plants are, of course, members of the genus *Camassia* Lindl. They are particularly well known for their role in the diets of people indigenous to the Pacific Northwest (Kuhnlein and Turner, 1991; Sturtevant 2008) but are also among this region's largest and showiest native wildflowers and are important to pollinators and wildlife (Parachnowitsch and Elle, 2005; Craighead *et al.*, 1998). Because *Camassia* spp. are so charismatic, they may be keystone restoration species, meaning that the public may be interested in protecting and restoring native prairies for their benefit.

If indeed the future of prairie restoration hinges on both restoration successes and a compelling narrative, careful study of the propagation of *Camassia* spp. is warranted. Thus, this thesis aims to determine factors that affect the growth and development of *Camassia* spp., with the intention that through these studies, methods for quickly growing *Camassia* bulbs from seeds will become apparent.

The Biology of Camassia *spp.*

Phylogeny

Camassia is a genus of spring flowering bulbous perennials endemic to North America. All *Camassia* spp. have a basal whorl of linear leaves and a simple erect scape with a raceme of large showy flowers (Gould, 1942). The genus was recently placed within the subfamily Agavoideae, in the family Asparagaceae (Chase *et al.*, 2009). DNA

analysis suggests that their closest relatives are in the genera *Hastingsia* S. Wats. and *Chlorogalum* (Lindl.) Kunth (Archibald *et al.*, 2015).

The proposed center of dispersal for the genus *Camassia* is southwest Oregon, where three species and five subspecies persist (Gould, 1942; Fishbein *et al.*, 2010). Three early diverging lineages of *Camassia* have been identified, and within these lineages six species have been delimited (Fishbein *et al.*, 2010). Ranker and Hogan (2002) provide excellent descriptions of each species and subspecies.

The first of the early diverging lineages that Fishbein *et al.* (2010) identified is the largest in terms of range and diversity. It contains four species: *C. quamash*, *Camassia cusickii* S. Watson, *Camassia scilloides* (Rafinesque) Cory, and *Camassia angusta* (Engelmann and A. Gray) Blankinship. *Camassia quamash* ranges from British Columbia and Alberta to California, and from the Pacific Coast to Utah and Wyoming (Ranker and Hogan, 2002). It is the most variable of the *Camassia* spp. and has eight recognized subspecies: *C. q. linearis* Gould, *C. q. breviflora* Gould, *C. q. utahensis* Gould, *C. q. azurea* (A. Heller) Gould, *C. q. maxima* Gould, *C. q. walpolei* (Piper) Gould, *C. q. intermedia* Gould, and *C. q. quamash* (Ranker and Hogan, 2002). *Camassia quamash* is a paraphyletic species, and *C. scilloides* and *C. cusickii* were derived from within *C. quamash* (Fishbein *et al.*, 2010). *Camassia cusickii* flowers from mid to late spring and can be found on hillsides in the Wallowa Mountains of northeast Oregon, and on the slopes of the eastern bank of the Snake River in Idaho (Jewell, 1978). *Camassia scilloides* flowers in mid to late spring, and can be found on prairies from Texas, Alabama, and Georgia in the south, to Iowa, Wisconsin, Ontario and Pennsylvania in the North (Ranker and Hogan, 2002). The final species in this lineage, *C. angusta*, is derived from *C. scilloides* (Ranker and Schnabel, 1986). *Camassia angusta* flowers two to three weeks after sympatric populations of *C. scilloides*, in mid to late spring (Ranker and Hogan, 2002). Its range includes prairies from Texas, Oklahoma, and Arkansas in the south, to Illinois, Iowa, and Indiana in the north.

The next lineage contains one species, *C. leichtlinii*, and has two subspecies, *C. l. suksdorfii* (Greenman) Gould and *C. l. leichtlinii* (Fishbein *et al.*, 2010). Of the two

subspecies, *C. l. suksdorfii* has the largest range, which includes British Columbia, Washington, California, and Oregon (Ranker and Hogan, 2002). The other subspecies, *C. l. leichtlinii*, is endemic to the Umpqua Valley of southwest Oregon.

The third lineage is the smallest, containing one species, *Camassia howellii* S. Watson. The species is a candidate for federal protection, category 2, and is endemic to southwest Oregon, where it grows on dry, open slopes on serpentine soil (Gould, 1942; Knight and Seevers, 1992). *Camassia howellii* flowers in late spring, one to two weeks after sympatric populations of *C. leichtlinii* (Ranker and Hogan, 2002).

Within *Camassia* a considerable amount of work has gone into delimiting the different species. Gould (1942) recognized five species, but noted that while four species, *C. howellii*, *C. leichtlinii*, *C. quamash*, and *C. scilloides* were all genetically distinct, he had insufficient evidence to conclude that the same was true for *C. cusickii*. His original proposal has since been revised, as *C. cusickii* was shown to be a distinct species with many traits that distinguish it from *C. quamash* (Jewell, 1978). *Camassia angusta* was also shown to be a distinct species based on differences between it and *C. scilloides* in relative flowering time, morphological characteristics, and isozymic variation (Ranker and Schnabel, 1986). Finally, Uyeda and Kephart (2006) used allozyme analysis to support a species level distinction between *C. leichtlinii* and *C. quamash*. As for subspecies, while there is strong support for nesting *C. l. leichtlinii* within *C. l. suksdorfii*, there is no genetic evidence supporting phylogenetically distinct lineages corresponding with the recognized subspecies of *C. quamash* (Fishbein *et al.*, 2010).

Although this thesis will focus primarily on *C. leichtlinii* and to a lesser extent *C. quamash* propagation, the principles developed could likely be extended to other members of the genus. Furthermore, because *C. howellii* and *C. cusickii* are restricted to relatively small ranges, they may be particularly vulnerable to climate change and development, and thus worthy of future research efforts.

Anatomy

This section will review the various organs that make up *Camassia* spp. However, it is important to note that every organ (possibly excepting the basal plate) is replaced on an annual basis, in what is called an annual replacement cycle (Maclay, 1928; Thoms, 1989). This dynamic nature makes it difficult to consider their anatomy without also considering their annual replacement cycle. Information on the annual replacement cycle will be included where it is appropriate and will be covered in more depth in the next section.

The first organ to consider is the bulb. Bulbs are perennating underground storage organs that consist of a shortened stem called a basal plate, bearing several scales, which enclose one or more apical meristems (De Hertogh and Le Nard, 1993; Rees, 1972). Depending on the spring flowering bulb species, scales are either modified leaf bases or modified scale leaves (or both, e.g. *Lilium* L. bulbs), though Maclay (1928) was uncertain as to which made up the scales of *C. quamash* bulbs. These scales act as a store for food reserves, nutrients, and moisture, and thus they allow the plant to persist year after year in the face of environmental challenges such as summer drought, freezing winters, and fire (De Hertogh and Le Nard, 1993; Rees, 1972). Bulbs are dynamic, consisting at any time of both growing and senescing tissues (De Hertogh and Le Nard, 1993).

The bulbs of *Camassia* spp. consist of two parts: a mother bulb and its enclosed daughter bulb (Maclay, 1928; Thoms, 1989). The mother bulb acts as a source of food and nutrients for the growing daughter bulb and shrinks during its lifetime. The daughter bulb is a sink for energy and nutrients, fed by both the mother bulb and the leaves, and grows from its initiation until it becomes a mother bulb.

Every year, new leaves are developed as part of the daughter bulb, and they emerge in late winter or early spring. Leaves are linear, keeled, and often clasping at emergence, but form a basal whorl when open.

Camassia spp. have an adventitious root system. Adventitious roots are generally homogenous in size, and so are sometimes called fibrous roots (Esau, 1977). Roots

emerge from the basal plate, which is a modified shoot. The only non-adventitious root is the seedling taproot, i.e. the radicle, which emerges from the seed (Esau, 1977). However, in *C. leichtlinii*, this root is short-lived, and appears to senesce in the summer, after the leaves senesce and the plant is quiescent (personal observation). Branching in the seedling taproot and adventitious roots has been observed in *C. leichtlinii* (personal observation).

The inflorescence is an indeterminate raceme, with a sterile bract growing off each pedicle (Ranker and Hogan, 2002). Flowers can either be actinomorphic (radially symmetrical) or slightly zygomorphic (bilaterally symmetrical), depending on the species. Flowers have six tepals, which are lanceolate in shape and veined. The tepals are either white, blue, or violet. Flower have six stamens and an ovary with three locules. Septal nectaries are used to attract pollinators. The fruits are tri-locular capsules, and dehiscence of the capsules is loculicidal. The seeds are shiny and black and are ovoid, ellipsoid, or obpyriform.

Camassia leichtlinii employs droppers and contractile roots to ensure its bulb's subterranean position, and both have been observed in its second growing season (personal observation; Figures 1A and 1B). Droppers reposition the daughter bulb to lower soil depths and have been observed in other flowering bulb species (De Hertogh and Le Nard, 1993; Rees, 1972). Contractile roots are a special type of root, which shorten to pull the bulb deeper into the soil (Esau, 1977). Contractile roots have also been observed in *C. q. quamash* (Rimbach, 1929 *fide* Kawa and De Hertogh, 1992).

Figure 1: The anatomy of Camassia leichtlinii*. Organs are listed from the top most arrow down. (A)* Camassia leichtlinii *exhibiting a decomposing mother bulb, a dropper, a non-branching adventitious root and a contractile root. Note how the decomposed mother bulb is above the dropper. (B)* Camassia leichtlinii *exhibiting the tip of some sort of protective sheath, a decomposing mother bulb, a dropper, and a contractile root. (C)* Camassia leichtlinii *from July 2016, with a small terminal bud present (arrow), which will develop into next year's daughter bulb. From Thoms (1989, p.151), "By [late May or early June], a small terminal bud, the daughter bulb for next year, is present in the center of the carbohydrate-rich bulb." Note also in (C) the branching off the primary root.*

Some *Camassia* spp. are known to reproduce asexually through the generation of offset bulblets (Le Nard and De Hertogh, 1993b), however for other this appears to be quite rare. Thoms (1989) observed that single bulbs were the norm for *C. quamash* growing in the interior regions of the Pacific Northwest, with bulbs growing in clumps of two or three making up a small fraction of the total. He also mentions a variety of *C. quamash* that grows in clumps in the Snake River Valley in eastern Oregon (Thoms, 1989). In addition, he surmised that because *C. scilloides* and *C. cusickii* often grow in clumps they might regularly reproduce asexually. The best evidence for asexual reproduction in *C. leichtlinii* was provided by Beckwith (2004). In her nursery trials with *C. leichtlinii* from Vancouver Island, she found that roughly 15% of her bulbs reproduced asexually (produced at least one offset bulblet) during a five-year period. The number of offset bulblets produced each year increased over time, and the ability to produce multiple offset bulblets was associated with bulb weight (r=0.231; Beckwith, 2004). These results indicate that *C. leichtlinii* does indeed reproduce asexually, but that this is at least somewhat associated with bulb age or size.

Annual Replacement Cycle

Camassia spp. are spring flowering bulbs, meaning that their leaves emerge in late winter, they flower in the spring, and that they enter a summer rest period (Le Nard and De Hertogh, 1993b). Much of what is known about the periodicity of *Camassia* spp. comes from Maclay (1928), who first described the life cycle of *C. quamash*, and Thoms (1989). Maclay (1928) begins by describing the anatomy of *C. quamash* before its leaves emerge in late winter, starting with the outer most bulb layer. These layers can be divided into three generations, based on the year the tissues originated, and are called the mother bulb, the daughter bulb, and the granddaughter tissue. With each year that passes, the granddaughter tissue becomes the new daughter bulb, the daughter bulb becomes the new mother bulb, and the mother bulb shrinks and eventually rots away. These parts are all attached to the basal plate.

The outermost part of a *C. quamash* bulb is the mother bulb, which contains reserves that will feed the foliage and flowers that are about to emerge.

- It is covered by the tunic, which is a thin, brown layer of almost completely disintegrated tissue.
- Within the tunic are several white fleshy scales, possibly enlarged leaf bases, which store the bulk of the bulb's reserves.
- Within the mother bulb are the remains of last year's flowering stalk, surrounded by what Maclay (1928) calls the "scales of two".

Within the "scales of two" rests the daughter bulb, which contains the flowers and foliage that are about to emerge.

- The outer whorls of the daughter bulb are the leaves. Their bases possibly become next year's mother bulb, after the tops have senesced.
- Within the daughter bulb of a mature plant is a developed raceme; its flowers are not quite mature.

Finally, although it can hardly be considered a bulb, the tissue that will develop into next year's daughter bulb (i.e. the granddaughter tissue) is also present. It is made up of a mass of meristematic tissue forming a terminal bud and sits next to the daughter bulb's raceme.

Maclay (1928) describes the growth of *C. quamash* from granddaughter tissue through the development of the flower to pollination, while Thoms (1989) describes the development of both the daughter and mother bulb from flowering to death. Development of the meristematic mass begins with development of new leaves (Maclay, 1928). After they are developed, the meristematic region continues to elongate and forms flower primordia. By early summer the meristematic mass has developed into a small terminal bud, and this bud continues to grow into the summer, even after the aboveground foliage has senesced (Figure 1C; Thoms, 1989). The senescence of the aboveground foliage may be a good point of demarcation, after which the terminal bud can be considered as the daughter bulb. Its flower primordia continue to develop during autumn, with sporogenous tissue forming in the flowers in October (Maclay, 1928). Then the plant enters its winter rest period, which ends when the daughter bulb's leaves emerge the following spring, and then the flowers, and by the beginning of summer the flowers have withered and capsules

begin to form (Thoms, 1989). By mid-June, the daughter bulb attains its maximum size, and a little while later the capsules are fully formed. At this point in development, the daughter bulb has become a source of carbohydrates and nutrients, and thus can be considered as the new mother bulb. The mother bulb is a source of energy for the rest of the plant and expends carbohydrates from mid-June throughout the winter (Thoms, 1989). The following spring the carbohydrates of the mother bulb are largely expended, and by the beginning of summer the mother bulb has largely decomposed. This cycle is illustrated in Figure 2.

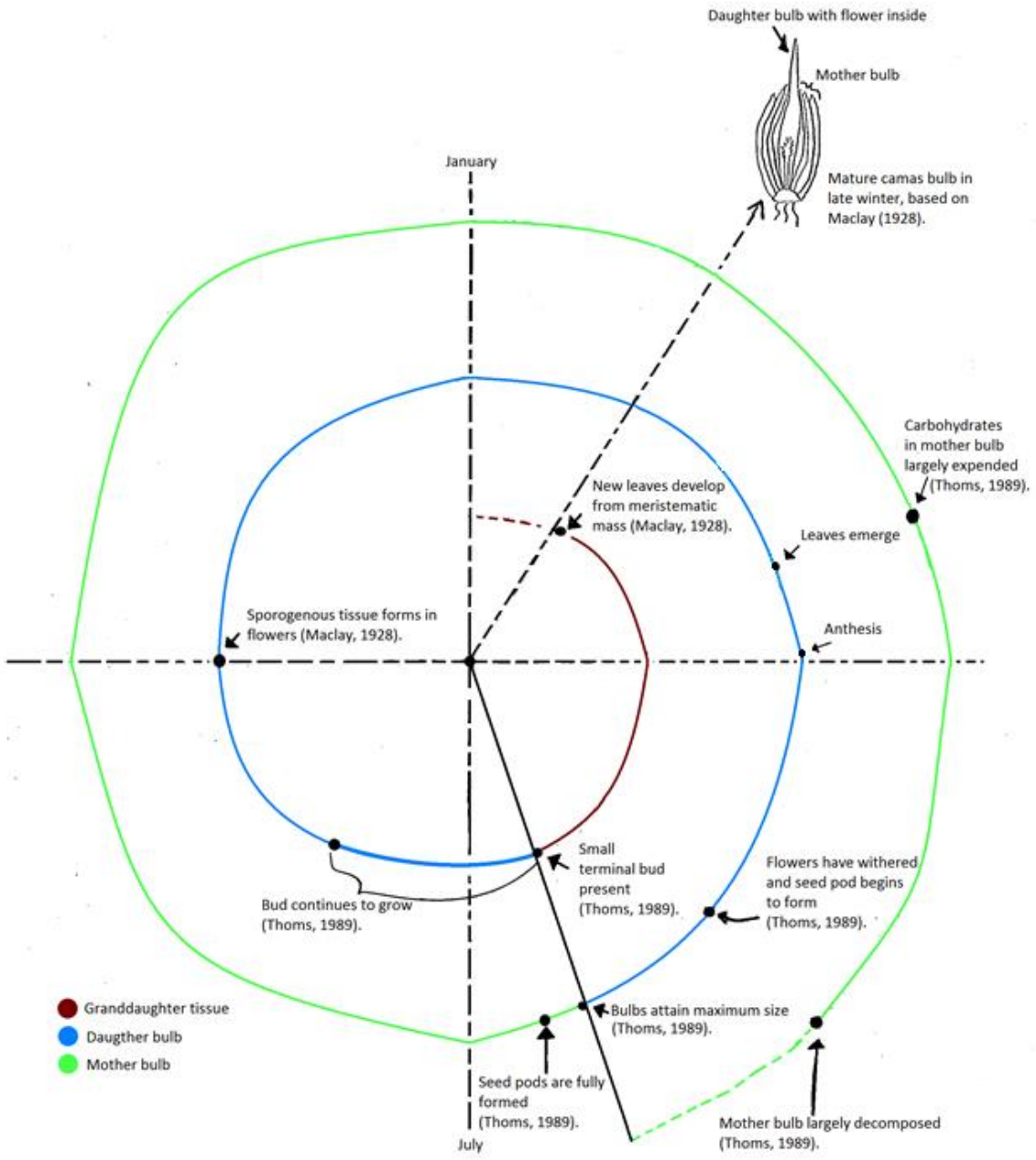

Figure 2: The annual replacement cycle of Camassia quamash*. The initiation of the granddaughter tissue occurs in late winter or early spring and this new organ persists for more than two years before it decomposes. Thus, at any given time of year, either two or three generations of tissue are present within the bulb. This figure is based on the written works of Maclay (1928) and Thoms (1989).*

The Cultural and Ecological Significance of **Camassia** *spp.*

Cultural Significance

Camassia quamash and *C. leichtlinii* (which will be referred to collectively as camas) are among the most culturally significant native plants of western North America. They were an important vegetable food for many of the native people living along the Pacific Northwest coast and on the Columbian (or Northwest) Plateau and were also used by some who lived in the Great Basin, modern day California, and the Great Plains (Kuhnlein and Turner, 1991; Sturtevant, 2008). Camas was used as either a staple, a supplement, or a condiment, depending on its availability (Thoms, 1989). *Camassia howellii* and *C. cusickii* may have been used as food as well (Beckwith, 2004; Jewell, 1978).

Camas was harvested by digging up the bulbs. This harvesting was frequently the responsibility of women, was often a group effort, and was performed using special digging sticks, crafted from *Taxus brevifolia* Nutt., *Holodiscus discolor* (Pursh) Maxim., or other woody plants (Kuhnlein and Turner, 1991; Turner and Kuhnlein, 1983). Bulbs were generally harvested in the summer, after flowering, though other times have been reported (Kuhnlein and Turner, 1991; Turner and Kuhnlein, 1983).

Camas was almost always cooked before it was eaten, and often this cooking was done in a steaming pit (Turner and Kuhnlein, 1983). Raw bulbs contain inulin, an indigestible polysaccharide, which is broken down into sweet and digestible fructose during the cooking process (Kuhnlein and Turner, 1991). Thus, camas was not only a source of carbohydrates, it was also a very flavorful food. Native groups are known to have distinguished between different types of camas by color, shape, location and flavor (Ray, 1933). Some places, including parts of what is now Idaho, were famous for the superior flavor, sweetness, and size of their camas bulbs (Turney-High, 1937).

Camas was a common trade item. In western Washington, no food, excepting choice varieties of dry salmon, was more widely traded (Gunther, 1973). Groups often traded camas to tribes that did not have access to camas grounds. For example, the Sooke and Songish from southern Vancouver Island traded bulbs to the Nuu-chah-nulth of western Vancouver Island, where camas was not grown (Gritzner, 1994). Camas was also traded to people who had access to camas grounds. For example, the Nez Perce traded their superior tasting camas to the Bitterroot Salish, even though camas was plentiful in Bitterroot Salish territories (Gritzner, 1994).

Native people in the modern era venerate camas. Because of this, some might be skeptical of propagation efforts, as they might be viewed as potentially leading to the commodification of a culturally significant plant. It is therefore imperative that growers, restoration specialists, and researchers be open to dialogue with native people, and to be sensitive and consider the possible ramifications of camas research.

Nutrition

Because of the importance of *C. quamash* and *C. leichtlinii* in the diets of the people indigenous to western North America, considerable work has been done on the nutrition of these species. Unfortunately, because these data were likely collected in accordance with indigenous practice, they may not reflect some of the dynamic changes that are occurring across the annual replacement cycle. Thus, they allow for only a cursory understanding of the chemical makeup of the bulbs. *Camassia leichtlinii* and *C. quamash* nutrition were summarized by Turner and Kuhnlein (1983; Table 1). For additional information on the nutrients of fresh *C. leichtlinii* and *C. quamash*, see Kuhnlein and Turner (1991).

Table 1: Nutrients of raw bulbs of Camassia quamash *and* C. leichtlinii.

Nutrient	Unit	*C. quamash*	*C. leichtlinii*
Moisture	%	83.4[a]	81.8[a]
Fat	%	0.01[a]	0.09[a]
Ash	%	0.84[a]	0.93[a]
Neutral detergent fiber	% DW	7.06[a]	6.03[a]
Acid detergent fiber	% DW	6.16[a]	6.02[a]
Inulin	% DW	41.1[b]	36.6[c]
Reducing sugar	% DW	6.8[b]	1.6[c]
Starch	% DW	0.0[b]	0.0[c]
Crude fiber	% DW	3.0[d]	-[c]
Nitrogen	% DW	0.89[a]	0.91[a]
Sulfur	% DW	0.115[a]	0.131[a]
Calcium	% DW	0.104[a]	0.105[a]
Phosphorus	% DW	0.270[a]	0.271[a]
Magnesium	% DW	0.052[a]	0.047[a]
Iron	ug gDW^{-1}	99[a]	31[a]
Copper	ug gDW^{-1}	3[a]	4[a]
Zinc	ug gDW^{-1}	27[a]	22[a]
Strontium	ug gDW^{-1}	6[a]	6[a]
Barium	ug gDW^{-1}	7[a]	4[a]

[a] Turner and Kuhnlein (1983). Data is from raw camas, tunic removed, from the SW coast of Vancouver Island.
[b] Yanovsky and Kingsbury (1938). Data is a mean of six samples, one each from Utah, Oregon, Washington, and Montana, and two from Idaho.
[c] Yanovsky and Kingbury (1938). Data is from one Washington sample.
[d] Yanovsky and Kingsbury (1938). Data from one Utah sample.

Inulin, which *C. quamash* and *C. leichtlinii* bulbs contain in abundance (Table 1; Yanovsky and Kingsbury, 1938), deserves special consideration. It is a polysaccharide, in the class Fructan, and is made up mostly or exclusively of β-(1→2) fructosyl-fructose linkages (Roberfroid, 2005). From an anthropocentric point of view, inulin is significant because it is indigestible, however, cooking breaks it down into edible fructose (Turner and Kuhnlein, 1983). From the plant's perspective, inulin, which is often found stored in underground overwintering organs, may offer protection from freezing and drought (Roberfroid, 2005). Indeed, *C. cusickii* is known to survive temperatures of -13°C (Sakai and Yoshie, 1984). Additionally, during an artificially imposed winter rest period, *C. leichtlinii* had no mortality even though temperatures fell to as low as -7°C (Chapter 3).

Ecological Significance

Camassia spp. are also ecologically significant. They remain among the most abundant native species found on remnant prairies (Dunwiddie, 2002). They are also an important forage for wildlife. Deer, elk, and moose reportedly graze on *Camassia* spp. (Craighead *et al.*, 1998), and pocket gophers (including the aptly named camas gopher) are known to eat and larder hoard the bulbs (Watson, 1988 *fide* Thoms, 1989). Finally, they are an important food source for pollinators. For example, *C. scilloides* provides nectar to a diversity of insect pollinators, including bumblebees, solitary bees, and small flies (Macior, 1978). Also, in a study conducted in remnant Garry oak ecosystems, of all the wildflowers surveyed, *C. quamash* had the most diverse group of insect visitors and received roughly half all the observed visits, including nearly all the observed visits on its peak flowering day (Parachnowitsch and Elle, 2005). Considering their large showy flowers and septal nectaries, it is no surprise that there is a strong relationship between *Camassia* spp. and pollinating insects.

The Propagation of Camassia *spp.*

Although *Camassia* spp. are an ecologically and culturally significant part of the Pacific Northwest landscape, little is known about their propagation (Stevens *et al.*, 2001). What has been documented offers us three different perspectives on the propagation of *Camassia* spp. These are the perspectives of native plant nurseries in western North America, Dutch bulb growers, and the indigenous women and men who have managed *C. leichtlinii* and *C. quamash* for centuries.

Seed Collection, Cleaning, and Storage

Seeds of *Camassia* spp. are held in capsules along their raceme (Ranker and Hogan, 2002). Capsules are collected when the inflorescence has senesced (i.e. turned tan), the seeds are mature (shiny and black), and the capsules have begun to split open (Luna *et al.*, 2008). This time generally corresponds with the middle of summer, and

capsules can be collected from this point until the first rain, if not later. Care must be taken when making collections, as the seeds will easily drop from the capsules. The capsules should be stored in paper bags and processed by shaking the seeds out of the open capsules (Luna *et al.*, 2008). Seeds are orthodox and can remain viable for multiple years if stored in cool, ventilated, and dry conditions.

Seed Stratification and Germination

Seeds of *C. leichtlinii* and *C. quamash* must be cold moist stratified (i.e. subjected to both cold and moist conditions) before they will germinate (Russell, 2011). They can be stratified either outdoors in ambient winter conditions or at any time using refrigeration. In one trial, Kaye (personal communication) achieved nearly 100% germination for Willamette Valley seeds stratified in cool moist conditions (i.e. ~5^{o}C) for eight weeks for *C. leichtlinii* or twelve weeks for *C. quamash*, though seeds from cooler climates might require a longer stratification period (Luna *et al.*, 2008). Seeds should be sown near the surface of the soil/media; seeds planted deeper than two centimeters are unlikely to germinate. (Watson, 1988 *fide* Thoms, 1989). The development of *C. leichtlinii* seeds after germination is presented in Figure 3.

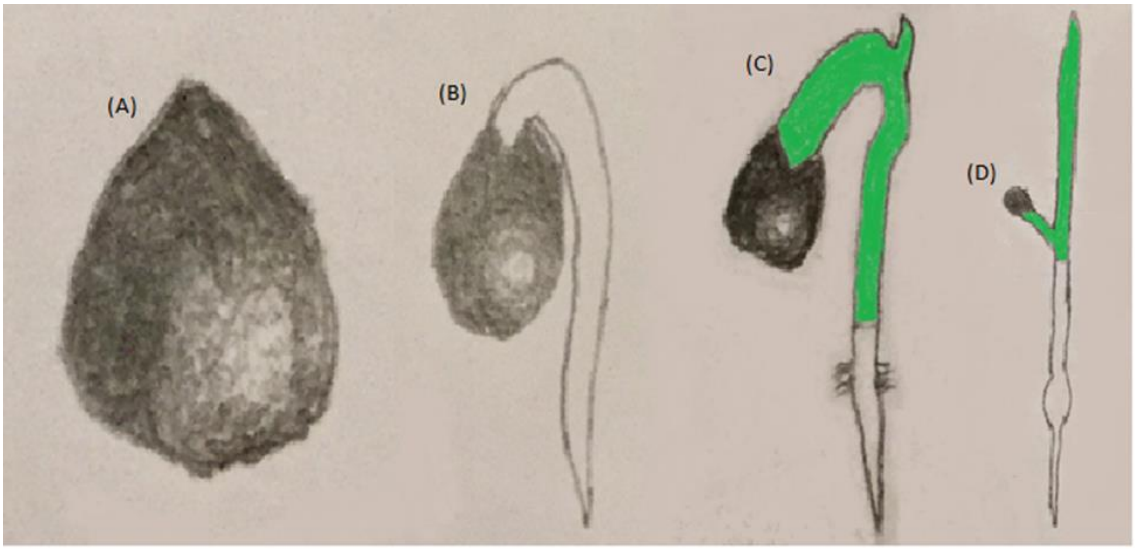

Figure 3: The growth of Camassia leichtlinii *seedlings. (A) The seed is glabrous, black, and teardrop shaped (drawing from three days after the end of stratification). (B) The first organ to emerge from the seed is the radicle (drawing from three days after the end of stratification). (C) A clear distinction between aboveground and belowground tissue starts to develop (drawing from twelve days after the end of stratification). (D) Leaf growth and bulb development occur (drawing from twelve days after the end of stratification).*

Irrigation

As the seeds germinate they should be misted at least once a day, as they are vulnerable to desiccation (personal observation). This vulnerability corresponds with the establishment period, which Luna *et al.* (2008) estimates to be four weeks. After the establishment period ends the irrigation frequency can be reduced, as the seedlings are able to access water held lower down in the substrate. At this point, seedlings can be irrigated in multiple ways, including overhead irrigation and subirrigation. One personal recommendation for the first growing season would be to irrigate the crop to field capacity every time that the water held in its media falls below 85% of its weight at field capacity (Dumroese *et al.*, 2015).

Soil, Artificial Media, and Fertilizer

Camassia spp. seem to perform well in both soil and artificial media, and can be grown in rows, nursery beds, or containers (personal observation). Some have proposed that it grows best in heavy soils (Kruckeberg, 1982). However, if grown in fields or nursery beds, sandy soil will make it easier to harvest the bulbs, which are known to reposition themselves to depths of up to 15 cm (Thoms, 1989). A published *C. quamash* propagation protocol recommends adding 1 gram of Osmocote 13-13-13 (The Scotts Miracle-Gro Company; Marysville, OH) to each container before sowing the seeds in the fall (Luna *et al.*, 2008). However, it is possible that this fertilization program may apply too much fertilizer and that much of this fertilizer may be released at a time when the plant is not taking up nitrogen (Chapter 2).

Leaf Senescence

Between late spring and midsummer, the leaves of *Camassia* seedlings will senesce. This is often the most perilous point in their cultivation, as some growers will assume that the plants have died and will throw them away (personal observation). However, the plants have simply entered their summer rest period. During this period, it is doubtful that the bulbs need much care. Once fall and winter return, they probably require two conditions to continue their life cycle; moisture, which allows for their roots to grow, and cool temperatures, which are required prior to reemergence in the spring. The effects of temperature manipulation on quiescent bulbs will be explored in Chapter 3.

Annual Growth Cycles

When *Camassia* leaves reemerge in late winter or early spring they will be noticeably larger and the plants will be hardier (personal observation). Once again, their leaves will senesce in the summer, and the cycle will continue to repeat itself until the bulb has enough C reserves (i.e. the bulb is big enough) to produce a flower. Flowering has been suggested to occur when the bulb is >6 cm in circumference for *C. quamash* and

>12 cm in circumference for *C. leichtlinii* and *C. cusickii* (Le Nard and De Hertogh, 1993b). At this point the plant is mature and highly merchantable.

The Dutch Method for Camassia *Propagation*

Interestingly, *Camassia* has been grown in The Netherlands as an ornamental bulb (Le Nard and De Hertogh, 1993b). Le Nard and De Hertogh (1993b) reviewed the Dutch method of *Camassia* propagation, for which the primary source is Langeslag (1989). The Dutch reportedly propagate *Camassia* asexually by planting mature bulbs in the fall and then harvesting their progeny (i.e. multiple offset bulblets) the following summer (Langeslag, 1989). This is interesting because much of the literature on *C. leichtlinii* and *C. quamash* suggests that asexual reproduction is not common, however it is possible that they are growing species, subspecies, or genotypes that readily produce multiple offset bulblets.

Bulbs should be planted in October or November, in sandy soil with a pH of between 6 and 7 and more than 2% organic matter (Le Nard and De Hertogh, 1993b; Langeslag, 1989). Planting densities recommended for *C. quamash* bulbs > 6 cm in circumference is between 5,000 and 7,000 kg ha^{-1}, while for bulbs of other *Camassia* spp. > 12 cm in circumference, the recommended planting density is between 15,000 and 175,000 kg ha^{-1} (Le Nard and De Hertogh, 1993b). Fertilizer should be delivered to the crop twice, once after planting in the fall and again in the spring. The fall fertilization should be a 7-14-28 fertilizer delivered at 49-70 kgN ha^{-1} and the spring fertilization should be a N fertilizer delivered at 60 kgN ha^{-1} (Langeslag, 1989). To encourage bulb growth, it is recommended that the raceme be removed after flowering (Le Nard and De Hertogh, 1993b).

The bulbs are harvested while the foliage is still green, with care taken to prevent mechanical damage. They are then stored in wood shavings in a dry ventilated room, with temperatures held between 17 and 20°C (Le Nard and De Hertogh, 1993b). Yields using these methods are reported to be 1,050,000 bulbs ha^{-1} for *C. quamash* and 42,000 bulbs ha^{-1} for the other *Camassia* spp. (Langeslag, 1989). Pests include the fungus *Rhizoctonia*

tuliparum (Kleb.) Whetzel & J.M. Arthur and the mosaic virus (Langeslag, 1989). Additionally, nematodes in the genus *Ditylenchus* Filipjev (Anguinidae) can infest bulbs, however they can be controlled with a hot (43.5 – 45^{o}C) water treatment for 4 h (Langeslag, 1989).

Indigenous Methods of Management

Indigenous methods of camas prairie management have been investigated and may inform growers and restoration specialists, especially those interested in pursuing organic practices. Indigenous people managed camas using a diversity of methods. Some simply foraged for camas, while others implemented more intensive cultivation practices, and how camas was managed varies by group, location, and time (Turner and Peacock, 2005; Beckwith, 2004).

Many tribes used cultivation practices to ensure access to camas, including selective harvesting, tilling, fertilization by top dressing with marine detritus and seaweed, weeding after digging bulbs, replanting of smaller bulbs, and the transplanting of bulbs into management plots (Suttles, 2005; Turner and Bell, 1971; Deur, 2000). These practices were often done in combination. For example, patches in southern Vancouver Island were often burned, cleared, maintained, and passed down from generation to generation within the families that owned them (Kuhnlein and Turner, 1991). The fact that the cultivation practices of indigenous people in the Pacific Northwest were not acknowledged by colonial authorities had profound consequences, as indigenous people were often dispossessed of the land on which they historically practiced cultivation (Deur, 2000). Perhaps the future of prairie restoration in the Pacific Northwest will be one where, guided by native people, we restore native practices in addition to native plants.

Fire was also used by indigenous people to ensure a sustainable supply of camas bulbs, and thus the landscape of the Pacific Northwest was altered for the benefit of these species (Beckwith, 2004). Because of this, modern research has been interested in the ways in which *Camassia* spp. respond to fire. One study showed that a one-time fall

burning increased the number of *C. quamash* in management plots, however, following that up with a second burn at either a two- or a three-year interval had little effect (Schuller, 1997).

Improving the Propagation of Camassia *spp.*

To restore native prairies in the Pacific Northwest, it may be necessary to produce large quantities of high quality *Camassia* bulbs. For the purposes of this thesis, a high-quality bulb will be defined as a bulb that is disease free and that survives outplanting and contributes to restoration success. It will be assumed, though it remains unknown, that a large bulb is more likely to survive outplanting, and thus is a high-quality bulb. Currently, one of the challenges facing growers of *Camassia* spp. is the long time required to produce mature (i.e. large and flowering) bulbs from seeds. For example, it has been reported that *C. quamash* requires three to four years of growth before it reaches maturity (Thoms, 1989). This disposition is not unique to *Camassia* spp.; many spring flowering bulb species are slow growing, requiring several years to reach maturity if grown from seeds (Fortanier, 1973).

The goal of this study was to develop methods for growing *Camassia* spp. more quickly and efficiently. To improve the propagation of these species, this study focused on two fields of inquiry, the use of N fertilizer and temperature manipulation. Nitrogen fertilizer was chosen because limited N availability can reduce productivity in plants. Thus, methods that provide the right amount of N at the right time should improve the growth of *Camassia* spp. in the nursery. Temperature manipulation was chosen because seasonal thermoperiodicity, i.e. seasonal changes in temperature, often regulates the growth and development of spring flowering bulbs (Le Nard and De Hertogh, 1993a). Thus, temperature manipulation may allow growers to control the life cycle of *Camassia* spp., and perhaps allow for multiple growing seasons in a single year.

Chapter 2: The Growth and N Use of *C. leichtlinii*

Humanity's use of N fertilizers has beneficial and deleterious effects on the biosphere. The benefits include increases in plant growth and crop yields, both of which are becoming essential pillars upon which the modern world rests. However, the overuse of N fertilizers and the techniques of their application can result in the loss of reactive N to the environment (Ingestad, 1977). This N pollution impacts drinking water, air quality, and freshwater and coastal ecosystems, and is in part driving biodiversity loss, ozone depletion, and climate change (Erisman *et al.*, 2013; Clark and Tilman, 2008).

The plant nursery industry is particularly culpable for N overuse and pollution. Ornamental nurseries often overfertilize container plants, sometimes applying 15 times more N than what is recommended for agronomic field crops (Chen *et al.*, 2001). Excessive fertilizer use appears to be common in native plant nurseries as well. However, one must have sympathy for native plant growers. They face numerous challenges that come with cultivating a diversity of understudied species, often in small quantities, and with limited labor. Fortunately, many native plant growers, and the restoration projects that they support, are motivated by a sense of moral duty to repair the damage done to natural ecosystems by humans (Elliot, 2008). Thus, they have an interest in improving fertilizer practices.

One method of determining whether a plant is overfertilized is to consider its nitrogen use efficiency (NUE), defined here as the percentage of the N applied that is taken up by the plant. Under this definition, it appears that some native plant nurseries may be overfertilizing *C. quamash* during its first growing season. A published *C. quamash* propagation protocol recommends adding 1 gram of Osmocote 13-13-13 (The Scotts Miracle-Gro Company; Marysville, OH) to the media of each container before sowing the seeds in the fall (Luna *et al.*, 2008). This should provide 130 mgN to each seedling during the first growing season. Considering that *C. quamash* is known to have a N concentration of about 8.9 mgN gDW^{-1} (Turner and Kuhnlein, 1983), if a seedling grew to a dry weight (DW) of 50 mg during its first growing season, then it would have a

N content of around 0.5 mg and a NUE of about 0.4%. However, Turner and Kuhnlein (1983) collected their data using wild plants, harvested in a manner reflecting indigenous practice, and so their data is not necessarily representative of nursery crops. Nevertheless, even at higher plant N concentrations the NUE of this fertilization program would be low. For example, the highest N concentration that this author has observed in *C. leichtlinii* during its first growing season is approximately 26.6 mgN gDW^{-1}. Therefore, using the same assumptions, the expected NUE would be ~1%. Furthermore, Luna *et al.* (2008) recommends that the containers be thoroughly leached during irrigation. So not only does this program have a low NUE, much of the N may be leaving the nursery to pollute the local environment.

This suggests that the fertilization program for *Camassia* spp. could be improved to allow growers to maintain or even enhance growth and nutrition while minimizing N overuse and pollution. Thus, two experiments were conducted with the intention of improving the fertilization program for *Camassia* spp. The first tested the efficacy of alternative fertilizer application practices during *C. leichtlinii*'s first growing season. The second investigated *C. leichtlinii*'s N uptake and allocation patterns during its second growing season.

Experiment 1: Investigations into Alternative Fertilizer Application Practices and the Growth and C and N Dynamics of C. leichtlinii

Introduction

Alternative fertilizer application practices may be effective in increasing plant growth and NUE while conserving resources and protecting the environment. One of these alternative practices is exponential fertilization, which aims to match N application with plant N demand. Because the amount of N required by a plant increases with increasing biomass, N demand increases exponentially during the plant's exponential growth phase (Ingestad and Lund, 1986). By using frequent small applications of fertilizer with exponentially increasing dose sizes, exponential fertilization programs match N application to plant growth and thus plant N demand (Ingestad and Lund, 1986).

Exponential fertilization is quite a departure from conventional fertilization, in which seedlings are fertilized using one large application at the start of the growing season or several applications of the same amount throughout the growing season (Imo and Timmer, 1992).

In exponential fertilization, the rate of increase for the dose size is the relative addition rate, defined as the amount of nutrient to be added to a plant, per unit time, in relation to the amount of nutrient present in the plant (Ingestad and Lund, 1986). The relative addition rate is calculated using Equation 2, rearranged from Equation 1.

$$N_T = N_s(e^{rT} - 1) \quad (1)$$

$$r = \left[\ln\left(\frac{N_T}{N_s} + 1\right)\right]/T \quad (2)$$

Where r is the relative addition rate for T number of applications required to raise the initial seedling N content N_s to the final N content $N_T + N_s$. After solving for the relative addition rate, the amount of N to be applied at a given application can be determined using Equation 3.

$$N_t = N_s(e^{rt} - 1) - N_{t-1} \quad (3)$$

Where N_t is the amount of N to be applied at time t and N_{t-1} is the cumulative amount of N that has been applied up to and including the most recent application.

Exponential fertilization increased growth and NUE in container grown *Pinus resinosa* Sol. ex Aiton when compared with conventionally fertilized controls (Timmer and Armstrong, 1987). However, exponential fertilization is not always associated with higher growth rates and NUE, likely because initially the roots do not have complete access to the media (Timmer *et al.*, 1991; Burgess, 1991). Thus, exponential fertilization needs to be modified to account for incomplete root exploitation of the media by increasing the amount of fertilizer applied early in the growing period (Timmer *et al.*, 1991). This is called the compensation period, and because root systems grow exponentially, Timmer *et al.* (1991) reasoned that the amount of compensating N should be delivered following a negative exponential function (Equation 4).

$$N_C = N_o(e^{-rT} - 1) \quad (4)$$

Where N_C is the additional N applied during the compensation period, which Timmer *et al.* (1991) decided should be the difference between the penultimate and the final N applications calculated using Equation 3, *T* is the number of applications during the compensation period (which should be the number of application until full root exploitation of the media), and N_o is the final amount of additional nutrient added during the compensation period (approaching zero). Timmer and Aidelbaum (1996) provide additional information on how to design a modified exponential fertilization program.

Evidence suggests that modified exponential fertilization produces seedlings with similar DWs to conventionally fertilized controls (Imo and Timmer, 1992; Timmer *et al.*, 1991). Furthermore, seedlings grown using modified exponential fertilization are often shown to have a higher NUE than seedlings grown using conventional fertilization (Dumroese, 2003; Imo and Timmer, 1992) or exponential fertilization (Imo and Timmer, 1992). Thus, modified exponential fertilization may be an appropriate method for improving NUE in native plant nurseries.

Another concept worth examining is the optimum relative addition rate, which is the relative addition rate that maximizes plant growth. In experiments testing various relative addition rates, researchers have been able to increase the relative growth rates of the plants that they study up to a maximum (Ingestad, 1982; Ingestad, 1987). Any increase in the relative addition rate after this optimum relative addition rate no longer results in a corresponding increase in the relative growth rate. Thus, growers who want to maximize plant growth during the exponential growing period while minimizing the amount of N applied should be interested in determining this optimum relative addition rate.

Modified exponential fertilization provided at the optimum relative addition rate may improve overall NUE and growth, but it is also important to reduce the amount of fertilizer lost to the environment. One way that fertilizer is lost to the environment is due to irrigation practices. Overhead irrigation often leaches nutrients out of plant containers

(Dumroese *et al.*, 1991; Dumroese *et al.*, 1995). However, by combining subirrigation (i.e. applying water from below) with the use of controlled-release fertilizers, less fertilizer is lost to leaching, leading to an increase in NUE and a decrease in fertilizer pollution (Landis and Dumroese, 2009; Dumroese *et al.*, 2006; Pinto *et al.*, 2008). To combine subirrigation with modified exponential fertilization however, it would be necessary to apply nutrients using the subirrigation water, like in hydroponics. This method of fertilization will be referred to as subfertigation.

This experiment tested alternative fertilizer application practices on *C. leichtlinii* to develop a fertilization program that both ensures maximum growth and reduces N overuse. It was hypothesized that modified exponential fertilization would improve the growth and NUE of *C. leichtlinii* when compared with conventional fertilization. This hypothesis would be supported if a modified exponential fertilization treatment that receives the same amount of N as a conventional fertilization treatment has a higher mean seedling DW and mean N content at the end of the experiment. It was also hypothesized that *C. leichtlinii* has an optimum relative addition rate. This hypothesis would be supported if one or more relative addition rates maximizes seedling DW at the end of the experiment. Finally, it was hypothesized that *C. leichtlinii* can be fertilized using subfertigation. Subfertigation will be assumed to be effective if plant N concentrations and contents for the different treatments correspond with the N supplied. Additionally, the growth and C and N dynamics of *C. leichtlinii* during the course of the growing season were characterized. For the experiment six treatments were applied, a control that received no fertilizer, four modified exponential fertilization treatments with different relative addition rates, and a conventional fertilization treatment. The experiment was arranged in a completely randomized design with repeated measures.

Methods

Materials: Materials used in this experiment were *C. leichtlinii* seedlings. Seeds of *C. leichtlinii* were collected in northwest Oregon by Inside Passage (Port Townsend, WA) and were sown on 22 October 2015 at the Oxbow Farm and Conservation Center's

native plant nursery (Carnation, WA; 47.69, -121.98). Facilities at the native plant nursery include a Cravo Greenhouse (Cravo Equipment Ltd.; Brantford, ON), which is where plant materials were kept from their sow date until 2 July 2016. The sown seeds were cold moist stratified using ambient winter conditions and germinated between 2 February and 16 March 2016. These seedlings were fertilized twice before the experiment began, once on 16 March and again on 1 April. Both times they were fertilized with Fish and Poop liquid fertilizer (9-6-2; Monterey Lawn and Garden; Fresno, CA) applied at the label rate for soil drench (3.75 g L^{-1}).

From 11 to 14 April, 2,940 seedlings were transplanted from this main crop into Ray-Leach SC10 containers (Stuewe and Sons, Inc.; Tangent, OR). Sunshine Mix #2 / LBS (Sungro Horticulture; Agawam, MA) was used as media. Containers were watered to field capacity on the day that they were transplanted and each subsequent day until 17 April. Once all seedlings were transplanted, containers were assigned to random cells within one of 30 Ray Leach 98 trays (Stuewe and Sons, Inc.; Tangent, OR), with a container in every cell. Trays 1-6 were assembled on 14 April, trays 7-18 on 15 April, and trays 19-30 on 16 April.

Treatments: On 18 April, day 1 of the experiment, trays were assigned to one of six treatments, with five trays for each treatment, in a completely randomized design. Four of the treatments received N applied at a modified exponential rate, with treatment names (2, 4E, 6, and 8) indicating the total amount of N to be supplied to each seedling during the 13-week experiment (2, 4, 6, and 8 mgN $container^{-1}$). The relative addition rates for these treatments were 0.18, 0.23, 0.26, and 0.29 mgN mgN^{-1} $week^{-1}$ respectively (and N_s was assumed to be 0.2 mgN). A control received 0 mgN $container^{-1}$ (treatment 0). The final treatment (treatment 4C) was to receive 4 mgN $container^{-1}$ delivered conventionally (i.e. 0.31 mgN $container^{-1}$ $week^{-1}$). The intended programs are displayed on Figure 4; however, fertilization was discontinued after week 8 of the 13-week program because most seedlings had entered their summer rest period and as such seedlings did not receive the full amount of N intended.

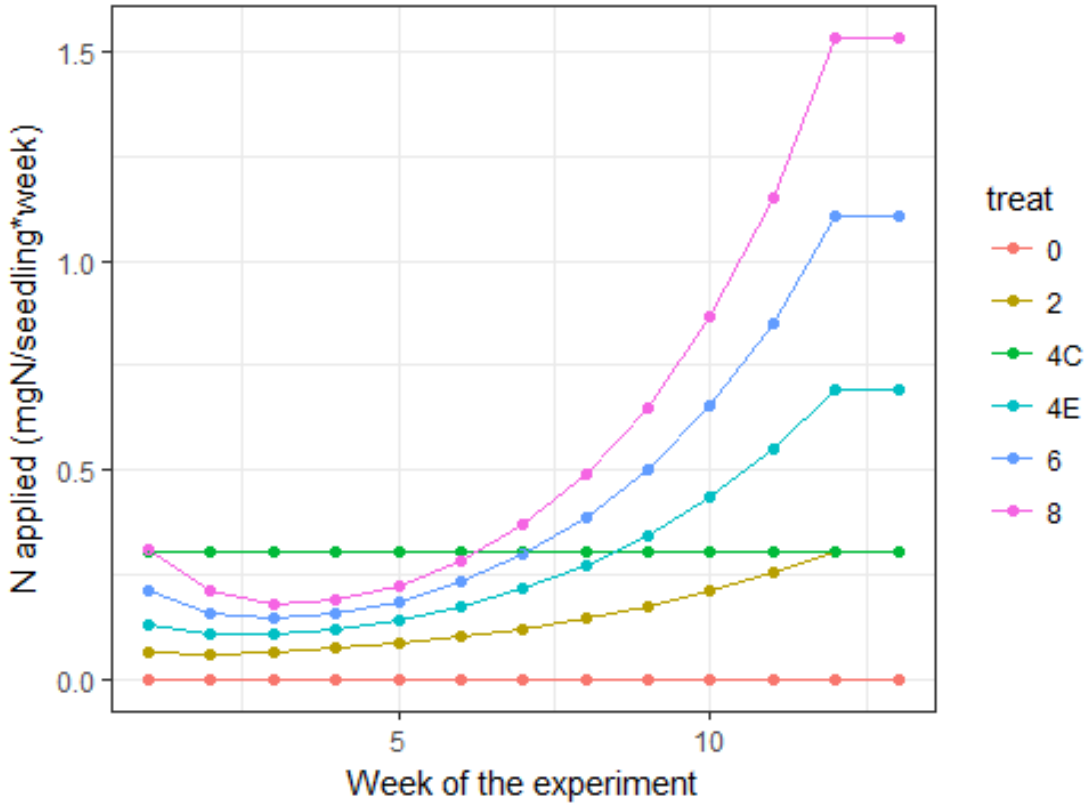

Figure 4: Intended N application for each week, derived using the Timmer and Aidelbaum (1996) formulas, for each of the treatments. Lines show the trends for the treatments.

Irrigation: From day 1 to day 75 of the experiment, if the water held in the media of a tray fell below 85% of its weight at field capacity, then that tray was subirrigated (Dumroese *et al.*, 2015). Trays were kept in subirrigation for 1 h, using 15 liters of water for each tray at each irrigation from day 1 to day 18 of the experiment. After day 18, this was changed to 7.5 liters of water to reduce the amount of waste water produced. After day 76 of the experiment, trays were no longer subirrigated, but were instead overhead irrigated.

Locations: Trays were randomized and rotated on days 8, 21, 34, 47, and 62 of the experiment.

On day 76 of the experiment, trays were moved to the mist irrigation greenhouse at the University of Idaho's Pitkin Forest Nursery (Moscow, ID; 46.73, -116.96). Three days later, these trays were accidentally fertilized using Wil-Sol® Pro-Grower (20-7-19; Wilbur-Ellis Company; San Francisco, CA). The trays were then watered for 2 h with a boom to leach out as much of this fertilizer as possible. Then, on day 82 of the experiment, trays were moved to the Rocky Mountain Research Station (RMRS; Moscow, ID; 46.72, -117.00). Seedlings were kept at RMRS through the summer and into the winter and if the water held in the media of a tray fell below 60% of its weight at field capacity, then that tray was overhead irrigated (Dumroese *et al.*, 2015).

Fertilization: Trays began to receive weekly doses of fertilizer on day 3 of the experiment. The amount applied depended both on the treatment, and in the case of the modified exponential fertilization treatments, the week of the program (Figure 4). Because fertilizer was applied using irrigation water, individual trays were fertilized on the first day, after each Wednesday, that they needed to be irrigated.

For each tray that needed to be fertilized, it was necessary to determine how much fertilizer to add to the irrigation water to provide the correct amount of fertilizer to each container. First, the tray was weighed to determine its "pre-irrigation weight," and then this weight was subtracted from its expected weight at field capacity to create an estimate for the volume of water that it would take up during subirrigation (Equation 5).

$$\text{Expected Weight at field capacity (kg)} - \text{Pre-irrigation Weight (kg)} = \text{Expected Water Uptake (l)} \quad (5)$$

Next, the amount of fertilizer to apply to the irrigation water was determined. It was assumed that the concentration of the fertilizer solution taken up by the tray would be the same as the concentration of the fertilizer solution in the flow tray (Equation 6). Thus, Equation 6 was rearranged to solve for the required fertilizer to add to the flow tray.

$$\frac{\text{Fertilizer}(\text{gN tray}^{-1})}{\text{Expected Water Uptake (l tray}^{-1})} = \frac{\text{Fertilizer (gN flow tray}^{-1})}{\text{Water (l flow tray}^{-1})} \quad (6)$$

FloraMicro (5-0-1; General Hydroponics; Santa Rosa, CA) and FloraBloom (0-5-4; General Hydroponics; Santa Rosa, CA) were then weighed out at a ratio of 3:2, at a rate that would provide the required amount of N to the flow tray. After 1 h of soaking and 1 h of dripping, the tray was reweighed to determine the amount of water taken up by the tray (Equation 7).

$$\text{Final Weight (kg)} - \text{Pre-irrigation Weight (kg)} = \text{Water Uptake (l)} \quad (7)$$

This volume was then used to determine the amount of fertilizer that was delivered to the tray (Equation 8).

$$\text{Fertilizer Uptake (gN tray}^{-1}) = \text{Fertilizer (gN flow tray}^{-1}) * \frac{\text{Water uptake (l)}}{\text{Water (l flow tray}^{-1})} \quad (8)$$

Using Equation 8, the amount of fertilizer added to each container at each fertilization was estimated, and this data is summarized in Table 2.

Table 2: Estimated amount of N applied to each container by week for each treatment (mgN container^{-1}).

	Week								
Treatment	1	2	3	4	5	6	7	8	Total
0	0	0	0	0	0	0	0	0	0
2	0.07	0.06	0.06	0.07	0.09	0.11	0.11	0.13	0.69
4C	0.31	0.28	0.24	0.32	0.30	0.32	0.27	0.29	2.33
4E	0.15	0.10	0.08	0.12	0.14	0.17	0.19	0.24	1.19
6	0.22	0.15	0.12	0.15	0.18	0.23	0.26	0.37	1.68
8	0.31	0.18	0.15	0.18	0.22	0.28	0.32	0.43	2.07

Data Collection: Fifteen random seedlings were destructively sampled during the first three days of transplanting (i.e. 11, 12, and 13 April). These were used to collect preliminary data for seedling DW, C concentration and content, N concentration and content, and C:N. Then, during the experiment there were five destructive samplings.

During each of these samplings, four containers were randomly subsampled from each experimental unit (tray) after they had received fertilizer (or in the case of the fourth and fifth samplings after they were irrigated), and measurements were taken for each seedling.

Seedlings were sampled on days 19 to 21, 31 to 34, 45 to 47, 59 to 62, and 92 to 95 of the experiment. Measurements were of the length of the leaf and the longest root. After measurements were taken, samples were oven dried at ~ 80ºC for ~12 h and weighed. For the first four samplings, if roots broke while being removed from the media they were measured in pieces. By the fourth sampling many of the leaves had completely senesced. If the petiole broke while the seedling was being sampled then the blade was salvaged, measured, and included in the measurements of seedling DW and C and N concentration. If the blade was absent at the time of sampling, it was excluded. By the fifth sampling (days 92 to 95 of the experiment) many of the roots had begun to senesce. If they broke while being sampled, that portion of the root was not measured, nor was it included in the seedling DW and C and N concentration measurements.

The number of quiescent seedlings were counted for each experimental unit on days 37, 44, 51, and 58 of the experiment. Seedlings were considered quiescent if the entirety of their leaf had senesced.

Seedlings sampled were tested for C and N concentration at the University of Idaho College of Natural Resources (Moscow, ID) using an ECS 4010 CHNS Analyzer (Costech Analytical Technologies, Inc.; Valencia, CA). These data were used to estimate C and N content by multiplying the concentration of each element to the corresponding seedling DW (Chapin III and Van Cleve, 2000).

Data Analysis: Data were analyzed using RStudio (R Core Team, 2017). Figures were produced using the package ggplot2 (Wickham, 2009). Other statistical packages used include nlme (Pinheiro *et al.*, 2017), car (Fox and Weisberg, 2011), emmeans (Lenth, 2018), multcomp (Hothorn *et al.*, 2008), geepack (Halekoh *et al.*, 2006; Yan and Fine, 2004; Yan, 2002), gridExtra (Auguie, 2017), cowplot (Wilke, 2017), and MASS

(Venables and Ripley, 2002). Data analysis was based on the methodologies presented by Zuur *et al.* (2009).

Throughout this thesis, the term "mean average" is frequently used. While this may seem redundant to some, it is necessary when describing the data analysis and results. This is because the response variables that were used in the data analysis were the average of four containers sampled from each experimental unit at each sampling. Thus, the mean that is calculated is for the average responses for the experimental units.

Means and standard deviations for the preliminary data collected prior to the start of the experiment were summarized for each of the responses measured. ANOVA F-tests were used to determine if treatments affected average seedling DW, average N concentration, average N content, and average C:N on the fifth sampling. Mean comparisons were made using the Tukey method for p-value adjustment.

Mixed effects modeling was used to compare the treatments across all five samplings. For these models, week of the experiment, treatments, and their interactions were tested as fixed effects, with experimental units included as random intercepts. Response variables tested include average leaf length, average length of the longest root, average seedling DW, average N concentration and content, average C concentration and content, and average C:N. Full models were fit for each response variable, and then residual plots for the main effects were examined to ensure that variances were homogeneous among the different treatment levels and by week of the experiment. If variances were determined to be heterogeneous for either, the homogeneous variance assumption was relaxed, and different variance covariates were included in the model. Models with and without the variance covariates were fit with the REML estimation method, and then compared using likelihood ratio tests, and the variance covariates that most improved the model were kept if the improvement was significant (α=0.05). The standardized residuals were then reexamined to ensure that the variance assumptions were met. Then, autocorrelation function plots were created to examine the possibility of autocorrelation within the experimental units, and models with and without a compound symmetry auto-correlation structure or a continuous AR(1) correlation structure were

compared using likelihood ratio tests. Finally, once the random part of the model was appropriate, the fixed effects were tested using backwards selection and likelihood ratio tests, with full and reduced models fit with the ML estimation method. If the interaction terms were not significant at $\alpha = 0.1$, they were dropped from the model. Likelihood ratio tests were then used to determine whether the main effects were significant. In addition, if the interactions were not significant, the main effects model was fit using the REML estimation method, and then the model was used to compare estimated marginal means between the different treatment levels using the Tukey method for p-value adjustment. These models were also used to characterize the trend across the five samplings and for graphical presentation.

To determine if there were differences in the proportion of seedlings quiescent by treatment and day, generalized estimating equations were used. For these tests, the mean proportion of quiescent seedlings in the i^{th} experimental unit on the s^{th} day was modeled as a function of treatments, day of the experiment, and their interactions, with a logit link. The correlation structure used was the auto-regressive correlation structure. Explanatory variables were tested for significance using backwards selection with the Wald test.

Results

At the start of the experiment, mean seedling DW was 22.5 mg (s.d. = 4.7), mean N concentration was 9.2 mgN gDW^{-1} (s.d. = 2.0), mean N content was 0.21 mgN (s.d. = 0.06), mean C concentration was 436 mgC gDW^{-1} (s.d.= 3), mean C content was 9.8 mgC (s.d. = 2.1), and mean C:N was 49 (s.d. = 8).

On the final sampling, treatment had a significant effect on average N content, but not average seedling DW, average N concentration, or average C:N (Table 3).

Table 3: ANOVA F-tests, means, standard errors, and mean separation for the different treatments on the fifth sampling.

Treatment	Average seedling DW (mg)	Average N content (mgN)	Average N concentration (mgN gDW^{-1})	Average C:N
0	45.9 (4.3)	0.46 (0.05) B	10.3 (0.7)	44 (2)
2	52.5 (4.3)	0.56 (0.05) AB	11.0 (0.7)	40 (2)
4C	55.5 (4.3)	0.71 (0.05) A	12.8 (0.7)	36 (2)
4E	43.7 (4.3)	0.48 (0.05) AB	11.4 (0.7)	40 (2)
6	46.3 (4.3)	0.54 (0.05) AB	11.7 (0.7)	40 (2)
8	52.1 (4.3)	0.57 (0.05) AB	11.2 (0.7)	40 (2)
ANOVA	$F_{5, 24} = 1.18$ p=0.3494	$F_{5, 24} = 2.69$ p=0.0457	$F_{5, 24} = 1.33$ p=0.2851	$F_{5, 24} = 1.30$ p=0.2951

Including week of the experiment as a continuous explanatory variable allowed for trends to be modeled across the five samplings, which all occurred during the second half of the first growing season. Across all response variables tested, autocorrelation function plots were never cause for concern, and including a compound symmetry auto-correlation structure or a continuous AR(1) correlation structure in the random effects part of the model never improved the mixed effects models tested. Including variance covariates for the different treatments improved the model for mean average C concentration ($p<0.0001$). Including an exponential of the variance covariate for the week of the experiment improved the model for mean average C content ($p=0.033$). Including a combination of variance structures, with variance covariates for the different treatments and a power of the variance covariate for the week of the experiment improved the models for mean average length of the longest root and mean average N content ($p=0.0215$ and $p=0.0204$, respectively).

Likelihood ratio tests showed that the interactions between week of the experiment and treatments were never significant at $\alpha = 0.1$. The trend for mean average leaf length was best described using a linear model, and a likelihood ratio test showed that week of the experiment had a significant effect on average leaf length ($p=0.0006$). The other response variables were best described using a second order polynomial for the week of the experiment, and a likelihood ratio test showed that keeping the polynomial

improved the models for mean average length of the longest root ($p<0.0001$), mean average seedling DW ($p<0.0001$), mean average N concentration ($p=0.0009$), mean average N content ($p<0.0001$), mean average C concentration ($p<0.0001$), mean average C content ($p<0.0001$) and mean average C:N ($p=0.0014$). Including the treatments improved the models for mean average N concentration ($p=0.0322$) and mean average C:N ($p=0.0128$).

Between week 3 and 9 of the experiment, mean average leaf length decreased by 0.6 mm week^{-1} ($t=-3.397$, $df=89$, $p=0.001$; Figure 5A). Mean average root length appeared to increase, though at a diminishing rate, from week 3 to 7 of the experiment, after which it began to decrease (Figure 5B).

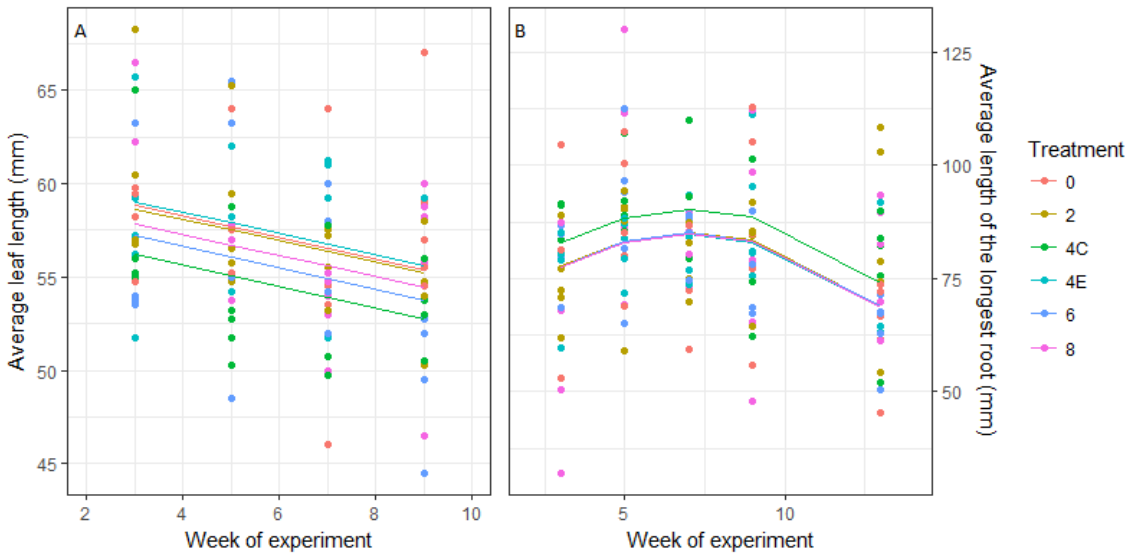

Figure 5: Changes in (A) mean average leaf length and (B) mean average length of the longest root during the course of the experiment. Parallel lines were included for the different treatments.

The trend in the data suggested that mean average seedling DW increased up until week 9 of the experiment (though at a diminishing rate) after which it began to decrease (Figure 6A). This same trend was found for the change in mean average N content and

mean average C content (Figures 6C and 6E respectively). The mean average C:N appeared to increase at a diminishing rate up until week 7 of the experiment, after which it began to decrease (Figure 6B). Mean average N concentration appeared to decrease at a diminishing rate up until week 7 of the experiment, after which it began to increase (Figure 6D). Mean average C concentration appeared to increase at a diminishing rate up until week 5 of the experiment, after which it began to decrease (Figure 6F).

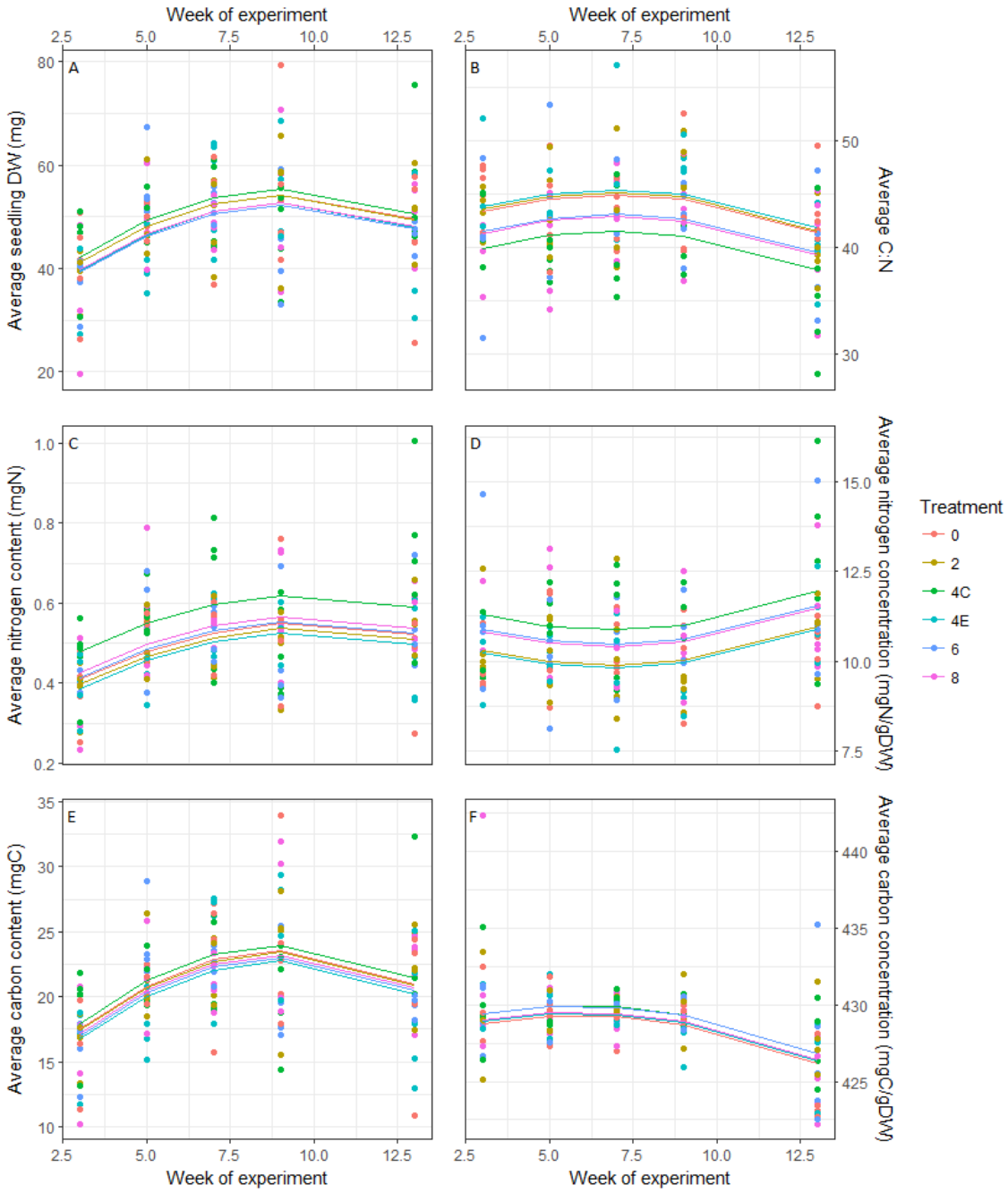

Figure 6: Growth and N and C dynamics across the experimental period. (A) Mean average seedling DW, (B) mean average C:N, (C) mean average N content, (D) mean average N concentration, (E) mean average C content, and (F) mean average C concentration all changed during the course of the experiment.

Significant differences between the estimated marginal means for average N concentration were not detected at either $\alpha = 0.05$ or $\alpha = 0.10$ (Table 4). One significant difference between the estimated marginal means was detected for average C:N, between the treatments 4E and 4C (p=0.0482). Table 4 includes the estimated marginal means for all response variables.

Table 4: Results from likelihood ratio tests, estimated marginal means, standard errors, and mean separation for each treatment for response variables across all five samplings.

	Likelihood ratio test	**0**	**2**	**4C**	**4E**	**6**	**8**
Average leaf length (mm)	L=7.26 df=5 p=0.202	57.1 (0.9)	56.9 (0.9)	54.5 (0.9)	57.3 (0.9)	55.5 (0.9)	56.2 (0.9)
Average length of the longest root (mm)	L=3.92 df=5 p=0.561	85.0 (3.5)	85.2 (3.1)	90.2 (2.7)	84.8 (2.6)	85.0 (2.6)	84.8 (4.5)
Average seedling DW (mg)	L=2.27 df=5 p=0.811	53.1 (1.9)	53.0 (1.9)	54.1 (1.9)	51.5 (1.9)	51.1 (1.9)	51.7 (1.9)
Average N concentration (mgN gDW^{-1})	L=12.20 df=5 p=0.032	9.9 (0.3)	9.9 (0.3)	10.9 (0.3)	9.8 (0.3)	10.5 (0.3)	10.4 (0.3)
Average N content (mgN)	L=8.56 df=5 p=0.13	0.53 (0.02)	0.52 (0.02)	0.60 (0.03)	0.51 (0.02)	0.54 (0.02)	0.55 (0.03)
Average C concentration (mgC gDW^{-1})	L=5.36 df=5 p=0.374	429.2 (0.3)	429.8 (0.3)	429.8 (0.3)	429.3 (0.3)	429.8 (0.3)	429.4 (0.3)
Average C content (mgC)	L=1.85 df=5 p=0.870	23.1 (0.8)	23.0 (0.8)	23.5 (0.8)	22.3 (0.8)	22.5 (0.8)	22.7 (0.8)
Average C:N	L=14.48 df=5 p=0.013	45 (1) AB	45 (1) AB	42 (1) A	45 (1) B	43 (1) AB	43 (1) AB

Generalized estimating equations were used to determine whether day of the experiment and treatments affected the mean proportion of seedlings that were quiescent. Model comparisons showed that the interactions were not significant at $\alpha = 0.10$.

However, treatments and day of the experiment were significant (p=0.0087 and p<0.0001 respectively). The estimated correlation parameter for the model without the interaction was α = -0.0346 and the scale parameter was 0.0302.

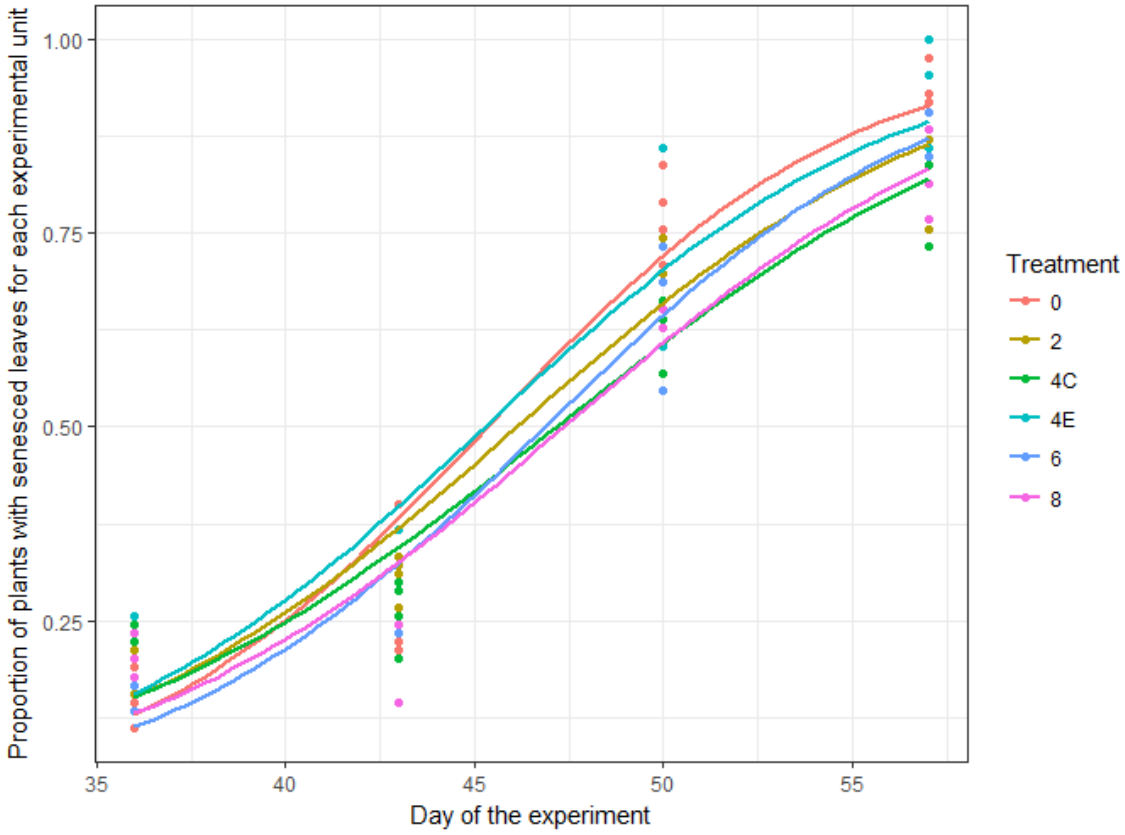

Figure 7: The proportion of senesced leaves by treatment. A Wald test for the effect of treatment showed that there were differences between the treatments in the mean proportion of leaves that had senesced.

Discussion

This study aimed to develop a fertilization program to improve the growth and the NUE of *Camassia* spp. during propagation. Unfortunately, due to flaws in the timing of fertilizer application, the study failed to provide any concrete conclusions on the efficacy of modified exponential fertilization, nor was it able to show any differences between the

treatments receiving different relative addition rates. Evidence suggested, however, that subfertigation provided N to the seedlings, indicating that it may be applicable in native plant nurseries. Other interesting data and observations, including information on the growth of *C. leichtlinii* in the second half of its first growing season, were also gathered.

Evaluating the growth and N content of the seedlings from treatments 4E and 4C was intended to allow for a comparison of modified exponential fertilization with conventional fertilization. Unfortunately, considering the circumstances of this experiment, comparing these two treatments would result in erroneous conclusions. Treatment 4E received approximately half as much N as treatment 4C because seedlings senesced before the entire fertilization program could be applied (Table 2). To compensate for this difference, a comparison could potentially be made between treatments 8 and 4C, which received close to the same amount of N. However, an even more egregious error was made (though this blunder builds into an understanding of the annual growth cycle of *Camassia* spp.). Because seedlings were already well into their exponential growth phase when the experiment started, it was wrong to start the modified exponential fertilization program from the beginning. This error resulted in the seedlings receiving their smallest fertilizer doses while they were actively growing, and they would not start to receive large doses until after their leaves had senesced. Future studies on exponential and modified exponential fertilization must be careful to match the fertilization program with the growth of the plant.

The study also failed to determine the optimum fertilizer relative addition rate for *C. leichtlinii*, with no significant differences detected between the treatments in seedling DW at the end of the experiment. However, these results should not be used to conclude that the optimum fertilizer relative addition rate for *C. leichtlinii* is no fertilizer. There were many errors in the fertilization program that may have resulted in a lack of treatment differences. As previously stated, the fertilization program was flawed in that it delivered most of the N during a period when little N uptake was observed. Also, considering that the most heavily fertilized treatment (treatment 4C) received an estimated 2.3 mgN container^{-1} during the experiment, it is possible that too little N was

delivered to create detectable differences. Another crop of *C. leichtlinii*, grown at the University of Idaho's Pitkin Forest Nursery, was tested concurrent to this experiment. The Pitkin Forest Nursery's crop appeared to be heavily fertilized and had a greater mean seedling DW (mean = 57 mg, s.d. = 23, n = 3) and mean N concentration (mean = 23.8 mgN gDW^{-1}, s.d. = 4.7, n = 3) than what was observed in this experiment. This small observation provides a modicum of evidence for a relationship between fertilization and increased seedling growth in *C. leichtlinii*. Thus, future studies might consider being more generous in their N application or focusing on the timing of fertilization to align nutrient delivery with plant uptake.

There was considerable evidence that subfertigation was an effective method of delivering fertilizer to plants. Treatment 4C, which received the most fertilizer, had a greater mean average N content than treatment 0 at the fifth sampling. Also, the estimated marginal mean for average C:N was greater for treatment 4E than for treatment 4C when compared across all five samplings. Finally, generalized estimating equations showed that treatments had a significant effect on the proportion of seedlings that were quiescent across the four weeks during which observations were made. Considering that fertilization often delays leaf senescence (Kumar *et al.*, 2005), this suggests that the fertilizer was being delivered to and taken up by the seedlings.

This experiment allowed for a better understanding of the growth and development of *C. leichtlinii* in the second half of its first growing season. The leaves of the seedlings appear to have had already reached leaf maturity by the first sampling (i.e. the leaves were no longer growing), and for some, the tips of the leaves were beginning to senesce. As the season progressed, the leaves continued to senesce and shrink, and by the fourth sampling nearly all the leaves had completely senesced. This trend is seen in other spring flowering bulb species, including tulips and *Narcissus* L., whose annual leaf growth is determinate (Rees, 1972).

The roots appear to have grown in length between the first and third samplings. However, after the third sampling they began to shrink. During the fifth sampling many of the roots appeared to be senescing, which may explain why they were observed as

shrinking. Alternatively, it is possible that the decrease in root length was a result of the change in the data collection protocol, as root fragments were no longer being salvaged if the roots broke. During the fifth sampling, it was noted that the seedlings had two types of roots; older roots that were senescing, gray, and limp, and younger roots that were white and turgid. These results suggest that *C. leichtlinii* roots senesce at the end of the growing season and new roots emerge while the plant is quiescent. This is consistent with other spring flowering bulb species, for which roots often senesce at the end of the growing season and then new roots emerge in late summer or autumn after planting (Rees, 1972, Niedziela *et al.*, 2015).

The growth data suggest that the crop had stopped growing exponentially before the first sampling. Mean average seedling DW appeared to increase up until the fourth sampling (though at a diminishing rate), after which it began to decrease. This is in line with the literature on other spring flowering bulbs. Tulip total DW increases for a 13-week period, with the total DW of the daughter bulbs increasing rapidly up until the leaves start to senesce, after which growth slows (Rees, 1972). Then after leaf senescence, the bulbs start to lose weight, as they continue to respire during the summer rest period. Carbon and N content appeared to follow a similar trend as seedling DW, possibly because increases in C and N content are driven by growth.

Considering that *C. leichtlinii* and other spring flowering bulb species exhibit determinate growth, they may not benefit from receiving nutrients in exponentially increasing amounts. Indeed, studies on tulips have found that their N uptake follows a cubic function (Niedziela *et al.*, 2015). Perhaps after developing an understanding of *C. leichtlinii*'s N uptake and allocation patterns, a better fertilization program can be designed.

Finally, the data suggest that *C. leichtlinii*'s C and N concentrations are dynamic across its annual replacement cycle. While C and N concentrations appeared to be stable during the second half of the growing season, after the leaves of the seedlings senesced their N concentration increased and their C concentration and C:N decreased. This is likely because the bulbs continue to respire during the summer rest period, as each bulb

develops a new daughter bulb for the next growing season (Rees, 1972; Thoms, 1989). Thus, after entering the summer rest period, C slowly leaves the bulb while the N content appears to remain constant.

These results allow for a reinterpretation of previous studies on *Camassia* nutrition. Turner and Kuhnlein (1983) found that the N concentration of wild *C. leichtlinii* was 9.1 mgN gDW^{-1}, which is lower than it was during the course of this experiment. While there are many possible explanations for this difference, the most likely reason that the *C. leichtlinii* in that study had a lower N concentration is that bulbs were from wild populations with limited access to N, while the bulbs from this experiment were grown in a greenhouse and fertilized. Furthermore, because Turner and Kuhnlein (1983) collected bulbs in accordance with traditional practices, bulbs may have recently undergone leaf senescence, and so were probably most similar with this experiment's fourth or fifth samplings. This suggests that when wild *C. leichtlinii* is actively growing, the plant N concentration might be lower than what was observed by Turner and Kuhnlein (1983).

Experiment 2: Investigations into the N Uptake and Allocation of C. leichtlinii

Introduction

Exponential fertilization and modified exponential fertilization have been recommended as ways of improving the NUE of crops (Ingestad, 1977; Imo and Timmer, 1992; Dumroese, 2003). However, these fertilization programs might not be universally appropriate, as they were developed using fast growing species with indeterminate growth, such as *Betula verrucose* Ehrh. (Ingestad, 1977). One example of a group of plants that might not benefit from these fertilization programs are those with determinate growth, including spring flowering bulbs such as *Camassia* spp.

Nitrogen uptake in spring flowering bulb species has been associated with two periods in their annual replacement cycle. The first of these is during the winter rest

period, a time when spring flowering bulbs lack aerial tissue, do not assimilate C, and lose DW due to respiration (Niedziela *et al.*, 2015). For example, after tulip bulbs are planted in the autumn, adventitious roots rapidly emerge from the basal plate. Then during the winter these roots accumulate N from the soil (Baba, 1967; Baba and Ikarashi, 1968). In one experiment, fall planted tulips grown with and without winter N application had very different root N concentrations when they were sampled on 13 December, with the +N treatment having a mean root N concentration of 85 mgN gDW^{-1} and the -N treatment a mean root N concentration of 22 mgN gDW^{-1} (Ohyama *et al.*, 1985). A hydroponic ^{15}N feeding experiment showed that during a month of winter fertilization, tulips took up ~20 mg of ^{15}N from the media, supplementing the 30 mgN stored in the bulb (Ohyama *et al.*, 1988). Much of this ^{15}N was later allocated to the leaves and then to the daughter bulbs, and plants grown without a winter ^{15}N treatment underwent leaf senescence earlier and yielded fewer daughter bulbs. In field grown tulips, winter N uptake was best described with a slightly positive linear function (0.10 mgN $plant^{-1}$ day^{-1}) from planting in the fall until 10 days prior to shoot emergence, after which the N uptake pattern became sigmoidal (Niedziela *et al.*, 2015). This resulted in a lower winter N uptake than what was presented by Ohyama *et al.* (1988) and may reflect a difference between hydroponic and soil culture.

While tulips exhibit a noticeable increase in N content during the winter, evidence suggests that they take up most of their N during the growing season (Niedziela *et al.*, 2015). This N uptake follows their sigmoidal growth pattern, with the inflection point between increasing and decreasing rates of dry matter and N accumulation at approximately the date of peak shoot dry matter. From shoot emergence to peak shoot dry matter, N sources include the mother bulb scales, the roots, basal plate, and the soil solution, while the daughter bulbs and the shoot are sinks. However, after the shoot reaches peak dry matter, it becomes a source and the daughter bulbs are the sole N sink.

Traits conducive to winter and spring N uptake may have resulted from the seasonal availability of N in the environments in which spring flowering bulb species evolved. In Willamette Valley and other Mediterranean prairies, it has been found that N

mineralization is greater in the winter and spring than in summer (Pfeifer-Meister and Bridgham, 2007). Thus, *Camassia* spp. may have evolved to luxury consume N during these nutrient flushes, creating reserves for periods of low nutrient availability (Chapin III, 1980).

Soil feeding of the stable isotope ^{15}N in the form of KNO_3-^{15}N, followed by sampling for dry matter accumulation and N content may reveal *C. leichtlinii's* N uptake and allocation patterns. Once taken up by the plant, there are three potential sinks for N in *C. leichtlinii*: the leaves, the daughter bulb, and the roots. Leaves often have the highest concentrations of N, because of N's importance for metabolism (Chapin III *et al.*, 2011). Nitrogen in the daughter bulbs is stored for the next growing season (Niedziela *et al.*, 2015). Roots are often intermediate in their nutrient concentrations (Chapin III *et al.*, 2011). Prioritization between these plant parts can be determined by calculating Partitioning ($\%P_N$), defined as the proportion of the total newly acquired N allocated to the different plant parts, using Equation 9 (Unkovich *et al.*, 2001).

$$\%P_N = \frac{(N_{pp} * A\%\ excess\ {}^{15}N_{pp})}{(N_{plant} * A\%\ excess\ {}^{15}N_{plant})} \quad (9)$$

Where N_{pp} is the N content of the plant part, N_{plant} is the N content of the whole plant, $A\%\ excess\ {}^{15}N_{pp}$ is the excess ^{15}N in the plant part, and $A\%\ excess\ {}^{15}N_{plant}$ is the excess ^{15}N in the whole plant (Unkovich *et al.*, 2001).

Measuring the growth and N uptake and allocation of *C. leichtlinii* during its growing season will allow for a fuller understanding of its annual replacement cycle and may be useful in developing efficient and environmentally friendly fertilizer management programs for bulb production. It was hypothesized that *C. leichtlinii* takes up N during the winter. This hypothesis would be supported if plants have a greater N content at the end of the winter rest period than at its beginning. It was also hypothesized that *C. leichtlinii* grows and takes up N in the spring following a cubic function. This hypothesis would be supported if the trends in DW and N content for *C. leichtlinii* either follow a cubic function or do not conflict with the possibility of a cubic function. Additionally, it was hypothesized that *C. leichtlinii*'s growth and N allocation are affected by N

availability. This hypothesis would be supported if there are differences between treatments receiving different amounts of N fertilizer in the growth and N content of their various organs. Finally, it was hypothesized that *C. leichtlinii* allocates much of its N to the leaves up until leaf maturity, after which N is allocated to the daughter bulb. This hypothesis would be supported if the N content of the leaves increases until leaf maturity and bulb N content increases after leaf maturity. Thus, an experiment with a completely randomized design and repeated measures was conducted.

Methods

Materials: Materials used for this experiment were *C. leichtlinii*, with the experiment beginning at the start of their second growing season. On 31 December 2016, 65 days prior to the start of the experiment, the remaining *C. leichtlinii* from Experiment 1 were transported from the Rocky Mountain Research Station to the West Greenhouses at Oregon State University (Corvallis, OR; 44.57, -123.29). There they were stored in a walk-in cooler, which was set to maintain temperatures at 3^{o}C. During the storage period, if the water held in the media of a tray fell below 65% of its weight at field capacity, then that tray was overhead irrigated (Dumroese *et al.*, 2015). On 5 March 2017, leaves were observed emerging from the media and the trays were moved to the Oak Creek Forestry Greenhouse at Oregon State University (Corvallis, OR; 44.56, -123.29).

Treatments: On 6 March, day 1 of the experiment, 16 new trays were assembled, with 46 containers in each tray, using the remaining plants from the treatments 0 and 4C from Experiment 1. The containers were randomly distributed among the trays, however containers from treatments 0 and 4C were assigned to separate trays. Then, one of four two-factor treatments were randomly assigned to each tray. The first of these factors was the previous season N treatment, i.e. the amount of N applied in Experiment 1. There were two levels for this factor: 0, which received no N fertilizer, and 4C, which received ~2.33 mgN container^{-1}. The other factor was the current season N treatment, i.e. the amount of N applied during the growing season of this experiment. Again, there were two levels for this factor: Low, which received ~45 mgN container^{-1}, and High, which received ~98 mgN container^{-1}.

Fertilization: On day 2 of the experiment, Osmocote Plus (15-9-12; The Scotts Miracle-Gro Company; Marysville, OH) and Apex NPK (14-14-14; J.R. Simplot Company; Boise, ID) were mixed into the top 1 cm of the media of each container. The Low treatment received two prills of Apex NPK (~7 mgN) and four prills of Osmocote Plus (~21 mgN) per container. The High treatment received four prills of Apex NPK (~15 mgN) and nine prills of Osmocote Plus (~47 mgN) per container. Containers were then top dressed using OBC Northwest Seedling Mix Number 1 (OBC Norwest; Canby, OR).

In addition to receiving controlled-release fertilizer, containers received KNO_3 on days 24, 45, 66, and 87 of the experiment. During these fertilizer applications, three containers from each tray received 5.25 atom % ^{15}N-KNO_3 (4.88 atom % excess) while all other containers received unlabeled KNO_3. For trays receiving the Low treatment, 33 mg (± 5 mg) of KNO_3 was delivered to each container at each fertilization (4.29 mgN ± 0.65mgN). For trays receiving the High treatment, 70 mg (± 5 mg) of KNO_3 was delivered to each container at each fertilization (9.1 mgN ± 0.65 mgN).

On the day before each KNO_3 application, containers were leached to remove as much fertilizer from the media as possible. The next day, trays were fertilized in a random order, but containers within each tray were fertilized sequentially. Fertilizer came as a salt, which was dissolved into 1-1.5 mL of water. This was then taken up in a syringe, the tip of which was plunged ~ 3 cm into the media before the solution was released to ensure uptake by the roots.

Irrigation: During the experiment, if the water held in the media of a tray fell below 75% of its weight at field capacity, then that tray was irrigated (Dumroese *et al.*, 2015). Trays were overhead irrigated prior to the first application of KNO_3. From the first KNO_3 application until the end of the experiment trays were subirrigated in small flow trays (Stuewe and Sons, Inc.; Tangent, OR) to minimize leaching (excepting days when they were leached).

Location: On day 29 of the experiment, the trays were moved from the Oak Creek Forestry Greenhouse to the Oxbow Farm and Conservation Center's native plant nursery, which is where plant materials were kept from day 30 of the experiment until its end.

Data Collection: Samplings were conducted at five times, once before the experiment began (i.e. 6 March) and at four times during the experiment. For the 6 March sampling, four containers were randomly selected from each of the experimental units (trays) that had previously received treatments 0 and 4C. Measurements included the length of the longest root. The other four samplings occurred on days 46, 66-67, 87-88, and 108-109 of the experiment. For these samplings, six plants were destructively sampled from each experimental unit, three that had received the labelled fertilizer (5.25% 15N) and three that had not (controls). During these samplings the length of the leaf and the longest root were measured.

After measurements were taken, each plant was then divided into a leaf, bulb, and root sample and these were oven dried at a minimum of 50°C for a minimum of 48 h until stable DWs were reached. All samples were oven-dried again at 65ºC for 24 h before they were weighed. Then, the three samples (or four, in the case of the 6 March sampling) for each experimental unit-isotope treatment-sampling-organ combination were mixed together to create one sample for N analysis, with effort made to ensure that the amount of material from each of the samples was in equal proportion to the other two (or three in the case of the 6 March sampling). These combined samples were then ground using a Wig-l-bug dental amalgamator (Dentsply Sirona USA; York, PA) and oven dried (24 h at 65ºC) before being packed into 8x5 mm tin capsules and sampled. N concentration and atom % ^{15}N data for each sample were obtained using a PDZ-Europa 20/20 Isotope Ratio Mass Spectrometer (PDZ Europa Ltd.; Northwich, Cheshire, UK) with a Sercon GSL prep unit (Sercon Limited; Crewe, Cheshire, UK).

Leaf growth was also monitored. Starting on day 31 of the experiment and at four-day intervals until day 76, leaves were measured from seven randomly selected plants per experimental unit. These data were used to determine the date of leaf maturity.

Data Analysis: Data were analyzed using RStudio (R Core Team, 2017). Figures were produced using ggplot2 (Wickham, 2009). Other statistical packages used include nlme (Pinheiro *et al.*, 2017), car (Fox and Weisberg, 2011), gridExtra (Auguie, 2017), cowplot (Wilke, 2017), and emmeans (Lenth, 2018). Data analysis was based on the methodologies presented by Zuur *et al.* (2009).

Welch two sample t-tests were used to determine whether the different levels of previous season N treatment differed in their mean average length of the longest root, mean average bulb DW, mean average root DW, mean bulb N concentration, mean root N concentration, mean average bulb N content, and mean average root N content at the 6 March 2017 sampling. Paired two sample t-tests were used to determine whether the plants changed during the summer and winter rest periods (between 20 July 2016 and 6 March 2017). Pairs tested the mean difference in average length of the longest root, average plant DW, and average N content and concentration between the two samplings. T-test and paired t-test assumptions were checked using Shapiro-Wilk normality tests and histograms. If the differences for the paired t-tests had a non-normal distribution, they were tested using Wilcox signed rank tests.

The average four-day leaf relative growth rate (mm mm^{-1} 4 $days^{-1}$) was calculated for each experimental unit using their average leaf length during each of the leaf measurements. This data was used to determine the date of leaf maturity, which was defined as the date of the previous measurement if the experimental unit's average relative growth rate for the four-day interval was under 1%. Days from the start of the experiment to leaf maturity were then calculated for each experimental unit, and these were used as the response variable in a Poisson GLM, with treatments and interactions as explanatory variables. Interactions and main effects were tested with drop-in-deviance tests with a χ^2 distribution.

Mixed effects modeling was used to compare the growth and N dynamics of the treatments across all four samplings. For these models, day of the experiment, previous season N treatment, current season N treatment, and their first and second order interactions were tested as fixed effects, with experimental units included in the models

as random intercepts. The response variables tested were the average leaf length, average length of the longest root, average leaf DW, average bulb DW, average root DW, average plant DW, average leaf N content, average bulb N content, average root N content, and average plant N content (averages of 6 samples for each experimental unit at each sampling); average leaf N concentration, average bulb N concentration, average root N concentration, and average plant N concentration (average of the labelled and control samples created for each experimental unit at each sampling); partitioning to the leaves ($\%P_N$ leaf), partitioning to the bulb ($\%P_N$ bulb), and partitioning to the roots ($\%P_N$ root).

Full models were fit for each response variable, and then residual plots were examined to ensure that variances were homogeneous and normal among the different factor levels, and across the four samplings. If variances were determined to be heterogenous for either of the factors, models with and without variance covariates for the factor were fit and then compared using likelihood ratio tests, and variance covariates were kept if they improved the model (α=0.05). If the spread of the residuals changed with the day of the experiment, two models, one with the power of the variance covariate for day of the experiment and one with the exponential of the variance covariate for day of the experiment were compared to the base model using likelihood ratio tests. The model that increased the log likelihood the most was kept if the improvement was significant at α=0.05. Standardized residuals were then reexamined to ensure that the variance assumptions were met.

Autocorrelation function plots were created to examine the possibility of autocorrelation within the experimental units. Autocorrelation was also tested by including the AR(1) correlation structure in the random effects part of the model, and comparing that model to the model without the correlation structure using a likelihood ratio test.

Finally, once the random part of the model was appropriate, the fixed effects were tested using backwards selection and likelihood ratio tests, with full and nested models fit using the ML estimation method. If inclusion of the interaction terms in the model were not significant at α=0.05, they were dropped from the model. Once the final model was

determined it was refit using the REML estimation method, and then the model was used to compare estimated marginal means between the different factor levels using the Tukey method for p-value adjustment. Also, if there was a trend with the day of the experiment, it was plotted and described.

Results

There were no significant differences between treatments 0 and 4C during the 6 March sampling, but paired t-tests showed that the plants changed during the winter rest period. Average length of the longest root was significantly longer on 6 March 2017 than on 20 July 2016, with a mean difference of 36.5 mm ([95% CI: 18.5, 54.4]; $t_{(9)}$=4.5953, p=0.0013). Also, average plant DW decreased between the two dates, with a mean difference of -15.5 mg ([95% CI: -25.3, -5.7]; $t_{(9)}$=-3.5737, p=0.0060). The differences between the two samplings in their average N concentration and content had non-normal distributions, and so were tested using the Wilcox signed rank test. The 6 March sampling had a significantly greater average N concentration (V=55, p=0.0020), but no difference was detected for average N content (V=28, p=1).

A Poisson GLM was fit to determine whether treatments differed in their mean number of days to leaf maturity. Neither the interactions nor the main effects were significant at $\alpha = 0.05$. The mean number of days to leaf maturity was 60 days [95% CI: 56.3, 63.9] after the start of the experiment, i.e. 4 May.

Mixed effects models were fit to determine whether previous season N treatment, current season N treatment, day of the experiment, and their first and second order interactions affected the responses measured across the four samplings. The response variables average length of the longest root, average bulb DW, average plant DW, average bulb N content, average plant N content, average plant N concentration, and $\%P_N$ root were modeled using linear regression for day of the experiment. The response variables average leaf length, average leaf DW, average root DW, average leaf N content, average root N content, average leaf N concentration, average bulb N concentration,

average root N concentration, $\%P_N$ leaf, and $\%P_N$ bulb were modelled using second order polynomial regression for the day of the experiment.

Across all the response variables tested, autocorrelation function plots were never a cause for concern, and including the AR(1) correlation structure in the random effects part of the model never improved the mixed effects models tested. Including variance covariates for the different levels of the current season N treatment improved the model for mean average leaf length ($p=0.0247$). Including a power of the variance covariate for day of the experiment improved the models for mean average bulb DW, mean average root DW, mean average bulb N content, mean average root N content, and mean $\%P_N$ root ($p<0.0001$, $p=0.0034$, $p<0.0001$, $p=0.0015$, and $p=0.0006$ respectively). Including an exponential of the variance covariate for day of the experiment improved the models for mean average plant DW, mean average plant N content, mean average leaf N concentration, and mean average plant N concentration ($p=0.0013$, $p=0.0143$, $p=0.0349$, and $p=0.0132$ respectively).

Previous season N treatment had a significant effect on average leaf length and average leaf DW (Table 5). The estimated marginal means for average leaf length and average leaf DW were greater if previous season N treatment was 4C ($p=0.0001$ and $p=0.0014$, respectively). Current season N treatment had a significant effect on average plant DW (Table 5). The estimated marginal mean for average plant DW was trending towards being greater if current season N treatment was High ($p=0.0758$). There was a significant interaction between current season N treatment and day of the experiment in their effect on average length of the longest root ($L=3.987$, d.f.=1, $p=0.0459$; Figure 8B). The mean average length of the longest root increased by 0.10 mm day^{-1} if the current season N treatment was Low and decreased by 0.16 mm day^{-1} if it was High. Day of the experiment had a significant effect on average bulb DW and average plant DW ($p<0.0001$ and $p<0.0001$ respectively; Figures 8E and 8F). Across the four samplings mean average bulb DW increased by 1.78 mg day^{-1}, while mean average plant DW increased by 1.64 mg day^{-1}. Day of the experiment also had a significant effect on average leaf length and average root DW ($p=0.0002$ and $p=0.0031$; Figures 8A and 8D).

The effect of day of the experiment on average leaf DW was not significant (p=0.1919; Figure 8C).

Table 5: Estimated marginal means and standard errors for growth data for the different levels of the main effects.

Previous Season N Treatment						
	Average leaf length (mm)	Average leaf DW (mg)	Average bulb DW (mg)	Average length of longest root (mm)	Average root DW (mg)	Average plant DW (mg)
0	133.2 (2.7) B	28.9 (1.4) B	82.6 (2.4)	105.5 (2.2)	15.67 (0.87)	126.5 (3.8)
4C	149.2 (2.7) A	34.8 (1.4) A	83.6 (2.4)	102.7 (2.2)	15.19 (0.87)	134.1 (3.8)
Likelihood ratio tests	L=20.247 df=1 p<0.0001	L=13.595 df=1 p=0.0002	L=0.284 df=1 p=0.5938	L=0.918 df=1 p=0.3381	L=0.262 df=1 p=0.6086	L=2.538 df=1 p=0.1111
Current Season N Treatment						
	Average leaf length (mm)	Average leaf DW (mg)	Average bulb DW (mg)	Average length of longest root (mm)	Average root DW (mg)	Average plant DW (mg)
Low	139.7 (2.4)	30.6 (1.4)	81.5 (2.4)	Interaction with day of the experiment; Figure 8B	16.13 (0.87)	125.6 (3.8)
High	142.7 (3.1)	33.2 (1.4)	84.7 (2.4)		14.73 (0.87)	135.0 (3.8)
Likelihood ratio tests	L=1.031 df=1 p=0.3099	L=3.481 df=1 p=0.0621	L=1.769 df=1 p=0.1835		L=2.274 df=1 p=0.1316	L=3.998 df=1 p=0.0455

Note: Although a likelihood ratio test indicated that there was a significant effect of current season N treatment on average plant DW, this difference was not significant when comparing estimated marginal means with a Tukey p-value adjustment.

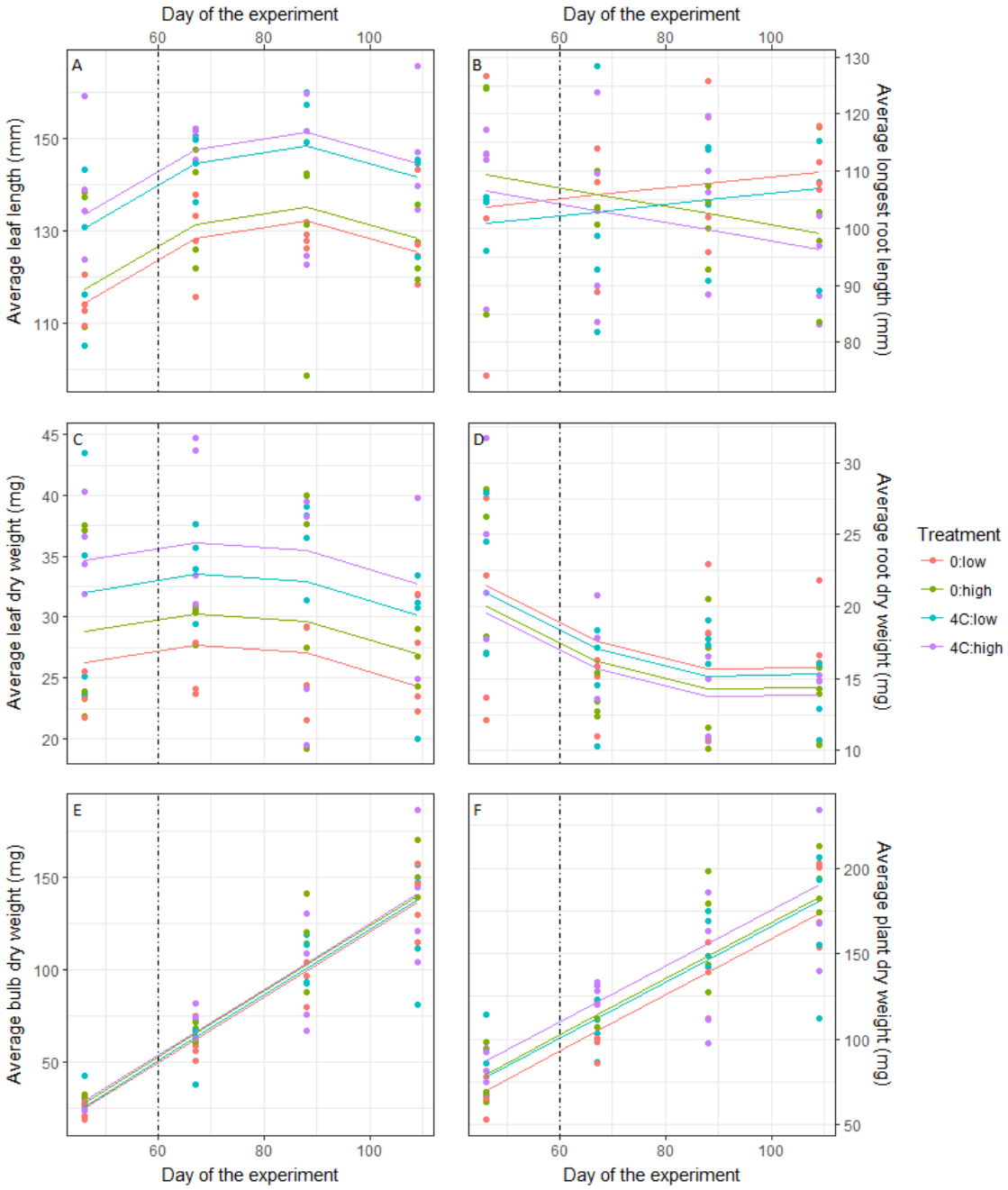

Figure 8: Plant growth during the 2017 growing season. The dotted lines indicate the date of leaf maturity. The day of the experiment had a significant effect on (A) average leaf length, (D) average root DW, (E) average bulb DW, and (F) average plant DW. (B) There was a significant interaction between current season N treatment and day of the experiment in their effect on the average length of the longest root. (C) Day of the experiment did not have a significant effect on average leaf DW.

Previous season N treatment and current season N treatment both had a significant effect on average leaf N content (Table 6). The estimated marginal mean for average leaf N content was greater for the treatment levels where more N was supplied ($p=0.0112$ and $p=0.0002$ respectively). Previous season N treatment and current season N treatment also had a significant effect on average plant N content (Table 6). The estimated marginal mean for average plant N content was trending towards being greater if previous season N treatment was 4C and was significantly greater if current season N treatment was High ($p=0.0639$ and $p<0.0001$ respectively). There was a significant interaction between current season N treatment and day of the experiment in their effect on average bulb N content ($L=13.724$, $df=1$, $p=0.0002$; Figure 9C). If the current season N treatment was Low the mean average bulb N content increased by 0.04 mgN day^{-1} and if High by 0.06 mgN day^{-1}. There was also a significant interaction between current season N treatment and day of the experiment in their effect on average root N content ($L=6.990$, $df=2$, $p=0.0304$; Figure 9E). Day of the experiment had a significant effect on average leaf N content, and across the dates tested it appeared to decrease at an increasing rate ($p<0.0001$; Figure 9A). Average plant N content was also affected by day of the experiment and mean average plant N content increased by 0.04 mgN day^{-1} ($p<0.0001$; Figure 9G).

There was a significant effect of current season N treatment on the average N concentration of the leaves, bulbs, and plants (Table 7). The estimated marginal means for these responses were greater if current season N treatment was High ($p=0.0001$, $p<0.0001$, and $p<0.0001$ respectively). Average plant N concentration was also affected by previous season N treatment (Table 7). The estimated marginal mean for average plant N concentration was greater if previous season N treatment was 4C ($p=0.0394$). There was a significant interaction between current season N treatment and day of the experiment in their effect on average root N concentration ($L=7.1996$, $df=2$, $p=0.0273$; Figure 9F). Average leaf N concentration was affected by day of the experiment ($p<0.0001$), and decreased at an increasing rate as the growing season progressed (Figure 9B). Average bulb N concentration was also affected by day of the experiment and

appeared to increase at a diminishing rate (p=0.0004; Figure 9D). Finally, average plant N concentration was affected by day of the experiment (p=0.0001; Figure 9H) and during the experiment mean average plant N concentration decreased by 0.08 mgN gDW^{-1} day^{-1}.

Table 6: Estimated marginal means and standard errors for the N content data separated by the different levels of the main effects.

Previous Season N Treatment				
	Average leaf N content (mgN)	Average bulb N content (mgN)	Average root N content (mgN)	Average plant N content (mgN)
0	1.11 (0.06) B	2.05 (0.06)	0.34 (0.02)	3.41 (0.12)
4C	1.30 (0.06) A	2.09 (0.06)	0.35 (0.02)	3.74 (0.12)
Likelihood ratio tests	L=8.085 df=1 p=0.0045	L=0.464 df=1 p=0.4957	L=0.140 df=1 p=0.7087	L=4.363 df=1 p=0.0367
Current Season N Treatment				
	Average leaf N content (mgN)	Average bulb N content (mgN)	Average root N content (mgN)	Average plant N content (mgN)
Low	1.03 (0.06) B	Interaction between treatment and day of experiment; Figure 9C	Interaction between treatment and day of experiment; Figure 9E	3.03 (0.12) B
High	1.38 (0.06) A			4.11 (0.12) A
Likelihood ratio tests	L=17.796 df=1 p<0.0001			L=23.796 df=1 p<0.0001

Note: Although a likelihood ratio test indicated that there was a significant effect of previous season N treatment on average plant N content, this difference was not significant when comparing estimated marginal means with a Tukey p-value adjustment.

Table 7: Estimated marginal means and standard errors for the N concentration data separated by the different levels of the main effects.

Previous Season N Treatment				
	Average leaf N concentration (mgN gDW^{-1})	Average bulb N concentration (mgN gDW^{-1})	Average root N concentration (mgN gDW^{-1})	Average plant N concentration (mgN gDW^{-1})
0	37.5 (1.1)	24.7 (1.0)	22.5 (0.8)	26.9 (0.7) B
4C	37.2 (1.1)	26.5 (1.0)	23.3 (0.8)	28.9 (0.7) A
Likelihood ratio tests	L=0.069 df=1 p=0.7926	L=3.212 df=1 p=0.0731	L=1.124 df=1 p=0.2891	L=5.409 df=1 p=0.02
Current Season N Treatment				
	Average leaf N concentration (mgN gDW^{-1})	Average bulb N concentration (mgN gDW^{-1})	Average root N concentration (mgN gDW^{-1})	Average plant N concentration (mgN gDW^{-1})
Low	33.9 (1.1) B	22.0 (1.0) B	Interaction between treatment and day of experiment; Figure 9F	24.3 (0.7) B
High	40.9 (1.1) A	29.1 (1.0) A		31.5 (0.7) A
Likelihood ratio tests	L=20.159 df=1 p<0.0001	L=24.217 df=1 p<0.0001		L=29.623 df=1 p<0.0001

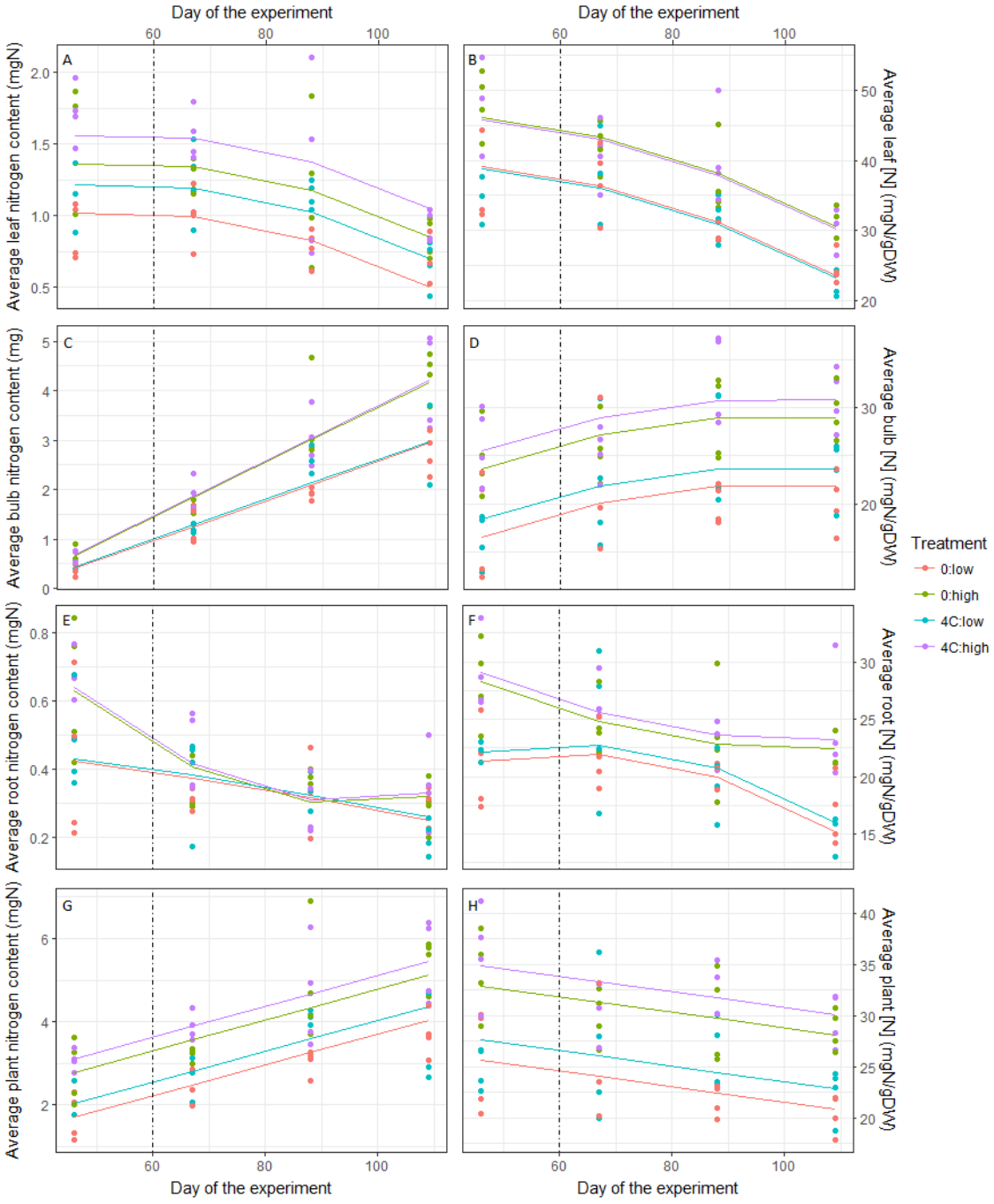

Figure 9: Nitrogen content and concentration during the 2017 growing season. The dotted lines indicate the date of leaf maturity. There was a significant relationship between the day of the experiment and (A) mean average leaf N content and (B) concentration, (C) mean average bulb N content and (D) concentration, (E) mean average root N content and (F) concentration, and (G) mean average plant N content and (H) concentration.

Partitioning to the leaves (%P_N leaf) was affected by current season N treatment (Table 8). The estimated marginal mean for %P_N leaf was trending towards being greater if the current season N treatment was High (p=0.0736). Partitioning to the roots (%P_N root) was also affected by current season N treatment (Table 8). The estimated marginal mean for %P_N root was greater if the current season N treatment was Low (p=0.0427). Day of the experiment had a significant effect on %P_N leaf (p<0.0001), which appeared to decrease between the first and second samplings and increase between the third and fourth samplings (Figure 10). Partitioning to the bulb (%P_N bulb) was also affected by the day of the experiment (p<0.0001) and increased up to the third sampling, after which it began to decrease (Figure 10). Finally, the %P_N root was affected by the day of the experiment, and decreased during the experiment (p<0.0001; Figure 10).

Table 8: Estimated marginal means and standard errors for the partitioning of new N (%P_N) data separated by the different levels of the main effects.

Previous Season N Treatment			
	%P_N leaf	%P_N bulb	%P_N root
0	0.291 (0.021)	0.502 (0.023)	0.217 (0.011)
4C	0.312 (0.021)	0.480 (0.023)	0.206 (0.011)
Likelihood ratio tests	L=0.9333 df=1 p=0.334	L=0.8138 df=1 p=0.367	L=0.7270 df=1 p=0.3938
Current Season N Treatment			
	%P_N leaf	%P_N bulb	%P_N root
Low	0.279 (0.021)	0.503 (0.023)	0.227 (0.011) A
High	0.324 (0.021)	0.479 (0.023)	0.197 (0.011) B
Likelihood ratio tests	L=4.0890 df=1 p=0.0432	L=0.9789 df=1 p=0.3225	L=5.2758 df=1 p=0.0216

Note: Although a likelihood ratio test indicated that there was a significant effect of current season N treatment on %P_N leaf, this difference was not significant when comparing estimated marginal means with a Tukey p-value adjustment.

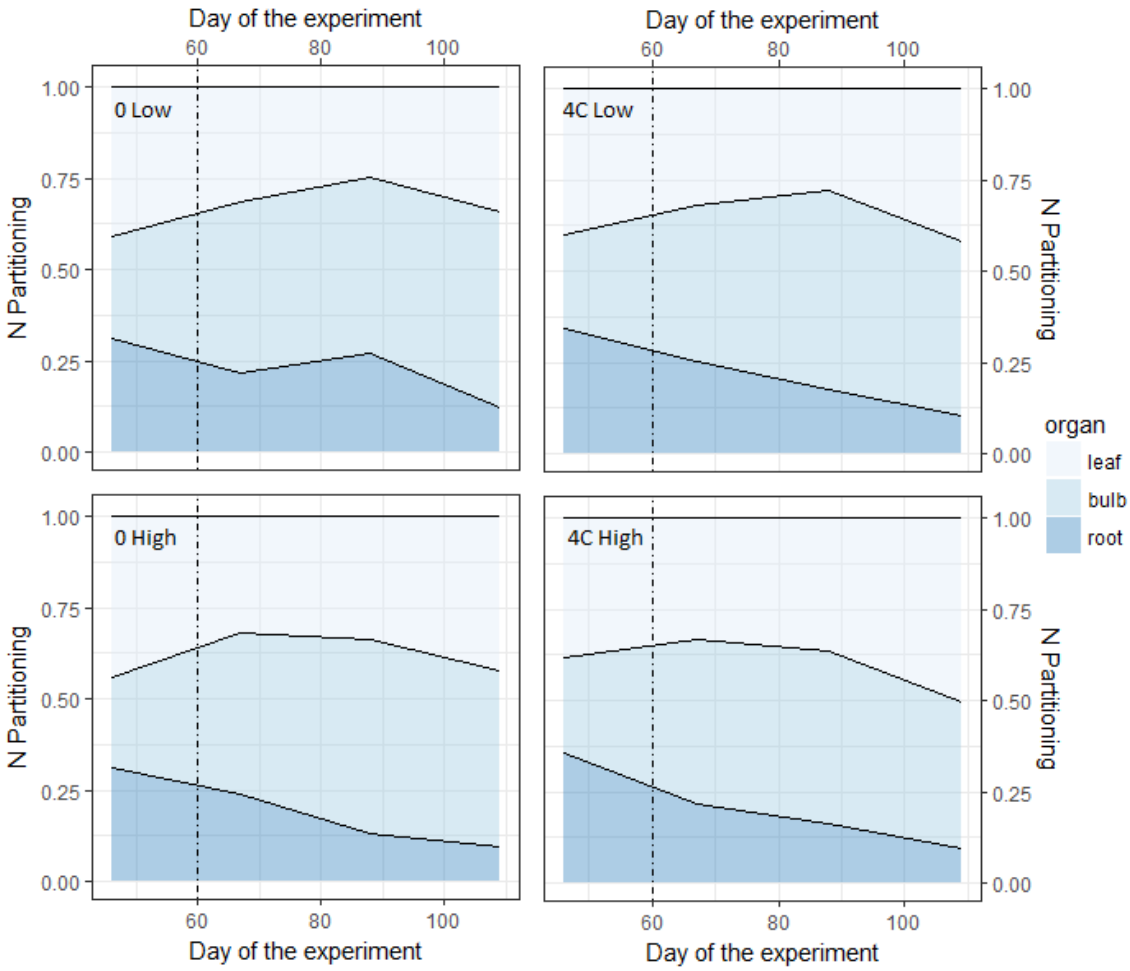

Figure 10: The partitioning of new N during the 2017 growing season. The dotted line indicates the date of leaf maturity.

Discussion

This experiment aimed to describe the growth and N uptake and allocation of *C. leichtlinii* from before its leaves reached maturity until the onset of leaf senescence, including trends in DW, N concentration and content, and the partitioning of the new N. Whether these trends were affected by N availability was also of interest. Previous season N treatment and current season N treatment stood in as proxies for mother bulb N availability and soil N availability respectively. However, previous season N treatment is an imperfect proxy, as there was undoubtedly a difference between the two treatment

levels in their daughter bulb DW (seen in the difference in mean average leaf DW between the two treatment levels). Nitrogen availability in the previous and current growing seasons affected N uptake and allocation. Previous season N treatment and current season N treatment both affected N allocation to the leaves, while N allocation to the bulbs and roots was affected by current season N treatment.

Interestingly, the treatment levels differed only a little in DW. Previous season N treatment effected the size of the leaves, and current season N treatment had a small effect on plant DW. This suggests that *C. leichtlinii* is a luxury consumer of N. Plants that luxury consume N take up more N than their immediate growth requires, and this behavior is common in slow growing plant species (Chapin III, 1980). If *C. leichtlinii* does luxury consume N, it is possible that bulbs can be loaded with nutrients before outplanting, which may improve restoration outcomes.

Camassia leichtlinii appears to have the determinate sigmoidal growth pattern that is common in other spring flowering bulb species (Rees, 1972; Niedziela *et al.*, 2015). From just before leaf maturity to just after the start of leaf senescence, the total DW of *C. leichtlinii* increased following a linear function. Furthermore, Experiment 1 suggests that as the leaves senesce the growth rate of the plant declines. Total N uptake appears to follow plant growth, with the N content increasing at a steady rate from day 46 to 109 of the experiment.

Leaf N content and concentration appear to have reached their zenith at roughly the time of the first sampling, and they both began to decrease at an accelerating rate as the leaves senesced. However, even though leaf N content and concentration decreased during the course of the experiment, this belied what was perhaps the strangest result. Across all four samplings the plants continued to partition newly acquired N to their leaves, and this partitioning was greatest at both the first and the fourth samplings. Thus, at a time when the leaves were starting to senesce, and leaf N content and concentrations were decreasing, the plants apparently sent much of their newly acquired N to their leaves. This may indicate that after the leaves start to senesce they become a convenient place to store N before it is converted into storage proteins and translocated to the

daughter bulb. Alternatively, the leaves may be where nitrate is assimilated before it is converted into storage proteins in the daughter bulb. This would suggest that the increase in new N found in the leaves may be a result of leaf senescence, which potentially leaves a fraction of this newly assimilated N behind. It should be noted that Ohyama (1991) found that tulips assimilate nitrate into glutamine in their roots (and not the leaves) before it is transported to other organs.

The bulb N content increased at a constant rate across the four samplings, however it increased more rapidly if current season N treatment was High. Bulb N concentration increased as well, though at a decreasing rate, up until the third sampling, after which it appeared to stabilize. The partitioning of new N to the bulb increased between the first and third samplings, after which it decreased slightly, perhaps the result of increased allocation to the leaves. These results suggest that much of the N taken up after leaf maturity is translocated to the daughter bulb for storage, again evidence of luxury consumption.

Root N content decreased across the four samplings, though at High levels of current season N treatment this decrease slowed as the season progressed. The partitioning of new N to the roots also decreased as the experiment progressed.

Previous season N treatment resulted in this experiment's clearest growth response. Plants that received more N during the previous growing season had larger leaves. Previous season N treatment also affected the N content of the leaf, likely because the leaves were larger and so demanded a larger quantity of N.

Current season N treatment also affected plants. While the experiment did not detect significant differences between the two levels of current season N treatment in the DW of the various organs, the plants that received more N had a greater plant DW and were trending towards being larger in every way excepting their roots. The differences between the two levels of the current season N treatment are exceptionally clear when comparing the N content and concentration of the organs, and plants that received more N in the current growing season had a higher N content and concentration in all organs.

Most intriguing were the differences in the allocation of new N between the two levels of current season N treatment. Results suggest that under high levels of soil N availability, *C. leichtlinii* will preferentially allocate N to the leaves, while at low levels of N availability the roots are preferred. This suggests the optimal partitioning theory, i.e. that the plant is allocating resources (in this case N, not biomass) to whatever organ is responsible for acquiring the most limiting resource (McCarthy and Enquist, 2007; Bloom *et al.*, 1985).

Finally, there was no change in the N content of the plants over the winter rest period. Thus, it appears that unlike tulips, *C. leichtlinii* does not take N up during the winter. However, it should be noted that the plants were not fertilized during the winter rest period, and indeed had been thoroughly leached of fertilizer near the end of Experiment 1, so this evidence is suspect. The plants did change in other ways during the winter rest period. Between the dates of 20 July 2016 and 6 March 2017 the roots grew in length, plant DW decreased, and plant N concentration increased. Tulips are an example of another spring flowering bulb species that exhibits root growth during the fall and winter, and this is thought to correspond with a return of cool moist conditions (Le Nard and De Hertogh, 1993a). This suggests that *C. leichtlinii* may need to be irrigated occasionally during the winter rest period, to provide adequate moisture for root growth.

Chapter 3: Seasonal Thermoperiodicity and Bulb-forcing

Introduction

The slow growth of spring flowering bulbs may be the result of a suite of adaptive traits that allow them to take advantage of a relatively short and poor growing season (Le Nard and De Hertogh, 1993a, Rees, 1972). The growing season, which starts in late winter or early spring, can be considered poor because temperatures and irradiance are low, though this may be a more favorable time to grow than summer in the Mediterranean climates where many spring flowering bulbs evolved. It can be considered short because not long after the summer drought begins the leaves of spring flowering bulbs senesce. The factors that drive leaf senescence in bulbs remain unclear, though it has been associated with adverse conditions such as drought (Le Nard and De Hertogh, 1993a). However, Lapointe (2001) postulated that the initiation of leaf senescence is driven by the cessation of sink demand once carbohydrate reserves in the bulb have been filled. This later explanation seems reasonable, as the leaves of *C. leichtlinii* will senescence even with generous irrigation (See Experiment 1). Furthermore, because spring flowering bulb species have a determinate growth form, it is possible that leaf senescence is triggered when sugar concentrations within the plant surpass an acceptable level (Lim *et al.*, 2007). In general, many biotic and abiotic factors can initiate premature leaf senescence in plants, including drought, nutrient limitation, extreme temperatures, oxidative stress, infection, and shading by other plants, though even under perfect conditions leaves have a lifespan (Lim *et al.*, 2007; Lim *et al.*, 2003).

After their leaves have senesced; development, dormancy, and reemergence for most spring flowering bulb species is controlled, at least in part, by seasonal thermoperiodicity, i.e. seasonal changes in temperature (Le Nard and De Hertogh, 1993a). For horticultural purposes, spring flowering bulbs are often described as requiring a warm-cold-warm cycle before they will reemerge (Le Nard and De Hertogh, 1993a). The summer rest period is the first part of this cycle, and during this period some species initiate and develop new organs within the bulb, including root initiation and

differentiation of the flower and vegetative buds. The second part of the cycle occurs during the fall and winter. During this time the cold requirement, frequently defined as an amount of time spent chilling at or below a certain temperature, must be met before the leaves and flowers of quiescent bulbs will emerge. The fall and winter are also associated with root growth in certain species. Finally, when warm temperatures return in the spring, the leaves and flowers will emerge.

Because seasonal thermoperiodicity often controls the growth and development of spring flowering bulbs, scientists have tested whether the methods of bulb-forcers could be used to shorten the amount of time between seed and flowering bulb (Fortanier, 1970; May, 2007). Bulb-forcers use temperature manipulation to accelerate or delay flowering and their techniques were reportedly commonplace in the 18th century (Le Nard and De Hertogh, 1993a; De Hertogh, 1974). The general program for forcing spring flowering bulbs consists of three parts, corresponding to the warm-cold-warm cycle mentioned previously. The steps of this sequence will be referred to here as the heat treatment, chilling, and growth.

Bulb-forcing techniques have allegedly been used to minimize the summer and winter rest periods for tulips (Fortainer, 1970) and *Lilium philadelphicum* L. (May, 2007) allowing for multiple growing seasons in a single year. If taken to the extreme, bulb-forcing strategies could form the basis of shortening the annual replacement cycle of a bulb to eight or even six months (Fortanier, 1970). If *Camassia* spp. are controlled by seasonal thermoperiodicity, it may be possible to manipulate their annual replacement cycles using bulb-forcing techniques. Alternatively, these techniques may be used to improve the growth period by allowing growers to control the date of leaf emergence and to ensure that all life cycle requirements are met each year.

The first step of a bulb-forcing program is the heat treatment, which can last several weeks and is an essential stage for some but not all spring flowering bulb species (Le Nard and De Hertogh, 1993a). In commercial bulb-forcing programs, spring flowering bulbs are harvested in the summer. Afterwards, storage temperatures can affect the rate at which the bulbs develop. For example, tulip flower bud differentiation occurs

rapidly if temperatures are held between 17 and 20°C (Hartsema, 1961). When tulip bulbs are chilled after they have developed a completely differentiated flower bud, the low temperatures induce elongation of the flower bud and the transformation of vegetative buds into bulbs (Le Nard, 1983). However, if tulip bulbs are chilled before the flower bud has completely developed, other behaviors are observed. First, if chilling is applied to tulips prior to the flower bud reaching physiological maturity, the plant will not react to low temperatures, but will instead grow slowly (Le Nard, 1975). After reaching physiological maturity, if chilling is applied while the apical bud has only leaf primordia, these primordia will change into scales and produce an apical daughter bulb. If the cold temperatures are applied before the flower bud is completely differentiated, the flower bud will not elongate, and will instead desiccate. Only when bulbs are chilled after developing a completely differentiated flower bud will they grow properly (Le Nard, 1983). This shows that if tulip bulbs have reached physiological maturity, dormancy for the apical meristems is induced by cold temperatures, and whatever growth phase they are at will be arrested by these cold temperatures (Le Nard, 1983). Thus, an extended period of warm temperatures prior to chilling is essential for proper growth and development for some spring flowering bulb species.

Considering that *C. quamash* is known to develop it flowers into the fall, the necessity of a heat treatment is highly probable (Maclay, 1928). Nevertheless, the relationship between a "prechilling" heat treatment and subsequent growth has not yet been characterized for any *Camassia* spp. This relationship should be of high interest to growers who wish to use bulb-forcing techniques when growing *Camassia* spp.

The second step of a bulb-forcing program is chilling. In commercial bulb-forcing programs, chilling is applied after spring flowering bulbs have received their heat treatment, and can result in root growth, scape elongation, and the beginning of leaf growth, depending on the species (Le Nard and De Hertogh, 1993a). This chilling is usually applied for between 10 and 20 weeks, depending on the species and the market. After the chilling is complete, the plants are brought into a warm greenhouse where the leaves and flowers emerge.

Many plant species require an extended chilling period prior to germination, budbreak, or leaf emergence in the spring. These chilling requirements likely evolved as a mechanism to allow the plants to "know" that winter has passed (Glover, 2007). Tulips are one example of spring flowering bulbs with a chilling requirement. They need 12 to 16 weeks of low temperature before their leaves and flowers will emerge, and by increasing the duration of chilling, one can decrease the number of days between planting and flowering (Saniewski *et al.*, 2000). During chilling, the hormonal status of tulip bulbs change, with levels of abscisic acid, which is associated with dormancy, decreasing and levels of free gibberellins, which are associated with stem elongation and flowering, increasing (Saniewski *et al.*, 2000). Gibberellins have been found to peak twice in field grown tulips, once in December or January, before the cold requirement is met, and again around the time of leaf emergence (Hanks and Rees, 1980). Low temperatures also induce starch and protein solubilization in tulip bulbs, making the sugars and the amino acids stored in the scales available for shoot growth (Ohyama *et al.*, 1988).

Chilling is often measured in accumulated chilling hours, which refers to the number of hours at or below a certain temperature. Considering that the goal of this bulb-forcing program is to minimize the length time between periods of growth, the optimum amount of chilling should be the number of accumulated chilling hours that minimizes the length of the winter rest period, as long as negative effects on the crop are avoided. This differs from Worrall and Mergen (1967), where the chilling optimum was defined as the point at which ten additional days of chilling does not correspond with a one-day increase in the number of days to budbreak, or in the case of flower bulbs, leaf emergence.

Two experiments were conducted with the aim of developing a bulb-forcing program for *Camassia* spp. However, unlike traditional bulb-forcing programs, which aim to deliver a healthy flower to a particular market, the objective of this bulb-forcing program was to reduce the length of the summer and winter rest periods while maintaining normal plant growth and development.

The first experiment aimed to determine the effect of the length of chilling at 4.6°C on quiescent *C. leichtlinii*, *C. quamash*, and *Toxicoscordion venenosum* (S. Watson) Rydb. It was hypothesized that these species respond to seasonal thermoperiodicity, and that this response includes a chilling requirement prior to leaf emergence. This hypothesis would be supported if increasing accumulated chilling hours increases the likelihood of leaf emergence, decreases the time to leaf emergence in the growth room, and increases leaf growth rates after leaf emergence for these species. It was also hypothesized that each of these species has a chilling optimum, defined as an amount of chilling that minimizes the length of the winter rest period whilst not resulting in any physiological issues. This hypothesis would be supported if the trends in days between the start of chilling and 50% leaf emergence for treatments receiving different amounts of chilling have clear vertices, and if treatments do not differ in their height at leaf maturity. For this experiment, 19 treatments were applied, and these were weeks spent chilling at 4.6°C. The treatments ranged from 0 weeks to 18 weeks, with one-week intervals. Eight containers were used for each treatment-species combination, and these were arranged as a completely randomized design within the environment. After receiving their chilling treatment, containers were moved into the growth room and allowed to grow.

The second experiment aimed to determine the effect of the length of the heat treatment, applied to quiescent bulbs prior to chilling, on bulb growth and development. It was hypothesized that *C. leichtlinii*'s roots and daughter bulb develop at the expense of the mother bulb during the summer rest period. This hypothesis would be supported if plants that receive a longer heat treatment have a greater daughter bulb DW and root DW and a lower mother bulb DW at the cessation of chilling. This hypothesis would be further supported if plants that receive a longer heat treatment have longer leaves at leaf maturity and larger bulbs after leaf senescence. Finally, it was hypothesized that leaf senescence is triggered by a reduction in sink demand. This hypothesis would be supported if treatments with a more developed daughter bulb (due to the length of the heat treatment) take longer for their leaves to senesce. For this experiment, eight

treatments were applied to 48 trays grown in two environments with a three-block randomized complete block design within each environment. The treatments were weeks spent in the heat treatment and ranged from 0 weeks to 14 weeks, with two-week intervals. After receiving the heat treatment, trays were moved into chilling for 14 weeks and then brought back into the grow rooms and allowed to grow.

Methods

Chilling

Materials: Plant materials used for the chilling experiment were bulbs, grown by University of Idaho's Pitkin Forest Nursery, which had just finished their first growing season. The nursery acquired and then cold moist stratified the seeds of *C. leichtlinii* (collected in northwest Oregon), *C. quamash* (collected in Lewis County, WA), and *T. venenosum* (collected on Dinner Island, WA) starting in February 2016. Then on 7 March and 7 April, germinated seeds were planted into Styroblock 77/170s (Beaver Plastics; Acheson, AB) with a Sungro Custom Blend Media (Sungro Horticulture; Agawam, MA) and were grown until leaf senescence. Leaf senescence occurred between 23 June and 10 July for *C. leichtlinii*, between 23 June and 3 July for *C. quamash*, and between 23 June and 7 July for *T. venenosum*.

Treatments: From this original crop, 152 individuals were randomly selected from each species and assigned to one of 19 treatments. These treatments were weeks of chilling at 4.6ºC and ranged from 0 and 18 weeks, with eight individuals used for each treatment-species combination. All bulbs were removed from their media and transplanted into Sunshine Mix #2 / LBS media (Sungro Horticulture; Agawam, MA) in SC10 cones (Stuewe and Sons, Inc.; Tangent, OR). *Camassia quamash* and *T. venenosum* were transplanted on 9 July and *C. leichtlinii* was transplanted on 10 and 11 July. No fertilizer was added to the media.

Once all the bulbs had been transplanted they were randomly assigned to a place within one of two sets of Ray Leach 98 trays (Stuewe and Sons, Inc.; Tangent, OR). Control bulbs, which received no chilling, were randomly distributed among a set of

eight trays that were to go into the growth room. The rest of the bulbs were randomly distributed among seven trays that were to go into the vernalization chamber. These trays were moved into their corresponding rooms on 14 July 2016, day 1 of the experiment. From then until the 18th week, a treatment consisting of 8 containers for each of the species was removed from chilling every week and randomly distributed throughout the growth room. Date and time were recorded after moving to determine accumulated chilling hours.

Chambers: Two controlled environment rooms were used. The first was a GR48 growth room (Controlled Environments Ltd.; Winnipeg, Manitoba) programed to provide a 12 h photoperiod (600 - 1800 h) with diurnal temperatures and relative humidity of 16^{o}C and 73% during the day and 14^{o}C and 83% at night. Metal halide / high pressure sodium lights were used in the growth room. The second room was a vernalization chamber, constructed by the staff at University of Idaho. It also had a 12 h photoperiod, however fluorescent lights were used, temperatures were maintained at 4.6^{o}C, and relative humidity fluctuated between 88.6% during the day and 97% at night. Average irradiance, measured on 15 July 2016, was 194.5 µE m^{-2} s^{-1} (n = 16 observations) in the growth room and 5.68 µE m^{-2} s^{-1} (n = 14 observations) in the vernalization chamber. It should be noted that there was an irradiance gradient in the growth room, ranging from 157 µE m^{-2} s^{-1} at the ends of the bench to 226 µE m^{-2} s^{-1} at its center.

Irrigation and Locations: Trays were first watered on day 1 of the experiment. From then on, trays were watered to field capacity once a week. Tray locations were randomized every other week, with the first randomization occurring on day 14 of the experiment. The experiment concluded on day 158 of the experiment, which was before all the plants had reached leaf emergence.

Data Collection: Containers were checked twice a week to determine if the leaves of any of the plants had emerged (i.e. green leaf tissue had emerged from the media). For each container, date of emergence was recorded, and this data was used to determine the number of days between the cessation of chilling and 50% leaf emergence for each treatment-species combination. Additionally, this was used to determine the number of

days between the start of the experiment and 50% leaf emergence for each treatment-species combination. Leaf length was measured every week. This data was used to determine growth rates in the first week for individual plants by subtracting each plant's second leaf measurement from its first. Date of leaf maturity for individual plants was determined to be the previous week if the relative growth rate of the leaf for the week was below 2%. This data was also used to determine the height of the leaves at leaf maturity.

Heat

Materials: Plant materials used for the heat treatment experiment were bulbs whose leaves had just senesced after their second growing season. What follows is an account of their growth during that second growing season. On 31 December 2016, the remaining *C. leichtlinii* from Experiment 1 were transported from the Rocky Mountain Research Station to the West Greenhouses at Oregon State University. There they were stored in a walk-in cooler, which was set to maintain temperatures at 3^{o}C. During the storage period, if the water held in the media of a tray fell below 65% of its weight at field capacity, then that tray was overhead irrigated (Dumroese *et al.*, 2015).

On 5 March 2017, leaves began to emerge from the media. Thus, the trays were moved to the Oak Creek Forestry Greenhouse at Oregon State University. On 7 March, trays from the treatments 6 and 8 from Experiment 1 were selected to be used in this experiment. Eight prills of Apex NPK (14-14-14; J.R. Simplot Company; Boise, ID) were mixed into the top centimeter of the media of each container (~207 mg Apex container^{-1} or ~29 mgN container^{-1}). Containers were then top dressed using OBC Northwest Seedling Mix Number 1 (OBC Norwest; Canby, OR). Finally, trays were watered to field capacity, and from then until 3 July, if the water held in the media of a tray fell below 75% of its weight at field capacity, then that tray was overhead irrigated (Dumroese *et al.*, 2015). On 3 April, trays were transported from Oregon State University to the Oxbow Farm and Conservation Center's native plant nursery where they were grown until their leaves began to senesce.

Starting on 17 June, leaf senescence was measured for all plants, and this was repeated every four days. These measurements were used to determine the date of leaf senescence for each plant, defined as when leaves were first observed as either completely yellow, completely desiccated, with a disconnected leaf, or some combination. On 3 July, irrigation frequency was reduced, and if the water held in the media of a tray fell below 65% of its weight at field capacity, then that tray was overhead irrigated (Dumroese *et al.*, 2015). On 11 July, trays were brought back to Oregon State University and kept in the Oak Creek Forestry Greenhouse. This marks the end of the second growing season.

Treatments: On 27 July, day 1 of the heat treatment experiment, 48 new trays were assembled with nine containers in each tray. Containers were randomly distributed among the 48 trays, however, containers that had previously received treatment 6 were distributed among trays 1 – 24 and containers that had previously received treatment 8 were distributed among trays 25 – 48. Leaf senescence for the plants used was observed between 3 and 23 July. These new trays were irrigated, and from then until the end of the experiment, if the water held in the media of a tray fell below 65% of its weight at field capacity, then that tray was overhead irrigated (Dumroese *et al.*, 2015). Finally, the newly assembled trays were moved into their respective controlled environment rooms.

Treatments were the number of weeks of heat treatment applied prior to being chilled. The first treatment to be moved into refrigeration was a control (treatment 0), which received no heat treatment. Subsequent treatments received 2, 4, 6, 8, 10, 12 and 14 weeks of heat, respectively. Details for the treatments are displayed in Table 9.

Table 9: Bulb-forcing program details.

Treatment	Heat treatment start date	Length of heat treatment (days)	Chilling start date	Length of chilling period (days)	Growth period start date
0		0	7/27/2017	98	11/2/2017
2	7/27/2017	14	8/10/2017	98	11/16/2017
4	7/27/2017	28	8/24/2017	98	11/30/2017
6	7/27/2017	42	9/7/2017	98	12/14/2017
8	7/27/2017	56	9/21/2017	98	12/28/2017
10	7/27/2017	70	10/5/2017	98	1/11/2018
12	7/27/2017	84	10/19/2017	98	1/25/2018
14	7/27/2017	98	11/2/2017	98	2/8/2018

Note: 6 trays were used for each treatment, 3 for each of the controlled environment room combinations.

Chambers: Four controlled environment rooms were used in the experiment. Two were grow rooms at Oak Creek Complex: hereafter called Oak Warm and Middle Warm. Each grow room was 2.80 m x 2.82 m x 2.64 m. Plants were stored on table tops, with 107 cm between the floor and the top of the containers. The other two controlled environment rooms were walk-in coolers: one on the second floor of Richardson Hall (hereafter called Richardson Cold) and another at Oak Creek Complex (hereafter called Oak Cold). During the heat treatment and the growth period, trays 1 – 24 were kept in Oak Warm and trays 25 – 48 in Middle Warm. After their respective heat treatments, trays 1 – 24 were moved to Richardson Cold and trays 25 – 48 were moved to Oak Cold. Thus, there were two grow room – walk-in cooler pairs, Oak Warm – Richardson Cold and Middle Warm – Oak Cold. Within each grow room – walk-in cooler pair there were three trays for each treatment, arranged in a randomized complete block design.

Heat Treatment: From day 1 to day 99 of the experiment the heat treatments were applied. During this time, the grow rooms were neither heated nor illuminated. Figure 11 shows the change in temperature across the heat treatment period. Temperature was recorded from day 13 of the experiment to the end of the heat treatment period in both grow rooms using Kestrel Drop D2s (Kestrel Meters; Minneapolis, MN).

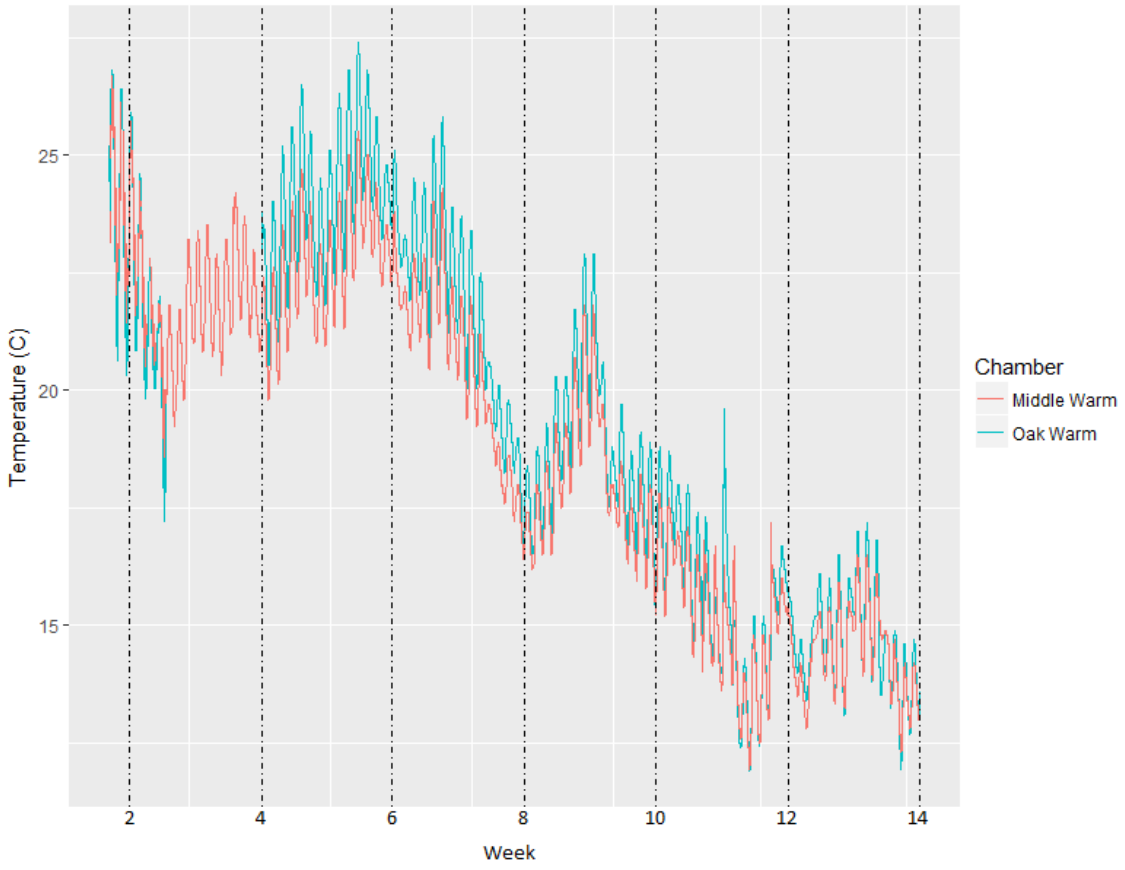

Figure 11: Grow room temperatures across the heat treatment period. Grow rooms were not temperature controlled during the heat treatment. Dashed lines indicate when treatments were moved out of the heat treatment and into chilling.

Chilling: Once trays had received their heat treatment, they were moved into their respective walk-in coolers where they were chilled for 14 weeks, accumulating approximately 2,352 chilling hours. Richardson Cold was set to keep temperatures at 4ºC, but on day 134 of the experiment, it malfunctioned, and temperatures fell to -7ºC. This incident was resolved the next day, however temperatures never returned to 4ºC, and instead fluctuated around a mean of 7.3ºC (s.d. = 0.5ºC). Although none of the plants died, treatments 6, 8, 10, 12, and 14 in Richardson Cold were compromised because the temperature changed while they were receiving their 98 days of chilling, and so each treatment received less chilling than the previous. Temperatures in Oak Cold were recorded from day 170 to 180 of the experiment using a Kestrel Drop D2 (Kestrel

Meters; Minneapolis, MN). The mean temperature for Oak Cold during this period was 3.2ºC (s.d. = 0.2ºC). It was assumed that this temperature was held for the entire chilling period.

Growth: On day 99 of the experiment, temperatures in the grow rooms were set to 21ºC. Heat was provided by 5,000-Watt Electric Compact Unit Heaters (GlenDimplex; Dublin, Ireland). Cooling was provided by 12,000 BTU Window-Mounted Room Air Conditioners (Frigidaire; Charlotte, NC). These were controlled using T6 Pro external programable thermostats (Figure 12A; Honeywell International Inc.; Morris Plains, NJ). Lighting was provided using 1233 Shoplights (Lithonia Lighting; Conyers, GA) with four fluorescent bulbs in each grow room, suspended 64 cm above the tops of the containers. These were set to provide a 12 h photoperiod (700 – 1900 h). Photon flux was measured at the level of the containers for each block on day 278 of the experiment. Within Oak Warm blocks received 19-21 μmol m^{-2} s^{-1}. Within Middle Warm, blocks received 17-21 μmol m^{-2} s^{-1}. CO_2 was monitored in Oak Warm starting on day 145 of the experiment using a CO2000 Carbon Dioxide CO_2 Data Logger and Monitor (Perfect Prime; Dayton, NJ), with measurements taken whenever the grow room was entered. No trend in CO_2 concentration with day of the experiment within the grow room was observed. Temperature and relative humidity was recorded during the growth period in both grow rooms using Kestrel Drop D2s (Kestrel Meters; Minneapolis, MN).

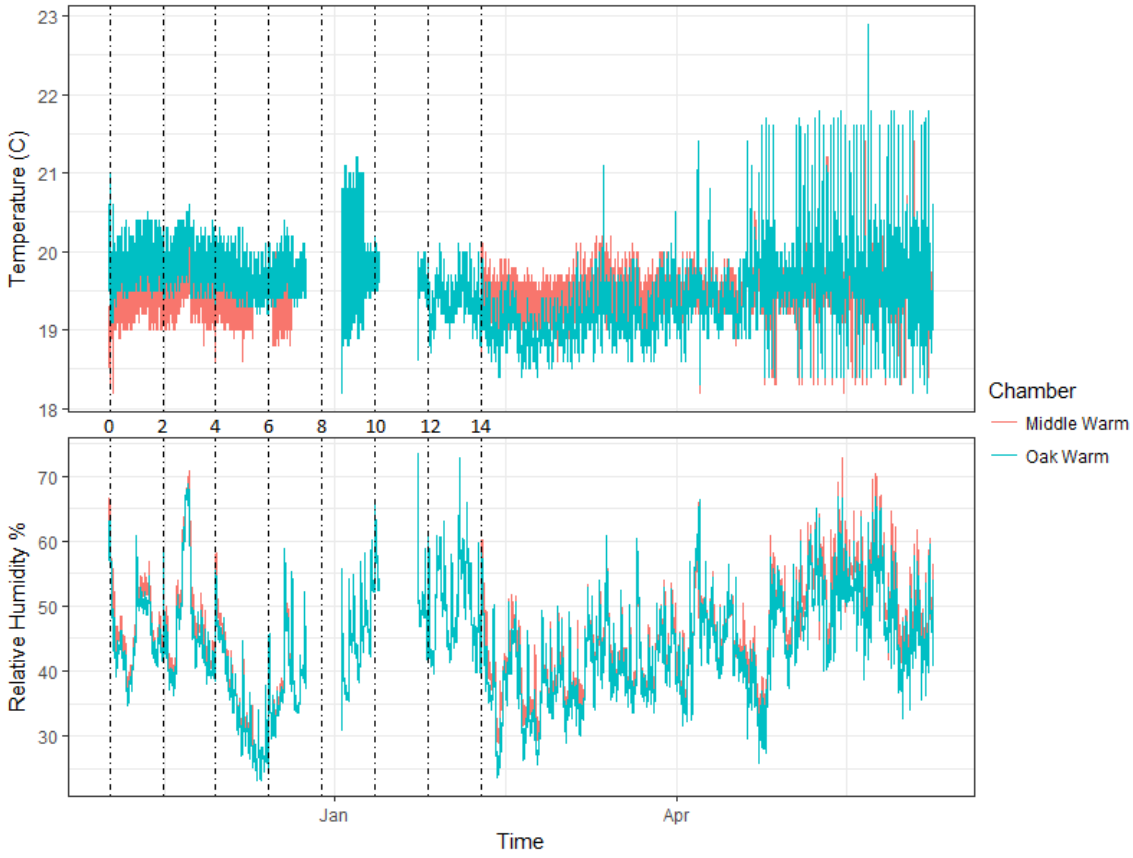

Figure 12: Temperature and relative humidity during the growth period. (A) The temperatures in the grow rooms remained relatively constant during the growth period. (B) Relative humidity seemed to fluctuate across the growth period. Dashed lines indicate when treatments were moved out of chilling and into the grow rooms.

Data Collection: Once an experimental unit (tray) had received the appropriate heat treatment and subsequent chilling, it was moved back into the grow rooms. At this point, the cessation of chilling, one container from each experimental unit was reserved and then dissected. Data collected includes the DW of the roots, daughter bulb, mother bulb, and the total plant.

Once in the grow rooms, containers were monitored twice weekly. Containers were checked for leaf emergence and senescence, and the longest leaf was measured. Leaf emergence was defined as the moment when the plant's leaf(ves) were first observed emerging from the media. Leaf senescence was defined as when the leaves were

completely chlorotic, desiccated, disconnected, or some combination thereof. These data were used to determine days between the cessation of chilling and 50% leaf emergence and the days between 50% leaf emergence and 50% leaf senescence for each experimental unit. Measurements of the longest leaf were used to determine leaf growth in the first week and the date of leaf maturity. Leaf growth in the first week was defined as the difference between the third and first leaf measurements, as leaves were measured twice weekly. The date of leaf maturity for individual plants was determined to be the previous week if the relative growth rate of the leaf for the week was below 2%. This data was used to determine the date of 50% leaf maturity and the average length of the leaves at leaf maturity for each experimental unit.

Once all plants had senesced, they were removed from their media and dissected. Bulbs were oven dried at 65^{o}C for 48 h and weighed to determine both the average and the total final bulb DW for each experimental unit.

Data Analysis

Data were analyzed using RStudio (R Core Team, 2017). Figures were produced using the package ggplot2 (Wickham, 2009). Other statistical packages used include emmeans (Lenth, 2018), MASS (Venables and Ripley, 2002), nlme (Pinheiro *et al.*, 2017), dplyr (Wickham *et al.*, 2017), purrr (Henry and Wickham, 2017), gridExtra (Auguie, 2017), cowplot (Wilke, 2017), and car (Fox and Weisberg, 2011). Data analysis was based on the methodologies presented by Zuur *et al.* (2009).

Chilling

The effect of accumulated chilling hours and species on the probability of leaf emergence by the final day of the experiment was tested using logistic regression analysis. A logistic regression model, which included species, accumulated chilling hours, and their interactions as explanatory variables was fit with a binomial leaf emergence response for individual bulbs. Then coefficients were tested for significance (at $\alpha = 0.05$) using drop-in-deviance tests with a χ^2 distribution. Finally, the mean odds of leaf emergence for bulbs that received 168 accumulated chilling hours and bulbs that

received 1680 accumulated chilling hours were estimated for each species using the full model, and these means were reported along with 95% confidence intervals.

The effect of accumulated chilling hours and species on the number days between the end of chilling and 50% leaf emergence was tested using a Poisson GLM. The model was fit using species, accumulated chilling hours, and their interactions as explanatory variables and the number of days between the end of chilling and 50% leaf emergence for each treatment-species combination as the response. Model assumptions were checked using residual plots and then coefficients were tested for significance (at $\alpha = 0.05$) using drop-in-deviance tests with a χ^2 distribution. Finally, the means for the number of days between the end of chilling and 50% leaf emergence for bulbs that received 1680 accumulated chilling hours and bulbs that received 3024 accumulated chilling hours were estimated for each species using the full model, and these means were reported along with 95% confidence intervals.

To determine the chilling optimum for leaf emergence, here defined as the number of accumulated chilling hours that minimizes the number of days between the start of chilling and 50% leaf emergence, a multiple linear regression model was fit, with species, accumulated chilling hours, accumulated chilling hours^2, and their interactions as explanatory variables. Then the chilling optima were determined by calculating the vertices of the fitted equations for each species. Confidence intervals for the vertices were determined for each species using bootstrapping techniques.

The effect of accumulated chilling hours and species on leaf growth after leaf emergence was tested using a generalized least squares model. The response variable was the total leaf growth in the first week for individual plants. Explanatory variables tested include accumulated chilling hours, species, and their interactions. Residual plots suggested that different species had different variances, so the homogeneous variance assumption was relaxed, and variance covariates for the different species were included in the model. Models with and without the variance covariates were fit with the REML estimation method, and then compared using a likelihood ratio test. The standardized residuals for the model with variance covariates were then examined to ensure that the

variance assumptions were met. Explanatory variables were tested using likelihood ratio tests with the ML estimation method. Estimated marginal mean separation between the species used the Tukey method for p-value adjustment.

Finally, to determine if there was a treatment effect on leaf length at leaf maturity, a linear model was fit with the leaf lengths at leaf maturity for individual plants as the response and species, accumulated chilling hours, and their interactions as the explanatory variables. *Toxicoscordion venenosum* was excluded from this analysis because the species had only one individual that reached leaf maturity. Model assumptions were checked using residual plots. Model coefficients were tested for significance (at $\alpha = 0.05$) using extra sum of squares F-tests.

Heat

Because Richardson Cold broke down during the chilling period, treatments in that grow room – walk-in cooler pair did not receive uniform chilling. Thus, the results were not reliable, and that controlled environment room pair was dropped from the analysis. Data used in the analyses for leaf length at leaf maturity, leaf growth for the first week after leaf emergence, and average final bulb DW were averaged for the eight plants in each experimental unit.

Mixed effects modeling was used to determine whether there was an effect of weeks of heat treatment on the response variables: mother bulb DW, daughter bulb DW, log of the root DW + 1, and total DW for the plants sampled at the cessation of chilling; average leaf length at leaf maturity; average leaf growth in the first week; and average final bulb DW and total final bulb DW. Weeks of heat treatment was included in each model as a fixed effect and blocks were included as random intercepts. Models were fit for each response variable, and then residual plots were examined to ensure that variances were homogeneous and normal. If the spread of the residuals increased with increasing weeks of heat treatment, another model, with the exponential of the variance covariate for weeks of heat treatment, was constructed. This model was then compared to the original model using a likelihood ratio test, and if the likelihood ratio test was significant $\alpha=0.05$

the variance covariate was kept in the model. Standardized residuals were then reexamined to ensure that the variance assumptions were met. For models best described using linear regression, the treatment effect was tested using a t-test. For models best described using polynomial regression, the treatment effect was tested using a likelihood ratio test with the ML estimation method.

The effect of treatment on days between the cessation of chilling and 50% leaf emergence, days between 50% leaf emergence and 50% leaf maturity, days between 50% leaf emergence and 50% leaf senescence, and days between 50% leaf maturity and 50% leaf senescence were tested using quasi-Poisson GLM.

Results

Chilling

A logistic regression model with species, accumulated chilling hours, and their interactions was tested to determine whether accumulated chilling hours affected the odds of leaf emergence. Drop-in-deviance tests showed that the interactions between species and accumulated chilling hours were not significant ($p=0.1413$). However, drop-in-deviance tests for species and accumulated chilling hours were significant ($p<0.0001$ and $p<0.0001$). Using the full model, the mean odds of leaf emergence by day 158 of the experiment for bulbs that received 168 accumulated chilling hours (1 week of chilling) was estimated to be 0.403 [95% CI: 0.195, 0.833] for *C. leichtlinii*, 0.434 [95% CI: 0.225, 0.838] for *C. quamash*, and 0.030 [95% CI: 0.009, 0.093] for *T. venenosum*. The mean odds of leaf emergence by day 158 of the experiment for bulbs that received 1680 accumulated chilling hours (10 weeks of chilling) was estimated to be 17.76 [95% CI: 6.66, 47.35] for *C. leichtlinii*, 5.66 [95% CI: 3.15, 10.18] for *C. quamash* and 0.28 [95% CI: 0.18, 0.45] for *T. venenosum*. The probability of leaf emergence by day 158 of the experiment as a function of accumulated chilling hours for each species is presented in Figure 13 and was plotted using the full model.

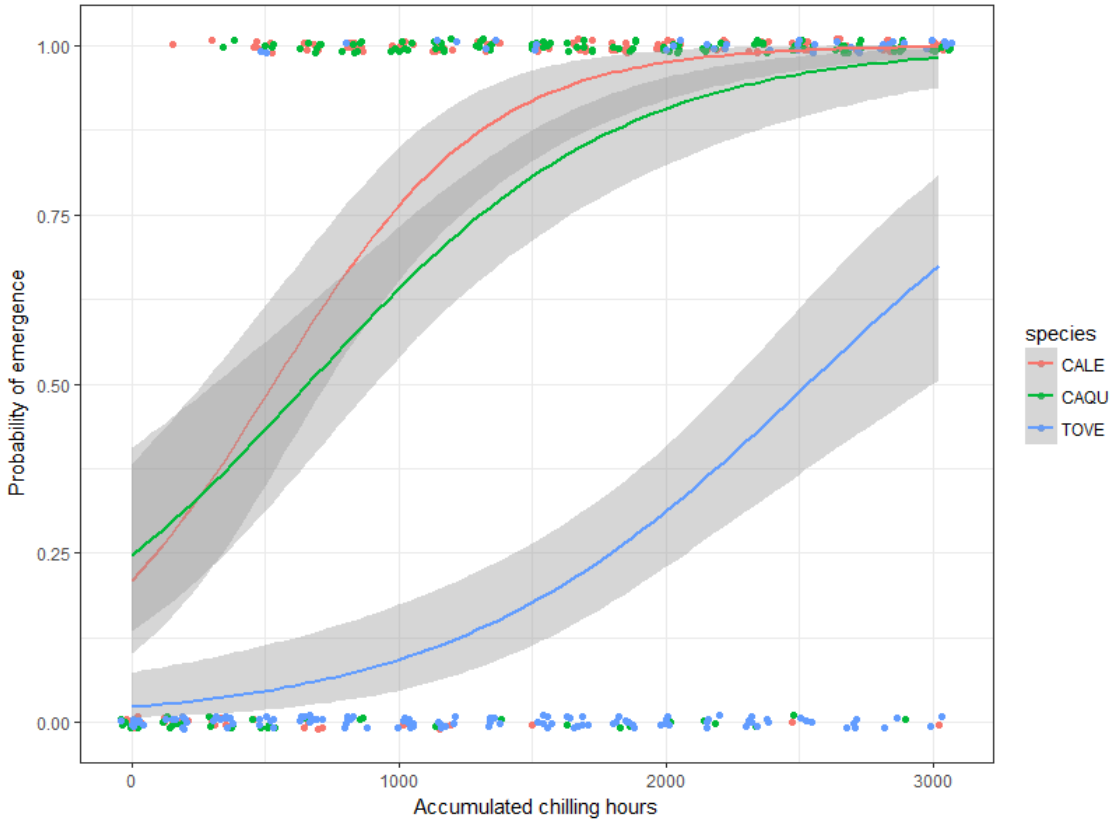

Figure 13: The probability of leaf emergence by day 158 of the experiment as a function of accumulated chilling hours with separate lines for each species. Camassia quamash *is presented in green,* C. leichtlinii *is presented in red, and* Toxicoscordion venenosum *is presented in blue. Points are individual plants, whose leaf either emerged (value=1) or did not emerge (value=0).*

To determine the effect of treatment on the days between the cessation of chilling and 50% leaf emergence, a Poisson GLM with species, accumulated chilling hours, and their interactions was tested. Drop-in-deviance tests showed that the interactions between species and accumulated chilling hours were not significant ($p=0.1396$). After dropping the interactions from the model, the coefficients for species and accumulated chilling hours were significant ($p<0.0001$ and $p<0.0001$). Using the full model, the mean number of days between the cessation of chilling and 50% leaf emergence for bulbs that received 1680 accumulated chilling hours (10 weeks of chilling) was estimated to be 34 [95% CI: 31, 37] for *C. leichtlinii*, 42 [95% CI: 39, 46] for *C. quamash*, and 117 [95% CI: 72, 191] for *T. venenosum*. Using the same model, the mean number of days between the cessation

of chilling and 50% leaf emergence for bulbs that received 3024 accumulated chilling hours (18 weeks of chilling) was estimated to be 6.5 [95% CI: 5.1, 8.2] for *C. leichtlinii*, 9.6 [95% CI: 7.7, 11.9] for *C. quamash*, and 14.9 [95% CI: 10.8, 20.5] for *T. venenosum*. The days between the cessation of chilling and 50% leaf emergence as a function of accumulated chilling hours for each species is presented in Figure 14 and was plotted using the full model. The fitted equations for each species are also presented (Equation 10, Equation 11, Equation 12).

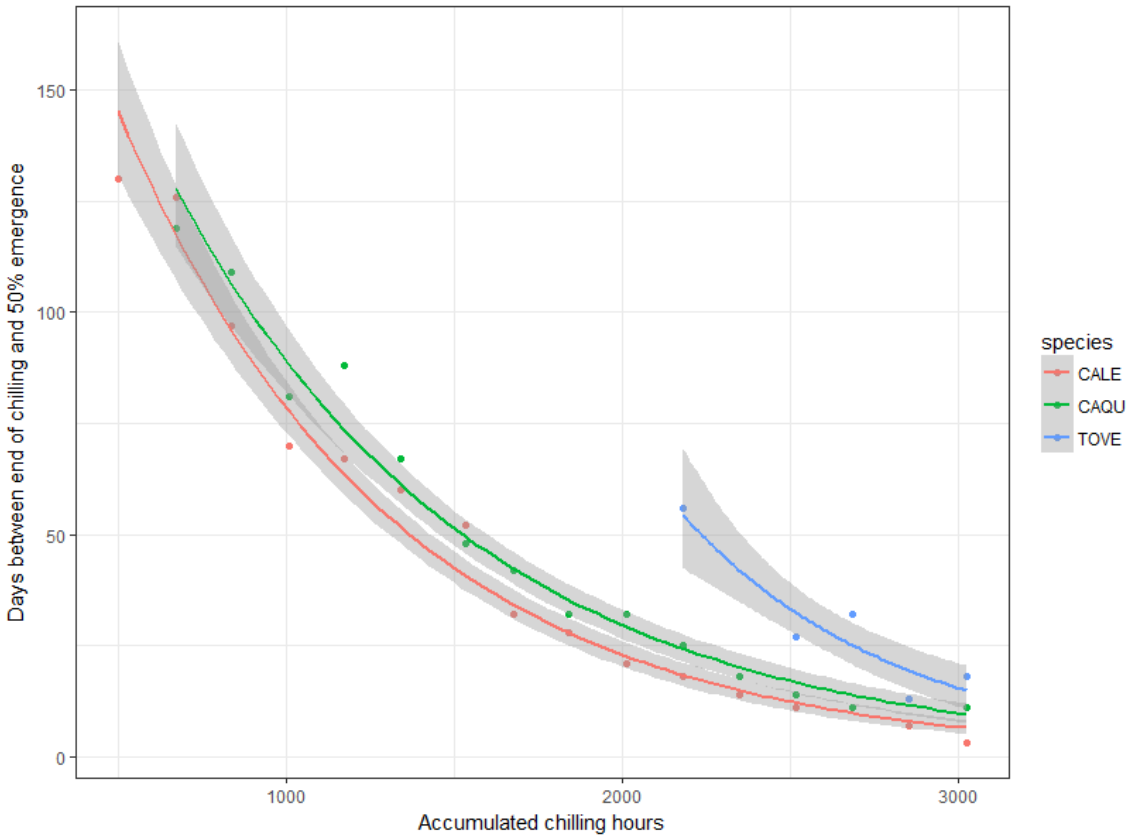

Figure 14: Accumulated chilling hours affects the number of days between the cessation of chilling and 50% leaf emergence for all three species (pseudo R^2 = 0.977). Camassia quamash *is presented in green,* C. leichtlinii *is presented in red, and* Toxicoscordion venenosum *is presented in blue.*

$$\textit{C. leichtlinii}: \ln\left(\widehat{days}\right) = 5.589 - 0.001228 * (ACH) \quad (10)$$

$$\textit{C. quamash}: \ln\left(\widehat{days}\right) = 5.587 - 0.001099 * (ACH) \quad (11)$$

$$\textit{T. venenosum}: \ln\left(\widehat{days}\right) = 7.34 - 0.00153 * (ACH) \quad (12)$$

The full model for the relationship between accumulated chilling hours and the number of days between the start of chilling and 50% leaf emergence is presented graphically in Figure 15, with separate lines for each species. The vertices for the species were estimated to be at 1921 [95% CI: 1791, 2001] accumulated chilling hours for *C. leichtlinii* and 1955 [95% CI: 1871, 2038] for *C. quamash*. Unfortunately, the bootstrapping technique could not determine the confidence interval for the vertex for *T. venenosum*.

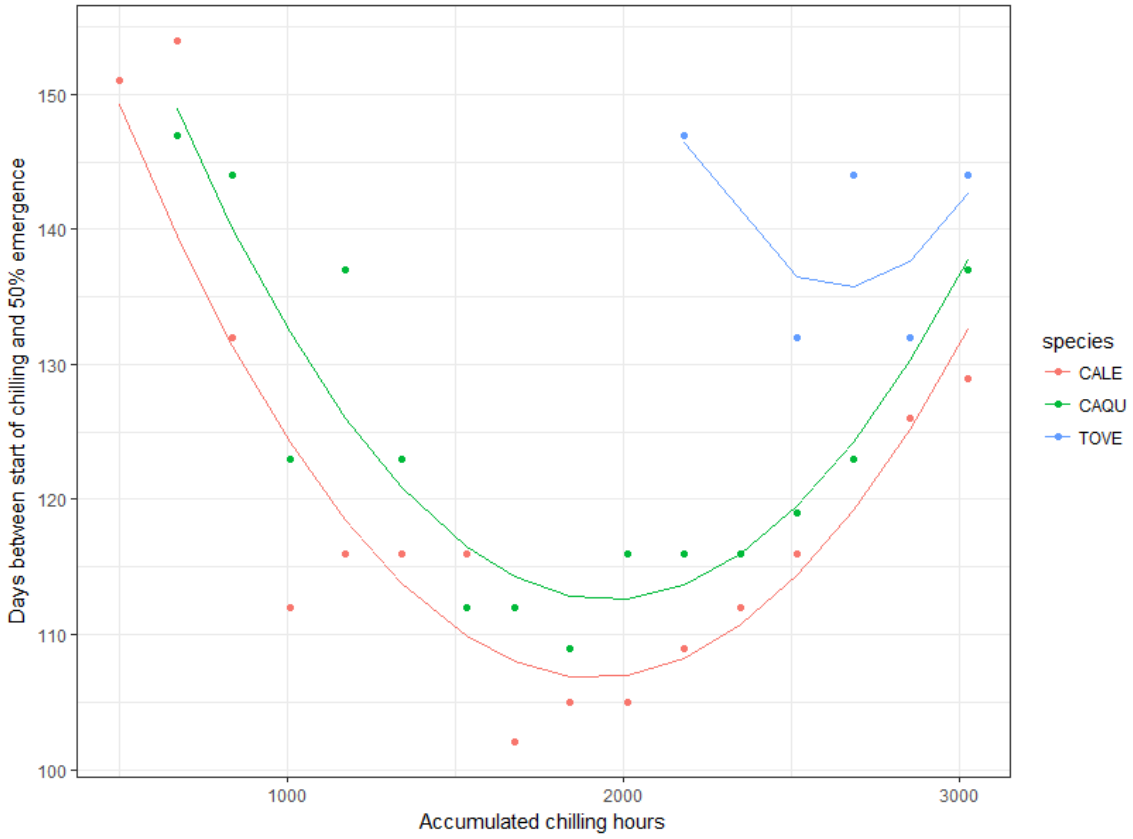

Figure 15: Accumulated chilling hours affects the number of days between the start of chilling and 50% leaf emergence for all three species. Camassia quamash *is presented in green,* Camassia leichtlinii *is presented in red, and* Toxicoscordion venenosum *is presented in blue.*

To determine whether accumulated chilling hours affected leaf growth rates, a generalized least squares model was fit with accumulated chilling hours, species, and their interactions as explanatory variables and the difference between leaf length at the second and first measurement for individual plants as the response variable. Including variance covariates for the different species improved the model ($p=0.0359$). Likelihood ratio tests showed that the interactions between species and accumulated chilling hours were not significant. However, after dropping the interactions, species and accumulated chilling hours were significant ($L=75.72$, $df=2$, $p<0.0001$ and $L=150.92$, $df=1$, $p<0.0001$ respectively). Estimated marginal means for the species were all significantly different. At any amount of accumulated chilling, the estimated marginal mean for leaf growth in

the first week for *C. quamash* was 8.7 (s.e. 1.7) mm greater than *C. leichtlinii* and 22.6 (s.e. 2.1) mm greater than *T. venenosum* ($p<0.0001$ for both), while *C. leichtlinii* was 14.0 (s.e. 2.1) mm greater than *T. venenosum* ($p<0.0001$). Increasing the amount of chilling by 168 hours (1 week of chilling) increased leaf growth in the first week by 2.5 mm [95% CI: 2.1, 2.8]. The full model is plotted in Figure 16.

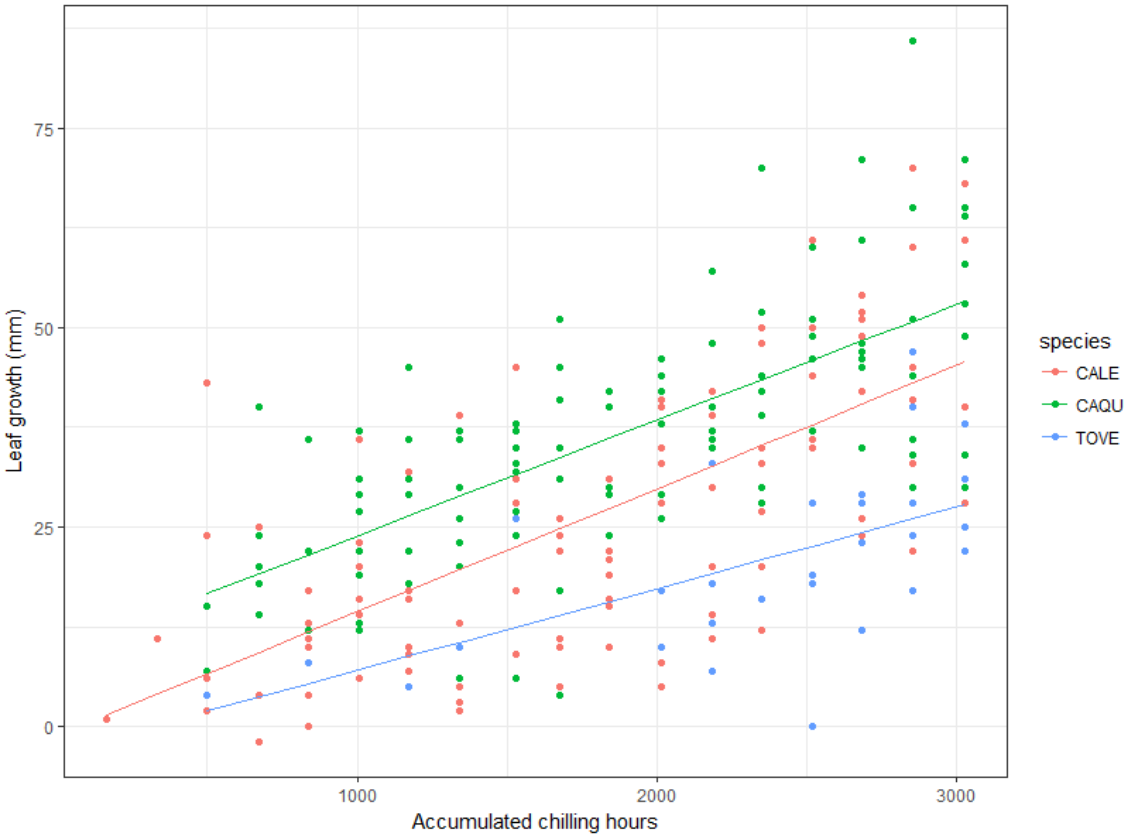

Figure 16: Accumulated chilling hours affects the total leaf growth (mm) between a plant's first and second measurement. Camassia quamash *is presented in green,* C. leichtlinii *is presented in red, and* Toxicoscordion venenosum *is presented in blue.*

Finally, extra sum of squares F-tests showed that there was no effect of accumulated chilling hours or species on leaf length at leaf maturity.

Heat

Including an exponential of the variance covariate for weeks of heat treatment improved the models for mean mother bulb DW at the cessation of chilling, mean daughter bulb DW at the cessation of chilling, and mean average final bulb DW ($p=0.0134$, $p=0.0327$, and $p=0.0443$ respectively).

Mother bulb DW at the cessation of chilling was affected by weeks of heat treatment, and with each additional week of heat treatment mean mother bulb DW decreased by 6.3 mg ($t = -2.541$, $df=20$, $p=0.0194$; Figure 17A). Daughter bulb DW at the cessation of chilling was also affected by treatment, and with each additional week of heat treatment mean daughter bulb DW increased by 2.7 mg ($t=4.837$, $df=20$, $p=0.0001$; Figure 17B). Log of the root DW +1 at the cessation of chilling was also affected by heat treatment and increased as weeks of heat treatment increased ($t=2.117$, $df=20$, $p=0.047$; Figure 17C). The effect of weeks of heat treatment on total DW at the cessation of chilling was not significant ($t=-0.755$, $df=20$, $p=0.4589$; Figure 17D), but for every week of heat treatment the model predicted that mean total DW would decrease by 2.4 mg.

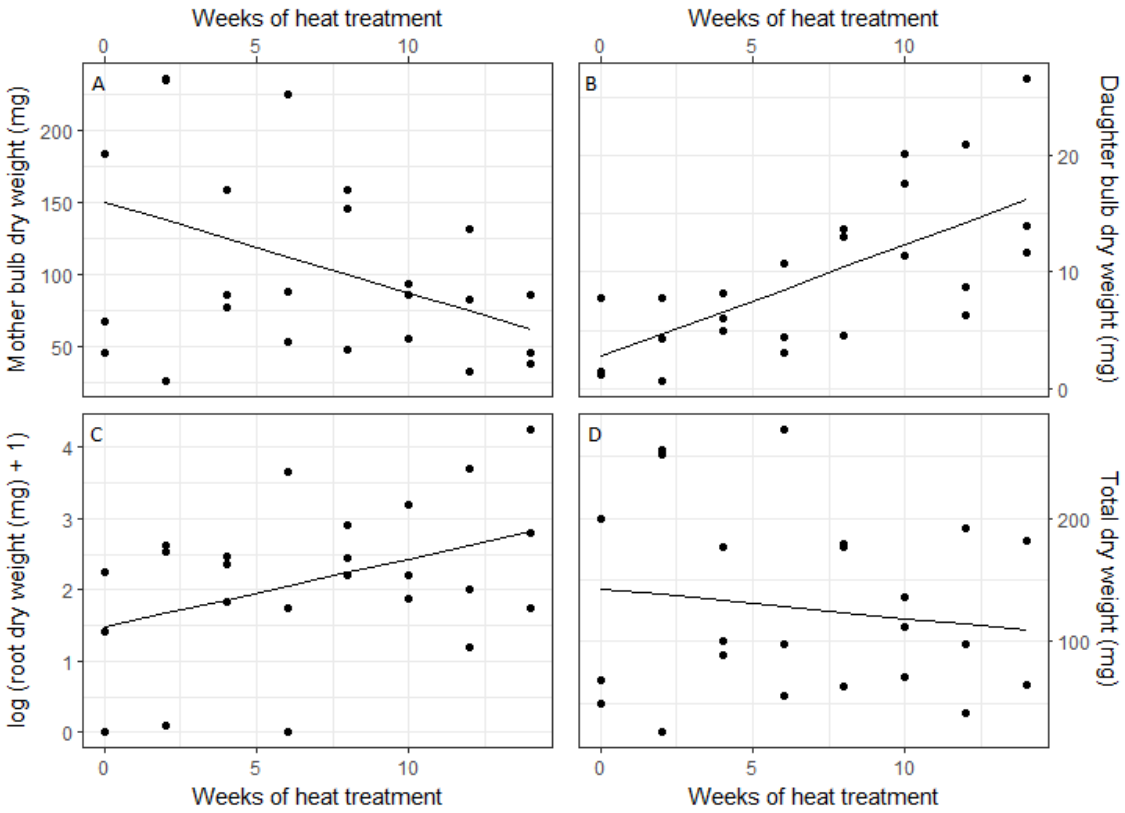

Figure 17: The relationship between weeks of heat treatment and the DW of the organs at the cessation of chilling for Camassia leichtlinii. *There was a significant effect of weeks of heat treatment on (A) mother bulb DW, (B) daughter bulb DW, and (C) log of the root DW +1 at the cessation of chilling. The effect of treatment on total DW at the cessation of chilling (D) was not significant but showed a negative trend.*

There was a significant effect of weeks of heat treatment on the number of days between the end of chilling and 50% leaf emergence ($F_{1, 22} = 87.843$, $p<0.0001$; Figure 18A), 50% leaf emergence and 50% leaf maturity ($F_{1, 22} = 25.316$, $p<0.0001$; Figure 18B), and 50% leaf emergence and 50% leaf senescence ($F_{1, 22} = 7.0212$, $p=0.01463$; Figure 18C). Weeks of heat treatment did not have a significant effect on the number of days between 50% leaf maturity and 50% leaf senescence ($F_{1, 22} = 1.2001$, $p=0.2852$; Figure 18D).

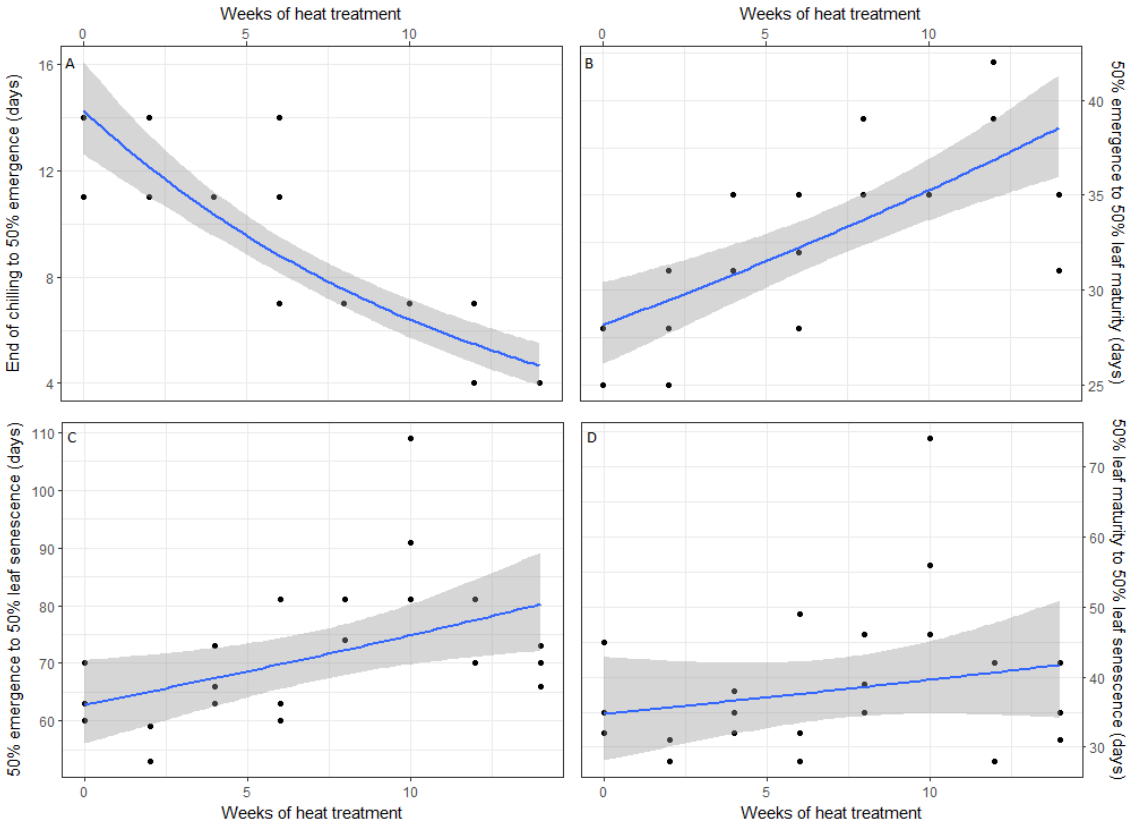

Figure 18: The relationship between weeks of heat treatment and the rates of leaf emergence and growth for Camassia leichtlinii. *Weeks of heat treatment affected (A) days between the end of chilling and 50% leaf emergence, (B) days between 50% leaf emergence and 50% leaf maturity, and (C) days between 50% leaf emergence and 50% leaf senescence. (D) Weeks of heat treatment did not affect the number of days between 50% leaf maturity and 50% leaf senescence.*

A likelihood ratio test suggested that a polynomial regression for the relationship between weeks of heat treatment and mean average leaf length at leaf maturity did not significantly improve the model over a linear regression (L=2.609, df=1, p=0.1063). For the linear model, average leaf length at leaf maturity was affected by weeks of heat treatment, and for each additional week of heat treatment, the mean average leaf length at leaf maturity increased by 7.6 mm (t=8.9567, df=20, p<0.0001; Figure 19).

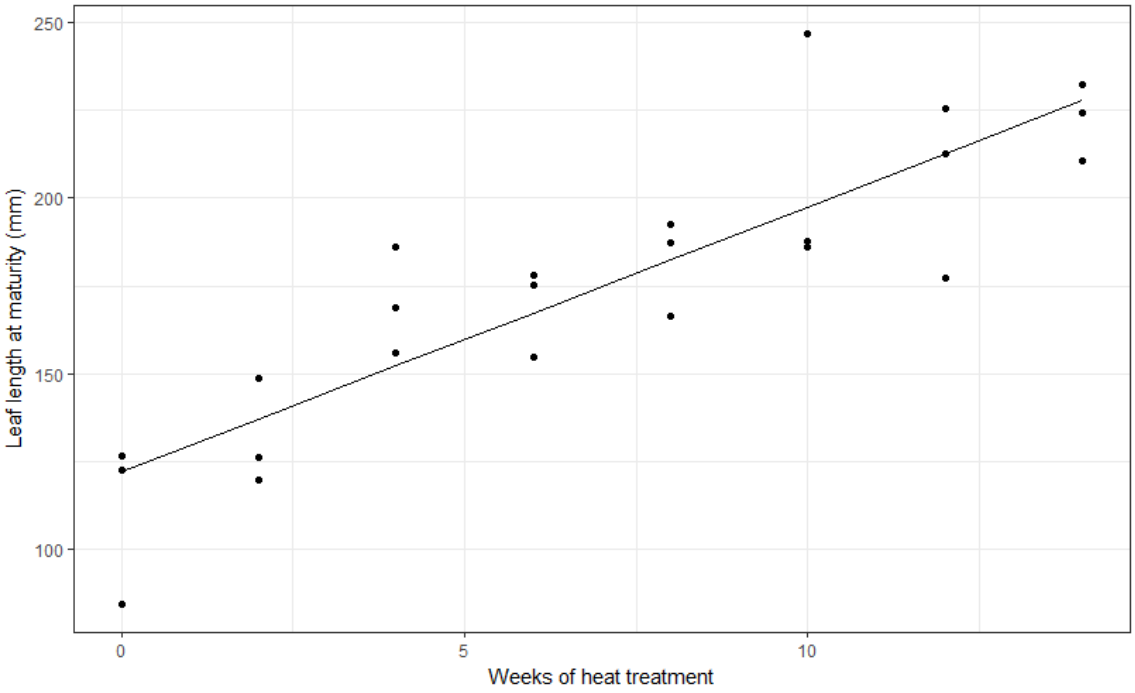

Figure 19: Average leaf length at leaf maturity was affected by weeks of heat treatment for Camassia leichtlinii.

Average leaf growth in the first week increased as the weeks of heat treatment increased. For each additional week of heat treatment, the regression showed that mean average leaf growth in the first week increased by 1.3 mm (t=4.636, df=20, p=0.0002).

For the final samplings, the relationships between weeks of heat treatment and both mean average final bulb DW and mean total final bulb DW were modeled using polynomial regression. Likelihood ratio tests showed that there was a significant effect of weeks of heat treatment on average final bulb DW (L=6.426, df=2, p=0.0402; Figure 20A). However, no relationship was detected between heat treatment and total final bulb DW (L=3.334, df=2, p=0.1889; Figure 20B). It was apparent from the results that only two experimental units had an average final bulb DW that was greater than their combined mother and daughter bulb DWs at the cessation of chilling, one from treatment 0 and one from treatment 2.

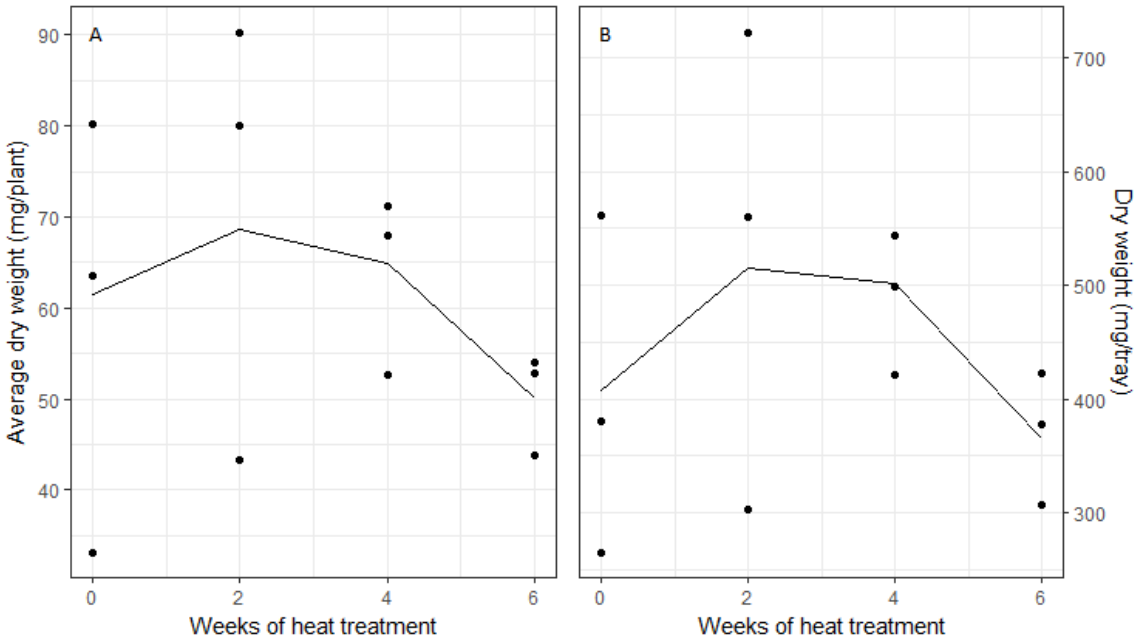

Figure 20: There may be a relationship between weeks of heat treatment and final bulb DW after leaf senescence. (A) This relationship was significant if the response variable tested was average final bulb DW. (B) This relationship was not significant if the response variable tested was total final bulb DW.

Discussion

Most bulb species respond to seasonal thermoperiodicity (Le Nard and De Hertogh, 1993a) and this appears to hold true for *C. leichtlinii*, *C. quamash*, and *T. venenosum.* Specifically, these species appear to have a chilling requirement, which needs to be met for timely leaf emergence. This is evidenced by the results: increasing chilling hour accumulation increased the likelihood of leaf emergence, decreased the number of days between the cessation of chilling and 50% leaf emergence, and increased leaf growth rates for all three species.

The interactions between the species and accumulated chilling hours were never significant, regardless of the response variable tested, suggesting that the species all responded to chilling in similar ways. However, for a given amount of chilling, the species showed clear differences. *T. venenosum* appeared to require more chilling prior to leaf emergence and emerged more slowly than *C. leichtlinii* and *C. quamash*. *Camassia*

leichtlinii and *C. quamash* were more similar, however *C. leichtlinii* emerged more quickly than *C. quamash* at 1680 accumulated chilling hours. Additionally, *C. quamash* leaves grew faster than *C. leichtlinii* and *T. venenosum* leaves regardless of the amount of chilling applied. Thus, although the species all respond to seasonal thermoperiodicity, expression of the response at a given level of chilling varies among species.

The differences between the species in their response to the same amount of chilling could be an effect of provenance, because seed collection for each species was restricted to a single location. Previous studies using other species have shown that seeds sourced from different parts of a species range will differ in their response to chilling (e.g. Wenny *et al.*, 2002). The seeds from *C. leichtlinii* were the southernmost in origin, and so may have evolved require less chilling, while the seeds from *T. venenosum* were the northernmost in origin, and so may require more. However, the ranges of these three species overlap across these locations, and thus it is possible that *C. leichtlinii* from latitude 46.6^{o} N might respond to chilling in the same way as *C. quamash* from that same latitude. Future studies should investigate the effect of chilling hour accumulation across the ranges of these species, to broaden the scope of inference.

Chilling optima have previously been defined as the point at which ten additional days of chilling does not correspond with a one-day increase in the number of days to budbreak (Worrall and Mergen, 1967), an admittedly arbitrary definition. In the interest of minimizing the length of the winter rest period, the chilling optimum was redefined for the purposes of this study as the number of accumulated chilling hours that minimizes the time between the start of chilling and leaf emergence, so long as adverse effects on the plant are avoided. Under this definition, the chilling optimum for leaf emergence for *C. leichtlinii* chilled at 4.6^{o}C appears to be 1921 hours (~80 days), while the chilling optimum for *C. quamash* appears to be 1955 hours (~81 days). Plugging these chilling optima into the fitted equations from the Poisson GLM suggests that with this amount of chilling, the number of days between the cessation of chilling and 50% leaf emergence should be 25 [95% CI: 23, 28] days for *C. leichtlinii*, and 31 [95% CI: 28, 35] days for *C. quamash*. These potential chilling optima are not that different from what is

recommended for forcing *L. philadelphicum* (9 weeks at 2-5ºC) or tulips (9 weeks at 5ºC) to achieve two growing seasons in a single year (May, 2007; Fortanier, 1970).

Unfortunately, because the experiment did not follow the bulbs until leaf senescence, there is uncertainty about whether too few or too many accumulated chilling hours may result in adverse effects on plant growth and development. For example, Risser and Cottam (1967) reported that for bulbs of *Erythronium albidum* Nutt. and *Dicentra cucullaria* (L.) Bernh., the length of time that their leaves remained green decreased if they received excessive chilling (chilling for 180 days), however they did not report whether this affected final bulb DW. They also found that different amounts of chilling resulted in different final heights in *Erythronium albidum* and *E. americanum* Ker Gawl. This experiment did not follow all treatments to leaf maturity, so it is possible that leaf length at leaf maturity may be different among the treatments. However, the trend in the data suggests that the amount of chilling does not impact the leaf length at leaf maturity for *C. leichtlinii* and *C. quamash*.

A heat treatment prior to chilling is often an essential part of a bulb-forcing program. This is because during the summer rest period some spring flowering bulb species undergo flower and vegetative bud differentiation, and/or root initiation (Le Nard and De Hertogh, 1993a). It appears that in the case of immature (i.e. too small to flower) *C. leichtlinii* bulbs, daughter bulb and root development occur during the summer rest period. Summer daughter bulb development is demonstrated by the relationship between weeks of heat treatment and both leaf length at leaf maturity and daughter bulb DW at the cessation of chilling. Summer root development is demonstrated by the relationship between weeks of heat treatment and root DW at the cessation of chilling. Thus, a heat treatment prior to chilling appears to be necessary for the growth and development of *C. leichtlinii*.

The effect of the heat treatment on the daughter bulb's development may inform our understanding of the factors that control bulb growth and leaf senescence. As the daughter bulb develops during the summer rest period, it is likely that the plant's total potential C assimilation for the following growing season increases. This would be the

result of the plant producing a longer leaf, which can assimilate more C, and an increasing number of cells in the daughter bulb, where assimilated C can be stored. Support for this can be found in the literature. Fortanier (1970) noted that tulips that were forced to grow at a 6-month cycle were 10-20% smaller than bulbs forced to grow on an 8-month cycle. Perhaps because chilling was applied earlier in the 6-month cycle, the leaves and daughter bulbs could not assimilate as much C. This suggests a tradeoff, where decreasing the total length of the summer rest period may result in decreased growth during the following growing season. Thus, future analysis should investigate the annual growth for bulbs grown under different bulb-forcing programs, to examine this tradeoff and determine the optimum cycle (i.e. the cycle that maximizes annual growth).

In this experiment however, increasing weeks of heat treatment did not result in larger bulbs, at least not between 0 and 6 weeks of heat treatment. Indeed, the trend in mean average final bulb DW indicated that the bulbs were potentially smaller with increasing heat treatment. Additionally, while increasing the duration of the heat treatment increased the number of days between 50% leaf emergence and 50% leaf senescence, it did not increase the number of days between 50% leaf maturity and 50% leaf senescence. However, these results may be due to an interaction between the heat treatment and the low irradiance in the grow room. It is possible that irradiance was too low for proper growth, and thus leaf senescence was initiated by unfavorable conditions. This would also explain why the average final bulb DWs for each experimental unit were frequently lower than the sum of the DWs of the mother and daughter bulb at the cessation of chilling for the same experimental unit.

If indeed the final bulb DW is decreasing with increasing heat treatment, this may demonstrate another potential tradeoff. This time the tradeoff is between a well-developed daughter bulb and the remaining reserves in the mother bulb. Because the heat treatment results in a lower mother bulb DW, and the mother bulb reserves could potentially be redirected to storage in the daughter bulb in the event of adverse conditions, daughter bulb development may come with potential risks. In the case of this experiment, the adverse condition was very low irradiance.

Finally, the relationship between leaf length at leaf maturity and the duration of the heat treatment appears to be linear, at least up to 14 weeks. Often, bulb-forcing programs try to minimize imposed rest (e.g. Fortainer, 1970), but in this case, there was no sign of imposed rest, and indeed the relationship did not appear to approach an asymptote. This makes it difficult to determine the optimum length of heat treatment to apply to bulbs before chilling. The linearity of the response may be evidenced in the literature. In tulips it has been found that when supplied with enough heat, even smaller bulbs that would not normally flower will initiate flowers (Le Nard, 1977). Perhaps there is no predetermined stopping point for daughter bulb development, and indeed they will continue to develop up until chilling is applied. This would not contradict with the determinate growth of spring flowering bulbs, as the daughter bulb growth would still be determinate in the spring.

Thus, the heat treatment experiment provides evidence that the summer rest period is anything but restful for *C. leichtlinii*. As weeks of heat treatment increase, daughter bulb and root DW measured at the cessation of chilling also increase, and this potentially results in a longer leaf at leaf maturity. However, whether this translates to a larger bulb at the end of the growing season has yet to be determined. Unfortunately, the optimum length of heat treatment for maximizing *C. leichtlinii's* annual growth remains undetermined.

Chapter 4: Conclusions

Fertilizer Practices

Experiments 1 and 2 showed that *C. leichtlinii* takes up N during its growing season following a cubic function. Nitrogen appears to be allocated to the leaves and the roots up until leaf maturity, after which it is allocated to the daughter bulb. However, newly acquired N does appear in the leaves throughout the growing season, which may be where it is assimilated prior to being allocated to the daughter bulb. *Camassia leichtlinii* also appears to be a luxury consumer of N, taking up large quantities of N even though this results in only a modest increase in its growth rate in the short term. It is possible that this luxury consumed N is stored for later use. Nitrogen uptake was not observed during the winter rest period, though this may be because no N was supplied.

Based on the results of these experiments, I have come to believe that the fertilization program for *Camassia* spp. can be improved in the following ways. First, growers of *Camassia* spp. (and perhaps other spring flowering bulb species) should avoid controlled-release fertilizers. Controlled-release fertilizers are frequently used in native plant nurseries to minimize labor; however, the release rates of controlled-release fertilizers are temperature dependent, with increased release rates under high temperatures (Lamont *et al.*, 1987). Our results showed that *C. leichtlinii,* which emerges in late winter or early spring, allocates soil N to its leaves from leaf emergence until leaf maturity. Thus, a mismatch between N allocation to the leaves and the release of N by controlled-release fertilizers is highly probable.

Instead, growers might benefit from fertilizing *Camassia* spp. following a Gaussian function. This program would be like exponential and modified exponential fertilization, but the change in the dose size would follow a Gaussian function rather than an exponential function. The program should aim to fertilize at an increasing rate up until leaf maturity, a constant rate after leaf maturity, and then a decreasing rate following the start of leaf senescence. This would concur with the results from Experiments 1 and 2, where it was shown that the N uptake of *C. leichtlinii* may be exponential up until leaf

maturity, constant after reaching leaf maturity, and decreasing as the leaves start to senesce. A program of a similar nature may be achieved by implementing the conifer fertilization programs frequently used in forest nurseries, where different nutrient ratios in “Starter”, “Grower”, and “Hardener” fertilizers match seedling growth phases (Driessche, 1990).

Once a fertilization program that accounts for increasing N uptake up to leaf maturity, constant N uptake after leaf maturity, and decreasing N uptake as the leaves senesce is developed, it could be used in future research to test different amounts of fertilizer application, to determine the rates that maximize the annual growth rates of *Camassia* spp. This would be similar to the optimum relative addition rate in exponential fertilization. However, it should be noted that the results presented in Experiment 2 suggest a large capacity on the part of *C. leichtlinii* to luxury consume N, and although this N may have little effect during the growing season in which it is taken up, it may have a significant effect in future growing seasons. Thus, the determination of the optimum rate at which to apply N may require multiple years of inquiry.

Temperature Manipulation

Camassia leichtlinii, *C. quamash*, and *T. venenosum* all appear to respond to chilling following leaf senescence; increasing the number of accumulated chilling hours applied results in an increasing likelihood of leaf emergence, fewer days between the cessation of chilling and 50% leaf emergence, and faster growth rates after leaf emergence. In addition, *C. leichtlinii* appears to respond to a heat treatment prior to chilling and increasing the number of weeks of heat treatment results in a more developed daughter bulb and root system, a longer leaf, fewer days between the cessation of chilling and leaf emergence, faster growth rates after leaf emergence, and possibly a longer period between leaf emergence and leaf senescence.

Based on these results, I would make the following recommendations to growers. First, storage conditions after *Camassia* spp. are harvested appear to be critical to bulb growth and development. If bulbs are harvested in the summer, they should be stored in a

warm ventilated room at between 17 and 20ºC (Le Nard and De Hertogh, 1993b). If the bulbs are instead placed into refrigeration after being harvested, this will undoubtedly affect the health of the crop, possibly for many years.

If readers of this document seek to use bulb-forcing techniques to force *Camassia* spp. into multiple growing seasons in a single year, it appears that the chilling optima for *C. leichtlinii* is 80 days and *C. quamash* 81 days at 4.6ºC. However, please be skeptical. These recommendations are based on the results of a single experiment, and the chilling optima might be different for bulbs of a different age, provenance, or if chilled at a different temperature. Furthermore, I cannot recommend a particular point at which to end the heat treatment, and indeed there is likely a tradeoff between weeks of heat treatment and growth in the following growing season. Also, although temperature manipulation may seem inaccessible to many growers, one course of action may be to germinate seeds in June, allow them to grow into the fall, be chilled during the winter, and then reemerge in the spring. I cannot vouch for the efficacy of this proposed program, but it is an example of how these principles might be applied to create two growing seasons in a single year on a limited budget.

Future researchers should investigate additional heat treatment temperatures. While it was shown that tulip flower bud differentiation occurs quickest if bulbs are held between 17 and 20ºC (Hartsema, 1961) more sophisticated heat treatments have since been developed. For example, if tulip bulbs are exposed to high temperatures for a short duration (~1-3 weeks at 30-35ºC) prior to storage at 20ºC, flower bud differentiation and rooting occur even more rapidly (Le Nard, 1972). Thus, future studies on *Camassia* spp. may achieve faster daughter bulb development if a short period of high temperature is provided prior to the heat treatment. Also, because increasing weeks of heat treatment and weeks of chilling both reduce the number of days between the cessation of chilling and leaf emergence, there may be an interaction between the heat treatment and chilling in the days from leaf senescence to reemergence. Thus, future research into the seasonal thermoperiodicity of *Camassia* spp., especially in the interest of bulb-forcing, should investigate the potential for an interaction between the heat treatment and chilling, and

test whether the development of the roots and the daughter bulbs is accelerated by providing a week of 30°C treatment. This could potentially be a three-factorial experiment (+/- 1 week at 30°C, weeks of heat treatment, and number of accumulated chilling hours).

In addition, future studies into the responses of bulbs to temperature manipulation should avoid pseudoreplication by using multiple controlled environment room pairs or by repeating the experiment. They should use plant materials from multiple populations. They should monitor temperature, irradiance, and CO_2 in all controlled environment rooms for the duration of the experiment. They should use lights with a high irradiance, such as high intensity discharge grow lights or LED grow lights, to ensure growth under more "natural" conditions (Apostol *et al.*, 2015). Finally, they should replace lamps when their irradiance begins to decay.

Using bulb-forcing techniques might result in greater annual growth, which could prove to be a valuable tool in meeting restoration targets. After identifying the optimum bulb-forcing program, future analysis should determine what the tradeoff is between the increase in annual yields and the cost of implementing bulb-forcing techniques in nursery production. Finally, it is important to consider how bulb-forcing techniques may lead to artificial selection. If certain genotypes perform better under a bulb-forcing program, growers may inadvertently select for bulbs with weaker dormancy (Dunwiddie and Delvin, 2006). Despite risks of this nature, recognizing the important role *Camassia* spp. should have in Pacific Northwest prairie restoration requires growers and researchers to collaborate in identifying economically and ecologically viable pathways to deliver quality plant materials to future projects.

Bibliography

Apostol, K. G., Dumroese, R. K., Pinto, J. R., & Davis, A. S. (2015). Response of conifer species from three latitudinal populations to light spectra generated by light-emitting diodes and high-pressure sodium lamps. *Canadian Journal of Forest Research*, 45(12), 1711-1719.

Archibald, J. K., Kephart, S. R., Theiss, K. E., Petrosky, A. L., & Culley, T. M. (2015). Multilocus phylogenetic inference in subfamily Chlorogaloideae and related genera of Agavaceae–informing questions in taxonomy at multiple ranks. *Molecular Phylogenetics and Evolution*, 84, 266–283.

Auguie, B. (2017). gridExtra: Miscellaneous functions for "grid" graphics (Version 2.0.0).

Baba, A. (1967). Mineral nutrition of tulip flowering phase (I). *Shokubutsu Seiri*, 6, 47–55.

Baba, A., & Ikarashi, T. (1968). Mineral nutrition of tulip flowering phase (II). *Shokubutsu Seiri*, 7, 13–20.

Baker, J. P., Hulse, D. W., Gregory, S. V., White, D., Sickle, J. V., Berger, P. A., … Schumaker, N. H. (n.d.). Alternative Futures for the Willamette River Basin, Oregon. *Ecological Applications*, 14(2), 313–324.

Beckwith, B. R. (2004). *The Queen Root of this Clime: Ethnoecological Investigations of Blue Camas (*Camassia leichtlinii *(Baker) Wats.,* C. quamash *(Pursh) Greene; Liliaceae) and its Landscapes on Southern Vancouver Island, British Columbia* (PhD dissertation). University of Victoria, Victoria, BC.

Bloom, A. J., Chapin III, F. S., & Mooney, H. A. (1985). Resource limitation in plants - an economic analogy. *Annual Review of Ecology and Systematics*, 16(1), 363–392.

Burgess, D. (1991). Western hemlock and Douglas-fir seedling development with exponential rates of nutrient addition. *Forest Science*, 37(1), 54–67.

Chapin III, F. S. (1980). The mineral nutrition of wild plants. *Annual Review of Ecology and Systematics*, 11(1), 233–260.

Chapin III, F. S., Matson, P. A., & Vitousek, P. (2011). *Principles of Terrestrial Ecosystem Ecology* (2nd ed.). New York, NY: Springer-Verlag.

Chapin III, F. S., & Van Cleve, K. (2000). Approaches to studying nutrient uptake, use and loss in plants. In *Plant Physiological Ecology* (pp. 185–207). New York, NY: Springer.

Chase, M. W., Reveal, J. L., & Fay, M. F. (2009). A subfamilial classification for the expanded asparagalean families Amaryllidaceae, Asparagaceae and Xanthorrhoeaceae. *Botanical Journal of the Linnean Society*, 161(2), 132–136.

Chen, J., Huang, Y., & Caldwell, R. D. (2001). Best management practices for minimizing nitrate leaching from container-grown nurseries. *The Scientific World Journal*, 1, 96–102.

Clark, C. M., & Tilman, D. (2008). Loss of plant species after chronic low-level nitrogen deposition to prairie grasslands. *Nature*, 451(7179), 712–715.

Craighead, J. J., Craighead, F. C., Craighead, J., & Davis, R. J. (1998). *A Field Guide to Rocky Mountain Wildflowers: Northern Arizona and New Mexico to British Columbia* (Vol. 14). Boston, MA: Houghton Mifflin Harcourt.

De Hertogh, A. A. (1974). Principles for forcing tulips, hyacinths, daffodils, Easter lilies and Dutch irises. *Scientia Horticulturae*, 2(4), 313–355.

De Hertogh, A. A., & Le Nard, M. (1993). Botanical aspects of flower bulbs. In *The Physiology of Flower Bulbs* (pp. 7–20). Amsterdam, The Netherlands: Elsevier Science Publishers.

Deur, D. E. (2000). *A Domesticated Landscape: Native American Plant Cultivation on the Northwest Coast of North America*. (PhD dissertation). Louisiana State University, Baton Rouge, LA.

Driessche, R. van den. (1990). *Conifer Seedling Mineral Nutrition*. Boca Raton, FL: CRC Press.

Dumroese, R. K. (2003). Growth of *Juniperus* and *Potentilla* using liquid exponential and controlled-release fertilizers. *Hortscience*, 38(7), 1378–1380.

Dumroese, R. K., Page-Dumroese, D. S., & Wenny, D. L. (1991). Managing pesticides and fertilizer leaching and runoff in a container nursery. In *Proceedings, Intermountain Forest Nursery Association* (pp. 12–16). Park City, Utah.

Dumroese, R. K., Pinto, J. R., Jacobs, D. F., Davis, A. S., & Horiuchi, B. (2006). Subirrigation reduces water use, nitrogen loss, and moss growth in a container nursery. *Native Plants Journal*, 7(3), 253–261.

Dumroese, R. K., Pinto, J. R., & Montville, M. E. (2015). Using container weights to determine irrigation needs: a simple method. *Native Plants Journal*, 16(1), 67–71.

Dumroese, R. K., Wenny, D. L., & Page-Dumroese, D. S. (1995). Nursery waste water. The problem and possible remedies (USDA For. Serv. Gen. Tech. Rep. No. PNW-GTR-365) (pp. 89–97). Fort Collins, CO: US Forest Service.

Dunwiddie, P. W. (2002). Management and restoration of grasslands on Yellow Island, San Juan Islands, Washington, USA. In *Garry Oak Ecosystem Restoration: Progress and Prognosis. Proceedings of the Third Annual Meeting of the BC chapter of the Society for Ecological Restoration* (pp. 78–87).

Dunwiddie, P. W., & Bakker, J. D. (2011). The future of restoration and management of prairie-oak ecosystems in the Pacific Northwest. *Northwest Science*, 85(2), 83–92.

Dunwiddie, P. W., & Delvin, E. (2006). Inadvertent selection in the propagation of native plants A cautionary note. *Native Plants Journal*, 7(2), 121–124.

Elliot, R. (2008). *Faking Nature: The Ethics of Environmental Restoration*. New York, NY: Routledge.

Erisman, J. W., Galloway, J. N., Seitzinger, S., Bleeker, A., Dise, N. B., Petrescu, A. R., … de Vries, W. (2013). Consequences of human modification of the global nitrogen cycle. *Phil. Trans. R. Soc. B*, 368(1621), 1–9.

Esau, K. (1977). *Anatomy of Seed Plants* (2nd ed.). New York, NY: Wiley and Sons.

Fishbein, M., Kephart, S. R., Wilder, M., Halpin, K. M., & Datwyler, S. L. (2010). Phylogeny of *Camassia* (Agavaceae) inferred from plastid rpl16 intron and trnD-trnY-trnE-trnT intergenic spacer DNA sequences: implications for species delimitation. *Systematic Botany*, 35(1), 77–85.

Fortanier, E. J. (1970). Shortening the period from seed to a flowering bulb in tulip. In *I International Symposium on Flowerbulbs 23* (pp. 413–420).

Fortanier, E. J. (1973). Reviewing the length of the generation period and its shortening, particularly in tulips. *Scientia Horticulturae*, 1(1), 107–116.

Fox, J., & Weisberg, S. (2011). *An R Companion to Applied Regression* (2nd ed.). Thousand Oaks, CA: Sage Publications.

Fuchs, M. A. (2001). Towards a recovery strategy for Garry oak and associated ecosystems in Canada: ecological assessment and literature review (Technical Report No. GBEI/EC-00-030). Environment Canada, Pacific and Yukon Region.

Glover, B. J. (2007). *Understanding Flowers and Flowering: An Integrated Approach*. Oxford, UK: Oxford University Press.

Gould, F. W. (1942). A systematic treatment of the genus *Camassia* Lindl. *The American Midland Naturalist*, 28(3), 712–742.

Gritzner, J. H. (1994). Native-American camas production and trade in the Pacific Northwest and northern Rocky Mountains. *Journal of Cultural Geography*, 14(2), 33–50.

Gunther, E. (1973). *Ethnobotany of Western Washington: The Knowledge and Use of Indigenous Plants by Native Americans* (Rev., Vol. 10). Seattle, WA: University of Washington Press.

Halekoh, U., Højsgaard, S., & Yan, J. (2006). The R package geepack for generalized estimating equations. *Journal of Statistical Software*, 15(2), 1–11.

Hanks, G. R., & Rees, A. R. (1980). Growth substances of tulip: the activity of gibberellin-like substances in field-grown tulips from planting until flowering. *Zeitschrift Für Pflanzenphysiologie*, 98(3), 213–223.

Hartsema, A. M. (1961). Influence of temperatures on flower formation and flowering of bulbous and tuberous plants. In *Handbuch der Pflanzenphysiologie* (Vol. 16, pp. 123–167). Berlin, Germany: Springer-Verlag.

Henry, L., & Wickham, H. (2017). purrr: Functional programming tools. (Version 0.2.4).

Hobbs, R. J., & Norton, D. A. (1996). Towards a conceptual framework for restoration ecology. *Restoration Ecology*, 4(2), 93–110.

Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical Journal*, 50(3), 346–363.

Imo, M., & Timmer, V. R. (1992). Nitrogen uptake of mesquite seedlings at conventional and exponential fertilization schedules. *Soil Science Society of America Journal*, 56(3), 927–934.

Ingestad, T. (1977). Nitrogen and plant growth; maximum efficiency of nitrogen fertilizers. *Ambio*, 6(2), 146–151.

Ingestad, T. (1982). Relative addition rate and external concentration; driving variables used in plant nutrition research. *Plant, Cell & Environment*, 5(6), 443–453.

Ingestad, T. (1987). New concepts on soil fertility and plant nutrition as illustrated by research on forest trees and stands. *Geoderma*, 40(3–4), 237–252.

Ingestad, T., & Lund, A.-B. (1986). Theory and techniques for steady state mineral nutrition and growth of plants. *Scandinavian Journal of Forest Research*, 1(1–4), 439–453.

Jewell, J. E. (1978). *A Biosystematic Study of* Camassia cusickii *S. Watson and its Allies (Liliaceace)* (PhD dissertation). University of Idaho, Moscow, ID.

Jobbágy, E. G., & Jackson, R. B. (2000). The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecological Applications*, 10(2), 423–436.

Kawa, L., & De Hertogh, A. A. (1992). Root physiology of ornamental flowering bulbs. In *Horticultural Reviews* (Vol. 14, pp. 57–88). New York, NY: Wiley and Sons.

Knight, L., & Seevers, J. (1992). *Special Status Plants of the Medford District BLM*. Medford, OR: Bureau of Land Management - Medford District.

Kruckeberg, A. R. (1982). *Gardening with Native Plants of the Pacific Northwest: An Illustrated Guide*. Seattle, WA: University of Washington Press.

Kuhnlein, H. V., & Turner, N. J. (1991). *Traditional Plant Foods of Canadian Indigenous Peoples: Nutrition, Botany, and Use* (Vol. 8). Philadelphia, PA: Gordon and Breach.

Kumar, V., Abdul-Baki, A., Anderson, J. D., & Mattoo, A. K. (2005). Cover crop residues enhance growth, improve yield, and delay leaf senescence in greenhouse-grown tomatoes. *HortScience*, 40(5), 1307–1311.

Lamont, G. P., Worrall, R. J., & O'Connell, M. A. (1987). The effects of temperature and time on the solubility of resin-coated controlled-release fertilizers under laboratory and field conditions. *Scientia Horticulturae*, 32(3–4), 265–273.

Landis, T. D., & Dumroese, R. K. (2009). Using polymer-coated controlled-release fertilizers in the nursery and after outplanting. *Forest Nursery Notes*, 6, 5–12.

Langeslag, J. J. J. (1989). *Teelt en Gebruiksmogelijkheden van Bijgoedgewassen* (Tweede Druk). Lisse, Netherlands: Ministerie van Landbouw, Natuurbeheer en Visserij en Consulentschap Algemene Dienst Bloembollenteelt.

Lapointe, L. (2001). How phenology influences physiology in deciduous forest spring ephemerals. *Physiologia Plantarum*, 113(2), 151–157.

Le Nard, M. (1972). Incidence de séquences de hautes et basses températures sur la différenciation des bourgeons, l'enracinement et la bulbification chez la tulipe. *Ann Amelior Plantes*, 39–59.

Le Nard, M. (1975). Studies on the possibility of delaying flowering of tulip. In *II International Symposium on Flower Bulbs 47* (pp. 251–258).

Le Nard, M. (1977). La différenciation florale chez la tulipe et l'iris bulbeux; Relations entre floraison et bulbification. *Sélectionneur Fr*, 23, 33–40.

Le Nard, M. (1983). Physiology and storage of bulbs: concepts and nature of dormancy in bulbs. In *Post-Harvest Physiology and Crop Preservation* (pp. 191–230). Boston, MA: Springer.

Le Nard, M., & De Hertogh, A. A. (1993a). Bulb growth and development and flowering. In *The Physiology of Flower Bulbs* (pp. 29–43). Amsterdam, The Netherlands: Elsevier Science Publishers.

Le Nard, M., & De Hertogh, A. A. (1993b). General chapter on spring flowering bulbs. In *The Physiology of Flower Bulbs* (pp. 705–739). Amsterdam, The Netherlands: Elsevier Science Publishers.

Lenth, R. V. (2018). emmeans: Estimated marginal means, aka least-squares means (Version 1.1).

Lim, P. O., Kim, H. J., & Gil Nam, H. (2007). Leaf senescence. *Annu. Rev. Plant Biol.*, 58, 115–136.

Lim, P. O., Woo, H. R., & Nam, H. G. (2003). Molecular genetics of leaf senescence in *Arabidopsis*. *Trends in Plant Science*, 8(6), 272–278.

Luna, T., Evans, J., & Wicks, D. (2008). Propagation protocol for production of container *Camassia quamash* (Pursh) Greene plants (172 ml (10 in3) Containers) (Native Plant Network). Moscow, ID: University of Idaho, College of Natural Resources, Forest Research Nursery.

Macior, L. W. (1978). Pollination ecology of vernal angiosperms. *Oikos*, 30(3), 452–460.

Maclay, A. M. (1928). *Studies of the Life History of* Camassia quamash *(Pursh) Greene* (MSc Thesis). State College of Washington, Pullman, WA.

May, L. L. (2007). Forcing cycles speed growth and flowering in western red lily (*Lilium philadelphicum* L.). *Native Plants Journal*, 8(1), 11–18.

McCarthy, M. C., & Enquist, B. J. (n.d.). Consistency between an allometric approach and optimal partitioning theory in global patterns of plant biomass allocation. *Functional Ecology*, 21(4), 713–720.

McDaniel, P., & Falen, A. (2014). Analysis of soils at the National Park Service Weippe Prairie site (Natural Resource Technical Report No. NPS/NEPE/NRTR-2014/914). Fort Collins, CO: National Park Service.

Niedziela, C. E., Nelson, P. V., & Dickey, D. A. (2015). Growth, development, and mineral nutrient accumulation and distribution in tulip from planting through postanthesis shoot senescence. *International Journal of Agronomy*, 2015, 1–11.

Ohyama, T. (1991). Assimilation and transport of nitrogen in tulip (*Tulipa gesneriana*) as pursued by 15N. *Japan Agricultural Research Quarterly*, 25(2), 108–116.

Ohyama, T., Ikarashi, T., & Baba, A. (1985). Nitrogen accumulation in the roots of tulip plants (*Tulipa gesneriana*). *Soil Science and Plant Nutrition*, 31(4), 581–588.

Ohyama, T., Ikarashi, T., & Baba, A. (1988). Effect of cold storage treatment for forcing bulbs on the C and N metabolism of tulip plants. *Soil Science and Plant Nutrition*, 34(4), 519–533.

Ohyama, T., Ikarashi, T., Obata, A., & Baba, A. (1988). Role of nitrogen accumulated in tulip roots during winter season. *Soil Science and Plant Nutrition*, 34(3), 341–350.

Parachnowitsch, A. L., & Elle, E. (2005). Insect visitation to wildflowers in the endangered Garry oak, *Quercus garryana*, ecosystem of British Columbia. *The Canadian Field-Naturalist*, 119(2), 245–253.

Pfeifer-Meister, L., & Bridgham, S. D. (2007). Seasonal and spatial controls over nutrient cycling in a Pacific Northwest prairie. *Ecosystems*, 10(8), 1250–1260.

Pinheiro, J., Bates, D., Deb Roy, S., Sarkar, D., & Team, R. C. (2017). nlme: Linear and nonlinear mixed effects models (Version 3.1-131).

Pinto, J. R., Chandler, R. A., & Dumroese, R. K. (2008). Growth, nitrogen use efficiency, and leachate comparison of subirrigated and overhead irrigated pale purple coneflower seedlings. *HortScience*, 43(3), 897–901.

R Core Team. (2017). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

Ranker, T. A., & Hogan, T. (2002). *Camassia*. In *Flora of North America* (Vol. 26, pp. 303–307).

Ranker, T. A., & Schnabel, A. F. (1986). Allozymic and morphological evidence for a progenitor-derivative species pair in *Camassia* (Liliaceae). *Systematic Botany*, 11(3), 433–445.

Ray, V. F. (1933). *The Sanpoil and Nespelem: Salishan Peoples of Northeastern Washington* (Vol. 5). Seattle, WA: University of Washington Press.

Rees, A. (1972). *The Growth of Bulbs: Applied Aspects of the Physiology of Ornamental Bulbous Crop Plants* (Vol. 1). London, UK: Academic Press.

Ries, L., Debinski, D. M., & Wieland, M. L. (2001). Conservation value of roadside prairie restoration to butterfly communities. *Conservation Biology*, 15(2), 401–411.

Rimbach, A. (1929). Die Verbreitung der Wurzelverkurzung im Pflanzenreich. *Ber. Dtsch. Bot. Ges*, 47, 22–31.

Risser, P., & Cottam, G. (1967). Influence of temperature on the dormancy of some spring ephemerals. *Ecology*, 48(3), 500–503.

Roberfroid, M. (2005). *Inulin-type Fructans: Functional Food Ingredients*. Boca Raton, FL: CRC Press.

Russell, M. (2011). Dormancy and germination pre-treatments in Willamette Valley native plants. *Northwest Science*, 85(2), 389–402.

Sakai, A., & Yoshie, F. (1984). Freezing tolerance of ornamental bulbs and corms. *Journal of the Japanese Society for Horticultural Science*, 52(4), 445–449.

Saniewski, M., Kawa-Miszczak, L., Wegrzynowicz-Lesiak, E., & Okubo, H. (2000). Role of ABA, gibberellins and auxin in dormancy and dormancy release of tulip bulbs. In *Dormancy in Plants: from Whole Plant Behaviour to Cellular Control*. (pp. 227–245). New York, NY: CAB International publishing.

Schuller, R. (1997). Vegetation response to fall prescribed burning within *Festuca idahoensis*-dominated prairie, Mima Mounds Natural Area Preserve, Washington 1985-1992 (Ecology and Conservation of the South Puget Sound Prairie Landscape) (pp. 207–216). Seattle, WA: The Nature Conservancy of Washington.

Stevens, M., Darris, D. C., & Lambert, S. M. (2001). Ethnobotany, culture, management, and use of common camas. *Native Plants Journal*, 2(1), 47–53.

Sturtevant, W. C. (Ed.). (1978). *Handbook of North American Indians* (Vols. 1–17). Washington, DC: Smithsonian Institution.

Suttles, W. (2005). Coast Salish resource management: incipient agriculture? In *Keeping It Living: Traditions of Plant Use and Cultivation on the Northwest Coast of North America* (pp. 181–193). Seattle, WA: University of Washington Press.

Taft, O. W., & Haig, S. M. (2003). Historical wetlands in Oregon's Willamette Valley: implications for restoration of winter waterbird habitat. *Wetlands*, 23(1), 51–64.

Thoms, A. V. (1989). *The Northern Roots of Hunter-Gatherer Intensification: Camas of the Pacific Northwest* (PhD dissertation). Washington State University, Pullman, WA.

Timmer, V. R., & Aidelbaum, A. S. (1996). Manual for exponential nutrient loading of seedlings to improve outplanting performance on competitive forest sites (NODA/NFP technical report No. 25). Sault Ste. Marie, Ont.: Great Lakes Forestry Centre.

Timmer, V. R., & Armstrong, G. (1987). Growth and nutrition of containerized *Pinus resinosa* at exponentially increasing nutrient additions. *Canadian Journal of Forest Research*, 17(7), 644–647.

Timmer, V. R., Armstrong, G., & Miller, B. D. (1991). Steady-state nutrient preconditioning and early outplanting performance of containerized black spruce seedlings. *Canadian Journal of Forest Research*, 21(5), 585–594.

Turner, N. J., & Bell, M. A. (1971). The ethnobotany of the coast Salish Indians of Vancouver Island. *Economic Botany*, 25(1), 63–99.

Turner, N. J., & Kuhnlein, H. V. (1983). Camas (*Camassia* spp.) and riceroot (*Fritillaria* spp.): two liliaceous "root" foods of the Northwest Coast Indians. *Ecology of Food and Nutrition*, 13(4), 199–219.

Turner, N. J., & Peacock, S. (2005). Solving the perennial paradox: ethnobotanical evidence for plant resource management on the Northwest Coast. In *Keeping It Living: Traditions of Plant Use and Cultivation on the Northwest Coast of North America* (pp. 101–150). Seattle, WA: University of Washington Press.

Turney-High, H. H. (1937). *The Flathead Indians of Montana* (Vol. 48). Menasha, WI: American Anthropological Association.

Unkovich, M. J., Pate, J. S., McNeill, A., & Gibbs, J. (2001). *Stable Isotope Techniques in the Study of Biological Processes and Functioning of Ecosystems* (Vol. 40). Dordrecht, The Netherlands: Kluwer Academic Publishers.

Uyeda, J. C., & Kephart, S. R. (2006). Detecting species boundaries and hybridization in *Camassia quamash* and *C. leichtlinii* (Agavaceae) using allozymes. *Systematic Botany*, 31(4), 642–655.

Venables, W. N., & Ripley, B. D. (2002). *Modern Applied Statistics with S* (4th ed.). New York, NY: Springer.

Wenny, D. L., Swanson, D. J., & Dumroese, R. K. (2002). The chilling optimum of Idaho and Arizona ponderosa pine buds. *Western Journal of Applied Forestry*, 17(3), 117–121.

Wickham, H. (2009). *Ggplot2: Elegant Graphics for Data Analysis*. New York, NY: Springer.

Wickham, H., Francois, R., Henry, L., & Müller, K. (2017). dplyr: A grammar of data manipulation (Version 0.7.2).

Wilke, C. O. (2017). cowplot: Streamlined Plot Theme and Plot Annotations for "ggplot2" (Version 0.9.2).

Worrall, J., & Mergen, F. (1967). Environmental and genetic control of dormancy in *Picea abies*. *Physiologia Plantarum*, 20(3), 733–745.

Yan, J., & Fine, J. (2004). Estimating equations for association structures. *Statistics in Medicine*, 23(6), 859–874.

Yan, Jun. (2002). geepack: Yet another package for generalized estimating equations. R-News, 2(3), 12–14.

Yanovsky, E., & Kingsbury, R. M. (1938). Analyses of some Indian food plants. *Journal of the Association of Official Agricultural Chemists*, 21, 648–665.

Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A., & Smith, G. M. (2009). *Mixed Effects Models and Extensions in Ecology with R*. New York, NY: Spring Science and Business Media.