THESIS

RESTORATION PLANTING OPTIONS FOR *PINUS FLEXILIS* JAMES IN THE SOUTHERN ROCKY MOUNTAINS

Submitted by

Anne Marie Casper

Graduate Degree Program in Ecology

In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Spring 2012

Master's Committee:

Advisor: William Jacobi

Anna Schoettle
David Steingraeber

ABSTRACT

RESTORATION PLANTING OPTIONS FOR *PINUS FLEXILIS* JAMES IN THE SOUTHERN ROCKY MOUNTAINS

Pinus flexilis James populations in the southern Rocky Mountains are severely threatened by the combined impacts of mountain pine beetles and white pine blister rust. *P. flexilis'* critical role in high elevation ecosystems heightens the importance of mitigating threats to its survival. To develop forest-scale planting methods, *s*ix *P. flexilis* seedling planting trial sites were installed. Planting sites extended from the Medicine Bow National Forest in southern Wyoming to the Great Sand Dunes National Park and Preserve in southern Colorado. Six plots were established at each site, with three plots under areas of high density canopy, and three plots in areas of low density canopy. Experimental treatments were implemented at each of the six plots included presence/absence of a nurse object and presence/absence of hydrogel. The hydrogel treatment was omitted at two sites due to planting logistics and National Park and Preserve regulations. There were six replicates of each treatment combination, with 432 seedlings planted at each of four sites with hydrogel treatment and 216 seedlings planted at each of the two sites without hydrogel treatment, totaling 2,160 seedlings. To determine *P. flexilis* natural regeneration periodicity and site requirements in surrounding *P. flexilis* stands, three random plots were installed with five, 4 x 25 m subplots in each. We recorded diameter at breast height (dbh), health classification, and species on all trees taller than 135 cm, and the age of a subset of *P. flexilis* trees within each subplot. For all trees between 30 and 135 cm tall, we recorded health classification, species, and height. At three set locations in each subplot and at each *P. flexilis* under 135 cm tall, we recorded nurse objects, percent canopy cover, and percent ground cover in a square, 1 m^2 microsite. In the seedling planting plots, 76% of all planted *P. flexilis* seedlings were alive three growing seasons after planting. Therefore, data were analyzed comparing healthy trees to those with some degree of foliar damage. When analyzed by orientation to nurse object, there was a higher percentage of healthy trees on the north (77%) and west (78%) side of the nurse object than on the east side (68%) or without an object (63%) ($p<0.05$). Denser canopy cover was positively correlated with healthier planted seedlings. There was no hydrogel effect for any of the parameters measured. Longer terminal growth length and longer needle length were positively correlated with healthy trees. Pith dates from trees in transects within established *P. flexilis* stands indicate regular recruitment in most decades in the last century. Neither natural regeneration presence nor age was correlated with site characteristics. Density of naturally regenerating seedlings was positively correlated with increasing *P. flexilis* basal area in the surrounding stand and percent groundcover of trees in the microsite. Presence of natural *P. flexilis* regeneration in transects was not correlated with planted seedling health. In conclusion, for best growth and survival in the first three years after planting, *P. flexilis* seedlings should be planted on the north or west side of a nurse object under canopy. Natural regeneration in established *P. flexilis* stands occurred regularly, and not in infrequent bursts relying on large disturbances.

ACKNOWLEDGEMENTS

I would like to acknowledge the many people who have been instrumental in helping with my thesis: firstly, my advisor, William Jaocbi, for all his help through every stage of my thesis, and my committee, Dave Steingraeber and Anna Schoettle for providing guidance throughout. Kelly Burns provided planning assistance and was the PI for our funding. Lea Pace and Jill Portillo each provided an amazing summer's worth of field assistance. Additionally, Lynn Scharf, Christy Cleaver, Erik Rotaru, Nate McBride, Meg Dudley, and Mariah Coler all made the planting of thousands of seedlings possible. Madeleine Lecocq provided assistance with the final field season, as well as help proofreading.

I would also like to thank Peter S. Brown who provided consultation on dendrochronology techniques and hard to read samples; James zumBrunnen for statistial analysis assistance; and Dave Hattis, Kevin Zimlinghaus, Bob Post, Floyd Freeman, Phyllis Bovin, and Chris Kuennen for assistance with logistics, particularly identifying possible planting locations.

This project was funded by the CSU Agricultural Experiment Station and through a USDA Forest Service Special Technology Development Program Region 2 grant awarded to Kelly Burns, William Jacobi, and Anna Schoettle.

TABLE OF CONTENTS

Abstract…………………………………………………………………..…….……..… ii

Acknowledgements……………………………………………………...…………..… iv

Chapter 1: Literature Review………………………………………………............. 1

Stand Structure and Growth……………………………………………………… 3

Limber Pine Morphology and Plasticity…………………………………………. 7

Growth Forms……………………………………………………………….…… 8

Bird Dispersal…………………………………………………………………..… 9

Bird and Squirrel Dispersal Interactions……………………...…………………11

Mountain Pine Beetle………………………………………….…………………12

White Pine Blister Rust…………………………………………..………………13

Climate Change…………………………………………………..………………15

Other Pathogens and Insects……………………………………………………… 17

Herbivory………………………………………………………...…………….... 18

Natural Regeneration and Seedling Establishment…………………..…………… 20

Limber Pine Response to Meteorological Conditions……………………….…… 22

Post-Disturbance Establishment………………………………………………….. 23

Planting Importance………………………………………………………….… 24

Soil…………………………………………………………………………….... 26

Water Stress…………………………………………………………….……… 26

Hydrogels……………………………………………………………………….. 27

  Seedling Protection: Canopy Cover, Nurse Objects, and Artificial Shelters…….28
  Other White Pine Planting Techniques………………………………………..…34
Chapter 2: Manuscript: Restoration Planting Options for *Pinus flexilis* James in the Southern Rocky Mountains………………………………………………………………. 36
  Summary..……………………………..…………………………………..…. 36
  Introduction……………………………………………………….……………….. 39
  Methods…………………………………………………………….………………. 44
    Planting Treatments……………………………………………………...……45
    Meteorological Data………………...………………...….………………47
    Soil Data……………………….................……………..................……..47
    Seedling Assessment…………………..………………..…………....48
    Natural Regeneration Survey………………...………………..…...…49
    Processing Tree Cores and Sections…………………...…………...……50
    Data Analysis…………………………...………………………….……….51
  Results……………………………………………………………………………54
    Seedling Planting Site Characteristics…………………………...……54
    Planted Seedling Survival and Growth…………………………………..56
    Modeling Planted Seedling Health…………………………………..…. 58
    Treatments…………………………………………………….………….... 58
    Planted Seedling Damages……………………………………..….. 59
    Natural Regeneration Transects………………………………..……….. 60
  Discussion………………………………………………………………………… 62
    Planting Treatments……………………………….………………………..63

Canopy……………………………………………………..….63

Nurse Object……………………………………………..65

Hydrogels……………………………………..……….66

Growth Parameters……………………………………………….67

Herbivory, Insects, Disease, and Abiotic Damages…………………...…69

Natural Regeneration Survey………………………………...……..69

Future Research…………………………………………………72

Conclusions……………………………………………..…………… 74

Tables and Figures……………………………….……………….………….. 75

Literature Cited………………………………………………….…………… 99

Appendix I: Detailed Methods...…………………………………….………… 108

Coring Methods……………………………………………...……109

Water Potential Readings……………………...……………….……….109

Needle Color and Burn……………….…………...……………...……... 109

Appendix II: Additional Tables and Figures…………………………...……110

Appendix III: Data Sheets………………………………………………115

Chapter I: Literature Review

Pinus flexilis James, hereafter referred to as limber pine, is a five-needle pine that grows at a wide range of elevations in xeric and mesic sites (Steele, 1990). Its range in North America extends in patches from northern New Mexico northwards to Alberta, and west to California, Oregon, and British Columbia (Tomback and Achuff, 2010). Limber pine grows in similar habitat and overlaps range with whitebark pine (*Pinus albicaulis* Engelm.) in the northern part of its range, and Rocky Mountain bristlecone pine (*Pinus aristata* Engelm.) in the southern part of its range (Steele, 1990). Great Basin bristlecone pine (*Pinus longaeva* D.K. Bailey) has strong similarities to Rocky Mountain bristlecone pine, but grows in isolated patches in further west mountain ranges in Nevada, Utah, and California (Steele, 1990).

Morphologically, limber pine is more similar to whitebark pine, and these two species are both dispersed primarily by Clark's Nutcracker *(Nucifraga columbiana*), a bird that collects and caches seeds in the ground. Due to the similarities between limber and whitebark pine, research done on one species may be informative for the other.

Limber pine grows from lower to upper tree line and has the widest elevation range of any tree species in the Rocky Mountains (Schoettle and Rochelle, 2000). It establishes in xeric environments at high elevation sites, often in areas where subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.), Engelmann spruce (*Picea engelmannii* Parry ex Engelm.), and lodgepole pine (*Pinus contorta* Douglas ex Loudon) are unable to grow (Shankman and Daly, 1988). In many areas of Colorado and Wyoming, it also grows at the low elevation tree line, interspersed with sagebrush (*Artemisia tridentate* Nutt.), Rocky Mountain juniper (*Juniperus scopulorum* Sarg.), and ponderosa pine (*Pinus ponderosa* Douglas ex. Lawson) (Tomback and Achuff, 2010).

Limber pine colonizes areas rapidly after fires, and can facilitate the establishment of subalpine fir and Engelmann spruce post-disturbance (Rebertus et al., 1991). Limber pine is a poor competitor, and in more moderate sites, is outcompeted over time by other species (Veblen, 1986; Shankman and Daly, 1988; Rebertus et al., 1991; Schoettle 2004).

Since limber pine grows in areas where many other tree species are unable to establish, it performs many important ecosystem functions (Schoettle, 2004). Limber pines help decrease erosion, which facilitates the growth of other plants and protects their watersheds (Lanner and Vander Wall, 1980). Limber pines are relatively avalanche resistant, and since they grow in areas that may be devoid of trees without them, they can help stabilize snow, moderate snow melt, and delay peak stream flows (Perla and Martinelli, 1976; Lanner and Vander Wall, 1980). Limber pines provide food for several bird and mammal species, and are also aesthetically pleasing. This pine is considered a keystone species as it is an essential food source for black bears (*Ursus americanus* Pallas) (McCutchen, 1996), corvid birds, *Tamiasciurus* squirrels, and other small mammals (Tomback, 1982). It may also be an important food source for grizzly bears (*Ursus arctos horribilis* L.) at lower elevations and when whitebark pine has poor seed years (Schallenberger and Jonkel, 1980). However, two more recent studies have not found grizzly bears to feed on limber pine (Kendell, 1983; McCutchen, 1996).

Limber pines face several major threats. They are being killed by the current massive mountain pine beetle (*Dendroctonus ponderosae* Hopkins, MPB) outbreak (Gibson et al., 2008), white pine blister rust fungus (*Cronartium ribicola* J.C. Fisch., WPBR*)*, and impacted by climate change (Burns, 2006; Kearns et al., 2008). Individually these factors would negatively impact limber pine growth and establishment; combined they may cause limber pine extirpation of some populations across the landscape.

Stand Structure and Growth

Limber pine stands can be grouped into two general categories. In more xeric sites, they grow in self-replacing stands, whereas in more moderate environments, they grow in broadly even-aged, non-regenerating stands (Rebertus et al., 1991). In more mesic environments, limber pines grow in mixed conifer stands, commonly with ponderosa pine and lodgepole pine. At higher elevations they grow intermingled in spruce-fir or Rocky Mountain bristlecone pine stands (Peet, 1981; Rebertus et al., 1991). Often these mixed stands are in transition, with limber pine being replaced by other species (Rebertus et al., 1991). Limber pines grow intermixed with this variety of species because they have the widest range of tree species in the central Rockies, growing from 1600 to >3300m in elevation, with a possible range of 870-3400m (Schoettle and Rochelle, 2000).

The longest-lived limber pines are found in on xeric sites (Schulman, 1954). These stands are open-spaced and lack crown fires and blowdowns. Additionally, these trees have a high resin content that decreases the harm of physical injury and may provide protection from biological damages (Shulman, 1954). Regeneration in these sites is probably limited to years with good conditions and in favorable microsites (Rebertus et al., 1991).

Broadly even-aged, non-replacing limber pine stands occur post-disturbance on more favorable sites, where they are eventually replaced by the other species mentioned above (Rebertus et al., 1991). While limber pines are usually the first trees to establish, their peak recruitment occurs several decades after disturbance (Rebertus et al., 1991; Coop and Schoettle, 2009). Since limber pine seeds are dispersed by Clark's Nutcrackers, they are suspected to be dispersed into burns further than wind dispersed species (Coop and Schoettle, 2009). Additionally, limber pine seedlings are believed to be exceptionally drought tolerant, allowing them to survive in harsher conditions than other species (Tomback and Linhart, 1990). Following establishment, limber pine ameliorates the site conditions, thus allowing other tree species to become established (Rebertus et al., 1991).

In the Kananskis Valley of Alberta in the Canadian Rocky Mountains, limber pine stand history was reconstructed based on both live and dead trees, including logs (Webster and Johnson, 2000). The limber pine stands used to develop the chronology grow in discrete local populations with open canopies (Webster and Johnson, 2000). In the Kananskis Valley, stand recruitment was found to be consistent in established stands. While trees rapidly recolonized burns post-fire (within 10 – 20 years), there was no evidence for an increase in survivorship in the post-fire cohort, since limber pine stands maintain a low density. However, burns may have an increase in seed caches due to possible preferences by Clark's Nutcracker (Lanner and Vander Wall, 1980; Tomback, 1982).

The consistency in recruitment within limber pine stands found in the Kananskis Valley (Webster and Johnson, 2000) contrasts with recruitment patterns in subalpine fir and Engelmann spruce in Alberta, where large waves of recruitment occurred post-fire (Johnson et al., 1994). Johnson et al. (1994) hypothesized that even without disturbance, recruitment occurs at low rates, but with high seedling mortality. The hypothesis of continued recruitment without disturbance runs counter to the hypothesis that there are chronological gaps in recruitment as part of the process of stand development. Johnson et al. (1994) hypothesized that the gaps in stand chronologies based on coring only live trees are due to missing data from dead trees. Stand chronologies that included both live and dead trees had fewer gaps, but these chronologies still lacked data from seedlings that had died and decomposed. Based on this data, Johnson et al. (1994) hypothesized that gaps in recruitment that do exist are related to environmental factors and tree mortality, as opposed to gaps in recruitment as an inherent part of stand development as a stand ages.

As Johnson et al.'s (1994) study emphasized, it is important to look beyond living trees for stand reconstruction, and stand structure and species are important in understanding stand dynamics. While one cannot infer the behavior of the understory cohort based on the overstory cohort in some species, such as lodgepole pine and Englemann spruce (Johnson et al., 1994), limber pine growth may be similar across cohorts, particularly in open-grown stands (Webster and Johnson, 2000).

While successful regeneration is often considered to be a rare, disturbance-dependent event (Rebertus et al., 1991), unsuccessful recruitment rates (death of germinated seedlings) is hard to estimate unless we monitor stands for seedling recruitment and mortality over time. Webster and Johnson (2000) found evidence of regular recruitment in limber pine stands without stand-removing disturbances. In mixed forest types, Knowles and Grant (1983) found regular, small-scale recruitment (at least 1 tree per 10 year age class) for limber pines that reach at least the sapling stage. In both limber pine dominated forests and mixed forests, Stohlgren et al. (1998) found limber pine seedling establishment in the Colorado Rocky Mountains. These results indicate that more research in undisturbed forests is important to understand limber pine establishment dynamics without stand-replacing disturbance events.

There are major differences in stand structure between lower and upper tree line limber pine stands. In a study east of the continental divide in Colorado, lower tree line trees differed in age and stand structure from upper tree line trees (Schuster et al., 1995). Lower tree line populations were established in the early 1900s and the seedings took ten years to reach the 30 cm coring height. The young age structure, combined with a lack of dead trees, indicated that a stand-replacing disturbance regime controled ages in these lower tree line stands. Schuster et al., (1995) hypothesized that stand establishment may have been a response to changes in fire regime and increased grazing caused by European settlement and that the poor recruitment in the 1930s and 1940s in these stands was due to regional drought and severe erosion.

The upper tree line stand in Schuster et al's (1995) study was characterized by older and larger trees. Four of the trees in the upper tree line stand were over 1,000 years old. The oldest tree was difficult to age but was estimated to be 1,547 years old. This harsh site lacked ground cover, which likely prevented fires, particularly stand-replacing fires, and also had had little impact from European settlement.

Limber Pine Morphology and Plasticity

Most tree species exhibit morphological and physiological differences across an elevation gradient. These differences include increased leaf life span, decreased shoot length and needle length, and changes in fascicle characteristics with increased elevation (Schoettle and Rochelle, 2000). In situations in which water stress is not a problem, stomatal density usually increases or remains unchanged with elevation due to decreasing carbon dioxide availability (Woodward, 1986; Korner et al., 1989). Therefore, one might expect limber pines to exhibit morphological and/or physiological variation across the wide elevation gradient of their range.

Counter to conclusions based on most other tree species, adult limber pines show little morphological variation across elevation gradients (Schoettle and Rochelle, 2000). Schoettle and Rochelle (2000) did not find changes in fascicle characteristics, needle length, or shoot length across elevation gradients, nor in leaf life span if only forested sites are included in analysis. Leaf life span did vary with elevation if the non-forested site was included in analysis. Stomatal densities in limber pine decreased with increasing elevation, indicating that water conservation is more important than carbon dioxide uptake. These results were similar to those found in the closely related Rocky Mountain bristlecone pine (Schoettle and Rochelle, 2000).

In a common garden experiment, first year limber pine seedlings grown from low and high elevation seed stock showed morphological variation between seed stocks, but did not show variation within seed stocks across a range of elevations. (Reinhardt et al., 2011).

The decoupling between growth and environmental conditions found in Schoettle and Rochelle's (2000) limber pine study is unusual, since growth processes are usually a function of temperature, and might indicate that limber pine may be a genetic generalist with a wide range of tolerance as a high capacity for physiological plasticity or physical tolerance; these characteristics are adaptive for species with long-distance dispersal across a wide elevation gradient. Reinhardt et al's (2011) seedling study demonstrated similar tolerances within seedlings from a set seed stock; however it also provided evidence for genetic adaptation to environmental stressors within limber pine stands. Further research looking at long term transplant studies would contribute to greater understanding of limber pine growth and plasticity within and among individuals and sites (Schoettle and Rochelle, 2000; Reinhardt et al., 2011).

Growth Forms

In many species of conifer, such as fir and spruce, multi-stemmed growth is clonal (Tomback and Linhart, 1990). However, in limber, whitebark, and swiss pines (*Pinus cembra* L.*),* which are bird dispersed, multi-stem clusters indicate either multi-stemmed growth from a single tree, or a cluster of individual trees (Tomback and Linhart, 1990). Multi-genet clusters are attributed to bird caches (Carsey and Tomback 1994). While this clustering leads to resource competition and decreased reproduction, the persistence of this growth form indicates that the benefits of nutcracker dispersal outweigh the costs of multi-genet growth form (Feldman et al., 1999). The high levels of relatedness within clusters may help ameliorate the competition costs to the individual trees (Schuster and Mitton, 1991). Additionally, tree clusters may graft roots, which would increase stability and acquisition of water and nutrient resources (Tomback and Linhart, 1990). In areas where trees are isolated, trees in multi-genet clumps have a higher rate of cross pollination, which greatly benefits reproduction, since limber pines are poor self pollinators (Tomback and Linhart, 1990.)

Bird Dispersal

Most pine species have winged seeds and are primarily wind dispersed. Limber pines are one of the 19 species of wingless or near-wingless seeded pines in the section *Strobus* (Tomback and Linhart, 1990). These species all live in habitats with stressful conditions, where large seeds are advantageous for successful germination and establishment (Tomback and Linhart, 1990). Birds take seeds long distances and deposit them in disturbed areas, providing an effective dispersal mechanism (Tomback and Linhart, 1990). Trees within this group have seeds that lack all or most of a wing, and these seeds are also heavier than those of wind dispersed pines. While several pines in the section *Strobus* trees retain ripe seeds within their cones, limber pines cones open and release their seeds upon ripening (Krugman and Jenkinson, 1974; Tomback and Linhart, 1990).

Seeds of wingless pines may be transported up to 22 km from the seed source, and across elevation gradients (Tomback and Linhart, 1990). This type of dispersion leads to unpredictable and widespread gene flow, unlike winged seed dispersion and gene flow (Tomback and Linhart, 1990). Even with this lack of geographical specialization in wingless, bird dispersed tree species, the geographic distributions of wingless seeds are wider or similar to those of pines with winged seeds (Tomback and Linhart, 1990).

There is evidence that limber pines and Clark's Nutcrackers, which disperse limber pine seeds, have a co-evolved mutualistic relationship (Tomback and Linhart, 1990; Benkman, 1995; Siepielski and Benkman, 2007). The range of limber pines falls entirely within the range of Clark's Nutcrackers (Lanner and Vander Wall, 1980). Both Clark's Nutcrackers and limber pines have traits that indicate co-evolution, such as the sublingual pouch in the Clark's Nutcrackers and cone morphology in limber pines that facilitate seed dispersal (Lanner and Vander Wall, 1980; Tomback, 1982; Siepielski and Benkman, 2007).

Clark's Nutcrackers get a high energy, efficiently harvested food from their caches of seeds, when they successfully relocate caches (Tomback, 1982). Their unique sub-lingual pouch, specific to their genus, allows them to carry approximately 125 limber pine seeds, which they cache in multi-seed stashes ranging from 1-15 seeds, with a mean of 3-4 seeds (Lanner and Vander Wall, 1980; Feldman et al., 1999). Their good spatial memories and ability to learn geometric relationships among landmarks allows them to re-find widely dispersed food caches (Kamil and Jones, 1997).

While Clark's Nutcrackers are the primary dispersal agent in core limber pine populations, in low elevation grasslands with peripheral limber pine stands, Clark's Nutcrackers are rare, and are unlikely to be the primary seed dispersal agents. Tomback et al.'s (2005) study found that nocturnal rodents cached limber pine seeds in locations suitable for germination, and that not all cached seeds were recovered by the rodents. This demonstrates that in the peripheral ranges of limber pine, other agents can disperse limber pine seeds.

Bird and Squirrel Dispersal Interactions

Pine squirrels (*Tamiasciurus hudsonicus* Erxleben) are pine seed predators, and compete with Clark's Nutcrackers for both limber and whitebark pine seeds. Pine squirrel habitat covers most, but not all of limber and whitebark ranges (Siepielski and Benkman, 2007). While other small mammals harvest seeds that fall on the ground, pine squirrels directly harvest cones and compete with Clark's Nutcrackers, providing a direct, competitive, selective pressure (Siepielski and Benkman, 2007). Most research on the relationship between limber or whitebark pines and Clark's Nutcrackers has not looked at the larger ecosystem picture, which includes the competitive pressure of pine squirrels (Benkman, 1995; Siepielski and Benkman, 2007).

Clark's Nutcrackers and pine squirrels select for similar features in limber and whitebark pines; however, the birds are a positive selective force and the squirrels are a negative selective force (Siepielski and Benkman, 2007). Despite these similarities, there were some differences in the cone characteristics for which the two species selected (Siepielski and Benkman, 2007). Clark's Nutcracker selection pressure led to cones with an increased volume of seeds per cone mass, whereas pine squirrel selection pressure led to cones with lesser cone mass (Siepielski and Benkman, 2007. Trees in areas with pine squirrels had cone morphologies that made predation by pine squirrels more difficult; however, this also made seed collection by Clark's Nutcrackers more difficult (Siepielski and Benkman, 2007). These differences in cone morphologies add evidence to the hypothesis that pressure from squirrels may have prevented evolution of bird dispersion in other species of pine (Benkman, 1995). While Benkman's 1995 study also found differences in Clark's Nutcracker beak morphology between areas with and without pine squirrels, more research is necessary to understand the possible reciprocal selection occurring between these species.

Since the morphological differences between the bird or squirrel dominated populations of limber pines are in the cones and not the seeds, seed size is more likely to be an adaptation for post-germination conditions, rather than a factor directly attributed to the mutualistic relationship with Clark's Nutcrackers (Siepielski and Benkman, 2007). Large seeds are an adaptation for survival in harsh conditions (Tomback and Linhart, 1990), and birds are better dispersers of large seeds than wind (Siepielski and Benkman, 2007).

Mountain Pine Beetle

The mountain pine beetle (MPB) is the most important beetle species when studying mortality of white pines because they target large, mature trees (Schwandt et al, 2010). The behavior of MPBs varies depending on region and host species (Perkins and Swetnam, 1996). MPB behavior ranges from targeting stressed or healthy trees, and MPB population dynamics vary from endemic to outbreak depending on region and host species (Schwandt et al., 2010). Outbreaks occur when there is an abundance of vulnerable hosts, conducive climate, and insect populations, and are a natural, episodic occurrence in western forests (Samman and Logan, 2000; Gibson et al., 2008), and limber pines are a particularly suitable host (Cerezke, 1995).

Historically, MPB outbreaks have been an important disturbance force for the renewal of mature pine stands (Wood and Unger, 1996). Episodic outbreaks throughout the western US have been reconstructed back to the 1730s (Perkins and Swetnam, 1996).

In some of these outbreaks, mature pines were nearly eliminated within the range of the outbreak, and these severe outbreaks were followed by wildfire, which opened up the landscape for limber pine regeneration (Schwandt et al., 2010).

The current MPB outbreak is surging across the western US and Canada in a range of different pine species. Research indicates that the extent and length of this outbreak may be related to drought and/or mild winters that have coincided with it (Logan and Powell, 2001; Bentz and Schen-Langenheim, 2007). While mortality of adult trees is not unprecedented in historical outbreaks, the presence of white pine blister rust in areas with white pines may interrupt the historical cycles of beetles/fire/regeneration, since MPB only attacks mature trees and white pine blister rust kills trees regardless of size. Wood borers, bark beetles, such as *Ips* species, and twig beetles may attack limber pine, causing mortaility or dieback (Furniss and Carolin, 1977).

White Pine Blister Rust

The fungus responsible for the disease white pine blister rust (WPBR) was introduced to western North America around 1910 (Schwandt et al., 2010; Tomback and Achuff, 2010). Over the course of the 20th century, WPBR spread throughout the white pines of western North America (Geils et al., 2010; Schwandt et al., 2010). The pathogen of WPBR has a complex lifecycle, cycling between white pines and an alternate host. While *Ribes* species have been long thought to be the only alternative hosts, *Pedicularis racemosa* and *Castilleja miniata* have recently been found to also serve as alternate hosts (McDonald et al., 2006). The fungus has minimal impact on its alternate hosts, but can form lethal stem cankers on white pines (Kearns et al., 2008).

White pine blister rust has three spore stages on its alternate host and two on its pine host (Kendrick, 2000). Alternate hosts are infected by aeciospores that are produced on white pines in the spring and early summer. Those spores germinate and hyphae enter through the leaf stomata. In two to three weeks uredinia form on the bottom of the leaf and produce urediniospores that re-infect the alternate host plant. Later in the summer, in the uredinia area telia form and produce teliospores, which form in long columns of spores that stay attached to the underside of the leaves. During periods of high humidity, basidiospores form on the telia spores and are wind dispersed to live pine needles, infecting them. On the pine tree, the fungus forms spermogonia (formerly pycnia), which produce spermatia. The next year aecia form and release aeciospores in early summer, completing the lifecycle.

Currently, seven of the eight white pine species in the western United States and Canada are affected by WPBR (Schwandt et al., 2010). Great Basin bristlecone pine is the only uninfected species; however, it is susceptible to the pathogen and there is no reason to believe that it will not become infected in the future (Hoff et al., 1980; Schoettle and Sniezko, 2007). WPBR is currently found in both western Canadian provinces and all of the western United States except for Utah, and is found throughout most of limber pine's range at varying severities (Schwandt et al., 2010).

As trees become infected, cankers girdle branches and stems, causing branch death and top-kill, which decreases seed production (Geils et al., 2010). Eventually, tree death occurs, which negatively impacts wildlife species dependent on the trees for survival, including their seed dispersal relationship with Clark’s Nutcrackers (Schoettle and Sniezko, 2007).

Although WPBR requires moisture for spore production and germination, even trees that live in xeric environments are not safe from it. Resler and Tomback (2008) found infection rates of 25 percent or higher in whitebark pine stands that grew in cold, dry conditions where rust was not expected to be viable. These high infection rates not only impact survival and reproduction of adult trees, but also rapidly infect and kill seedlings, leaving some areas at risk for species extirpation (Resler and Tomback, 2008).

Climate Change

Abiotic shifts due to climate change will influence plant species at the edges of their ranges first. As limber pines primarily live in harsh sites beyond the tolerances of other species, they are particularly at risk. In addition to the direct impact of climate change on limber pines, their survival is also influenced by how climate change impacts MPB, WPBR, and other pathogens (Logan and Powell, 2001; Schwandt et al., 2010). Due to limber pine's dependence on Clark's Nutcrackers for seed dispersal (Lanner and Vander Wall, 1980), shifts in Clark's Nutcracker range will directly influence where limber pine can regenerate. Even if adult trees survive, regeneration is particularly at risk, as seedlings are less able to handle abiotic changes than mature trees (Germino et al., 2002).

Studies that look at trees at the edge of their ranges provide us with insight into what may happen to limber pines as changes in climate alter their habitat. Germino et al. (2002) studied trees at upper tree line and found seedlings to be highly vulnerable to climate change, since germination is more difficult at the edges of tree ranges, and seedlings are less robust than adult trees in handling abiotic changes. While spruce and fir trees propagate vegetatively (Shea and Grant, 1985), successfully augmenting their seed-regeneration, limber pine does not (Germino et al., 2002). Therefore, limber pine may be less able to handle changes than spruce and fir species found nearby.

Climate change shifts may simulate conditions of lower elevations, since tree line is expected to shift higher due to warmer temperatures and less precipitation (Dullinger et al., 2004). Hogg and Schwartz (1997) looked at the success of natural regeneration from planted white spruce trees on private lands (farm yards, abandoned homesteads, shelterbelts) in Saskatchewan, Canada. The objective of this study was to try to understand the influence of climate change on the Canadian boreal forests by assessing regeneration success in very dry (aspen parklands) to very moist (boreal forest) vegetation zones. Very little natural regeneration was found in the drier climates, and the seedlings that were present were in poor condition. Since moisture deficiency has been long recognized as a factor limiting conifer seedling establishment, Hogg and Schwartz (1997) hypothesized that climate change may shift vegetation boundaries northward. The increasing frequency of fires may kill existing adult trees, decreasing seed sources and facilitating the loss of trees in their southern ranges (Hogg and Schwartz, 1997).

A study of black spruce (*Picea mariana* Mill.) seedling establishment at upper tree line in Labrador, Canada, found that planted seeds were able to germinate and grow above tree line, surviving throughout the five year study (Munier et al., 2010). Additionally, Munier et al. (2010) found that soil disturbance combined with temperature enhancement, performed by surrounding seeds with open top chambers made of greenhouse plastic attached to wooden stakes, significantly increased seedling emergence. These results indicate that climatic warming, when combined with seed dispersal and suitable substrate, may lead to upper tree line advancement.

It is unknown how climate change will influence the range of mountain pine beetle, white pine blister rust, and other insects and pathogens. However, predictive models can provide some insight. The range of MPB is expected to shift northward and to higher elevation stands. In MPB's southern range, it has two generations per year, and this may occur farther northward if the climate warms (Gibson et al., 2008; Bentz et al., 2010). Models predict that warming climate will increase habitat suitability for MPB at higher elevations in the Rocky Mountains (Hicke et al., 2006). The range of white pine blister rust is more difficult to model since predictions of currently unsuitable habitat have been incorrect (Resler and Tomback, 2008; Schwandt et al., 2010). However, the expansion of its range is expected to be influenced by climate (Kearns, 2005).

Other Pathogens and Insects

Along with the high profile issues of mountain pine beetle, white pine blister rust, and climate change that limber pines face, there are other challenges to their survival as well. Because the following pathogens and insects will also be influenced by climate change, their interactions with limber pines make predictions of the future even more challenging.

Limber pines are susceptible to root diseases including *Armillaria ostoyae* (Romagn.) Herink, *Heterobasidion annosum* (Fr.:Fr.) Bref., *Leptographium wageneri* (W.B. Kendr.) M.J. Wingf., *Phaeolus schweinitzii* (Fr.) Pat., *Phellinus sulphurascens* Pilat [syn. *Phellinus weirii* (Murr.) Gilb.], and *Perenniporia subacida* (Peck) Donk

(Schwandt, et al., 2010). They are also susceptible to an aggressive dwarf mistletoe (*Arceuthobium cyanocarpum*, A. Nelson ex Rydberg) and several foliar diseases including *Dothistroma septosporum* needle blight (Schwandt et al., 2010). Limber pine's reproductive success is challenged by cone and seed insects including the western conifer-seed bug (*Leptoglossus occidentalis* Heidemann), cone beetles (*Conophthorus ponderosae* Hopkins), and cone worms (*Dioryctria abietivorella* Grote) (Schoettle and Negron, 2001). In addition, they face wildlife damage and fire (Schwandt et al., 2010).

Some of the above factors may act in combination with MPB and WPBR to increase tree mortality. Others, such as Dothistroma needle blight, have been found to cause severe decline in limber pines on their own (Taylor and Walla, 1999). While fire is important for limber pine regeneration, fire can also kill mature trees that have survived mountain pine beetle outbreaks or WPBR infection, and fires open gaps in the forest that can promote *Ribes* growth (Schoettle and Sniezko, 2007; Coop and Schoettle, 2009).

Herbivory

The impact of vertebrate herbivory on conifer seedlings varies by species and habitat. McCaughey et al's (2009) planting guide for whitebark pine does not mention herbivore exclusion for ungulates, and simply recommends avoiding planting seedlings in areas with deep soil to minimize pocket gopher damage. However, ungulate and other vertebrate grazing negatively impact seedling growth in many tree species.

At tree line in the moist climate of Labrador, Canada, Munier et al.'s (2010) study found that the exclusion of vertebrates with hardware cloth increased tree growth and decreased damage and tree mortality in black spruce. In another non-water stressed climate in southern Sweden, Bergquist et al. (2009) performed a study of roe deer herbivory on Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* (L.) H. Karst.). Over the course of the four year study Bergquist et al.'s (2009) control trees lost approximately two years worth of growth to herbivory. Bergquist et al. (2009) also found that 60% of the Scots pine seedlings had multi-stem growth due to herbivory, while the Norway spruce showed little multi-stem growth. Due to the growth loss in both species and the multi-stem growth in Scots pine, Bergquist et al. (2009) recommended the use of exclusionary fences for at least the first four years of growth in Norway spruce and Scots Pine.

In contrast to these studies by Bergquist et al. (2009) and Munier et al. (2010), Gomez-Aparicio et al.'s (2008) study in the dry, Mediterranean mountains of southeast Spain found that ungulate presence or exclusion did not impact seedling height growth or survival in either European black pine (*Pinus nigra* Arn.), or in Scots pine. Gomez-Aparicio et al. (2008) hypothesized that ungulate presence was not significant because the abiotic stresses of drought and cold strongly influenced survival, overshadowing any herbivory effect. Gomez-Aparicio et al (2008) also found that nurse shrubs did not protect seedlings from ungulate grazing. However, they acknowledged that shrubs larger than those used in their study may protect seedlings from herbivory. Additionally, the five years of Gomez-Aparicio et al.'s (2008) study may not have been long enough to observe herbivore impact on the slow growing, relatively unpalatable European black pine and Scots pine seedlings, as they did observe some ungulate damage. While Ronco (1970a) did not study herbivory directly, he observed little herbivory in his planting study of Engelmann spruce and lodgepole pine in the Rocky Mountains of Colorado.

The variable results of these studies indicate that the impact of herbivores on seedlings varies by habitat and ecosystem. While a herbivore exclusion study with limber pine would be ideal, the Gomez-Aparicio et al. (2008) and Ronco (1970a) studies are the most applicable to limber pine planting. Gomez-Aparicio et al.'s (2008) studied planted pines with low palatability in a dry climate with cold winters. Limber pines grow in a similar habitat and have high levels of resin content that decrease their palatability and increase their ability to survive mechanical damage (Schulman, 1954). Ronco's (1970a) study was geographically close to limber pine habitat, including the same herbivores that limber pines experience. Therefore, unless seedlings are in a habitat with strong herbivore pressure, which is not indicated by Ronco's (1970a) observations, it is likely that stands will see no significant levels of seedling mortality or growth loss due to herbivory (Gomez-Aparicio et al., 2008).

Natural Regeneration and Seedling Establishment

Successful reproduction and establishment is vital to limber pine survival. Factors impacting survival vary across both macro and micro spatial scales, and by species. While literature on reproduction and establishment of other species can help frame limber pine research, it is important to simply use research on other tree species as a springboard for limber pine research.

Protection by either forest canopy or nearby objects at the micro or macro habitat scale is important in an array of species including: Engelmann spruce, subalpine fir, Scots pine, maritime pine (*P. pinaster* Ait.), Rocky Mountain bristlecone pine, and limber pine (Germino and Smith, 1999; Barbeito et al., 2009; Coop and Schoettle, 2009; Ronco

1970b; Rodriguez-Garcia et al. 2011 and Germino et al., 2002). Studies that looked at the orientation to a protecting object in other tree species found that planting on the north and/or west side provided the best protection (Germino et al., 2002; Germino and Smith, 1999; Ronco, 1970a).

Microsite conditions are vital for seedling survival in high altitude tree species (Germino et al., 2002; Colak, 2003; Castro et al., 2004; Coop and Schoettle, 2009). Since moderate temperatures and high precipitation are good for seedling establishment in harsh sites, microsites can create the specific environment needed for establishment (Germino et al., 2002). Even though competition from other vegetation is usually considered to be detrimental to seedling survival, the protection provided by other vegetation may outweigh the negative impacts of nearby plants for many tree species at high elevation or on harsh sites (Germino et al., 2002; Maher et al, 2005; Rodriguez-Garcia et al., 2011; Coop and Schoettle, 2009). In addition, growth near herbaceous plants was found to improve limber pine seedling survival in low elevation stands in the eastern grasslands of Colorado (Tomback et al., 2005).

In the Front Range of Colorado, limber pine seedling presence has been positively associated with leaf litter ground cover, standing tree trunks, shorter distances to the nearest object, shortest mean distance to the three nearest objects, and the percent of open sky (Coop and Schoettle, 2009). Mineral soil and cobble ground cover were inversely related to limber pine seedlings presence (Coop and Schoettle, 2009).

At the plot level of Coop and Schoettle's (2009) study, limber pine seedlings were positively associated with fireweed and kinnikinnick. However, this association is thought to be due to shared affinities between species, rather than intraspecific facilitation, due to the diversity of organisms in this association. Limber pine also had a negative relationship to leaf cover at the plot scale, and this was attributed to areas of dense conifer overstory.

On a macro level, patterns of seedling survival across the landscape inform us about overall habitat suitability (Coop and Schoettle, 2009; Webster and Johnson, 2000; Hogg and Schwartz, 1997). Limber pine regeneration ecology changes along the north/south gradient of Colorado, with less apparent regeneration in the south (Coop and Schoettle, 2009). This shift is attributed to decreasing habitat suitability in the south, which is near the southern limit of limber pine's range. In Alberta, Canada, open-canopy limber pine stands were found to have episodes of regeneration post-disturbance and continued recruitment in existing limber pine stands (Webster and Johnson, 2000.)

Limber Pine Response to Meteorological Conditions

Meteorological conditions are important to seedling survival. Irregular waves of establishment may occur in years with suitable conditions, especially sufficient moisture (Johnson et al., 1994). Microsite features ameliorate the surrounding abiotic conditions, decreasing the severity of wind damage, ice-blasting, temperature extremes, and photoinhibition due to intense solar radiation (Coop and Schoettle, 2009; Ronco, 1970b).

In the Sierra Nevada Mountains of California, a study by Millar et al. (2007) found that dense stands of 50 to 300 year old limber pine trees experienced high mortality during the persistent 1984 - 1992 drought. In addition to low precipitation, this drought was characterized by warm temperatures. By comparing chronologies of both live and dead trees to historical meteorological records, Millar et al. (2007) found that limber pines usually showed a positive growth response to both warmer minimum and maximum temperatures. However, during the drought, tree growth declined when high temperatures were combined with low precipitation. While Millar et al. (2007) did not specifically study seedlings, they cored trees at 0.5 m, therefore including some data from a tree's response to meteorological conditions from when the tree was a seedling. Additionally, since seedlings are usually more sensitive to abiotic and biotic conditions, they are likely to have responses similar to, though stronger than, those of mature trees.

Post-Disturbance Establishment

Limber pines often establish after disturbance, such as fire, particularly in areas with less xeric conditions (Rebertus et al., 1991; Coop and Schoettle, 2009). Coop and Schoettle (2009) found different establishment patterns in three different burns. In their northern Colorado site, limber pines established in the center of severe burns; whereas, in their southern two sites limber pine established more on the edges of burns. Within burns, they found that the densities of competing tree species seedlings, especially aspen (*Populus tremuloides* Michx.) and Engelmann spruce, were often one to two orders of magnitude higher than the numbers of limber pine seedlings. Therefore, even though the number of limber pines increased after a fire, their relative abundance decreased due to the prevalence of other species. Coop and Schoettle (2009) hypothesized that the difference in burn colonization could be due to changes in Clark's Nutcracker behavior or a decrease in the suitability of habitat in the farther south sites. Based on their results, Coop and Schoettle (2009) concluded that burn colonization patterns were due to habitat suitability and not Clark's Nutcracker behavior. Coop and Schoettle (2009) also found that regeneration was a slow process in all of their study sites, since 30-years post-burn the forests had not yet reached the density of the surrounding unburned forest.

Prescribed burns may be useful in promoting natural regeneration in limber pines by opening up areas for seedling establishment (Coop and Schoettle, 2009). Based on the number of naturally regenerating limber pine seedlings observed in three burns in Colorado, limber pine had the highest rates of regeneration in mixed severity burns in the two southern sites, and high severity burns in the northern site (Coop and Schoettle, 2009).

One potential disadvantage of prescribed burns is that openings created by fire facilitate growth of WPBR alternate hosts (Schoettle and Sniezko, 2007; Coop and Schoettle, 2009). It is unknown how this will influence WPBR severity and spread, but the growth of WPBR alternate hosts may not offset the benefit of burn treatments in facilitating limber pine regeneration (Coop and Schoettle, 2009). If these alternate hosts do increase the level of WPBR infection, then it may select for seedlings that are naturally resistant (Coop and Schoettle, 2009).

Planting Importance

Due to limber pine's adaptations to severe environments, their life history traits are not conducive to rapid adaption, and therefore management will be needed to help sustain populations in the presence of WPBR (Schoettle and Sniezko, 2007). Their traits, such as being long lived and regenerating infrequently, are maladaptive in surviving rapid selective forces, such as an invasive species (Schoettle and Sniezko, 2007). Therefore, by using management techniques to help establish WPBR resistant seedlings in limber pine stands, the impact of WPBR infection can be mitigated (Burns et al., 2008).

Outplanting resistant seedlings prior to rust outbreaks will boost populations and diversity of age classes (Schoettle and Sniezko, 2007). However, since regeneration is slow and prolonged in limber pine, responses to management may be slow, and it may take decades before results are visible (Coop and Schoettle, 2009). As there has already been high mortality of limber pine in Idaho, Montana, and Wyoming, and there is currently a large mountain pine beetle outbreak coinciding with the spread of WPBR, the death of mature trees in Colorado may lead to a lack of seed sources and limit management options. Therefore, for simulation of natural regeneration to be successful, it needs to occur soon (Coop and Schoettle, 2009).

Many factors, including soil moisture, wind exposure, snow loads and breakage, animal predation, solar protection, competition with other vegetation, and microtopographic features need to be considered when outplanting seedlings. Multiple studies have identified the use of lowland planting techniques and ignoring microsite variations as two major factors for reforestation failure (Colak, 2003; Elman and Peterson, 2003). Species that naturally cohabitate with limber pine may be used to indicate site suitability (Coop and Schoettle, 2009).

When seedlings are first planted, they experience planting check, or growth check, which is a reduction in shoot growth for the first one-to-three years after planting (Grosnickle, 2005). Growth check is caused by limited access to nutrients and water after planting (Grosnickle, 2005). It is vital to make sure seedlings have adequate resources to survive past this stage. Therefore, planting limber pine in suitable habitat and microsites will help facilitate survival.

Soil

Many soil characteristics influence suitability for root growth. Organic soil layers decrease temperature transfer and therefore are cooler than mineral soils in the rooting zone (Grossnickle, 2005). These cooler temperatures cause decreased root growth in conifers. In turn, this decrease in root growth leads to water stress, causing decreased photosynthesis, which limits growth, and thus feeds into a vicious cycle (Ronco, 1972). Additionally, cold soils increase resistance to water uptake, further increasing water stress (Grossnickle, 2005).When planting trees, it is important to limit air gaps because they decrease root/soil contact, which is very important for water update (Grossnickle, 2005). Conversely, if roots are confined in compacted soil, root growth is also limited. Container grown seedlings have better root growth than bare root seedlings (Grossnickle, 2005).

Water Stress

Water stress is a major cause of seedling death. Bernier (1993) found that planted black spruce seedlings experienced more water stress than naturally occurring seedlings, based on predawn water potential and midday stomatal conductance measurements. Typically, planted seedlings have restricted roots, low root system permeability, and poor root to soil contact, which limit water uptake from the soil (Grossnickle, 2005). To overcome planting stress, it is vital to plant seedlings at a time when they are physiologically able to grow new roots (Grossnickle, 2005). Therefore, in the case of limber pine seedlings, early spring plantings may be important to allow for suitable new root growth.

Trees planted in coarse soils have more trouble establishing themselves because these soils have less water storage than other soils. Dry soils exacerbate this problem. As water potential decreases, root growth decreases, and root growth is vital for seedling establishment (Grossnickle, 2005). Competing vegetation draws available water away from seedlings whenever roots are close enough to change each other's root environments (Grossnickle, 2005). However, as mentioned above, the benefits of shade and protection from competing vegetation may outweigh this drawback (Strand et al., 2006; Castro et al., 2004).

Hydrogels

Hydrogels, or polyacrylimide gels, are highly absorbent polymers that have been used in some studies to help ameliorate water stress by holding water in the soil near the tree roots (Rowe et al., 2005; Huttermann et al., 1999). Hydrogels are relatively inexpensive and have been used to improve tree survival in arid environments and in horticulture (Rowe et al., 2005). They are similar to substances used as a flocculant to reduce soil erosion; the difference is that these polyacrylmides are crosslinked (Rowe et al., 2005).

Huttermann et al. (1999) looked at survival of aleppo pine (*Pinus halepensis* Mill.) in sandy soils when hydrogel was added in varying percentages. They found seedlings that had 0.4% of hydrogel added to the soil survived twice as long as seedlings in control soils. In drought conditions, these seedlings grew approximately three times as much as the control seedlings. However, the influence of hydrogels is affected by soil type, with hydrogels having the strongest positive effect in sandy soils (Agaba et al., 2010).

Rowe et al. (2005) tested the effect of hydrogels added to soils when establishing trees on quarry waste sites. They used coarse-grade anionic polyacrylamide gels in different concentrations, and placed them dry at the bottom of the planting hole. The hydrogel treatment did not increase tree survival. However, hyrdogels did increase the growth rates of surviving trees, when compared to those without hydrogel. Overall, hydrogels had a positive effect.

Hydrogels may impact nutrient availability in seedling roots. While hydrogel prevented nutrient loss through leaching, they may reduce the availability of NO_3^- and NH_4^+, which may explain slower growth seen in some tree species (Rowe et al. 2005). The use of dry hydrogel in Rowe et al.'s (2005) study not cause tree mortality through absorbing the tree's moisture, because the tree roots had little contact with the hydrogel until it was wetted by rain. They recommended applying hydrogel when rain is expected; however, their technique of dry hydrogel application is probably not appropriate in xeric environments, such as limber pine habitat.

Seedling Protection: Canopy Cover, Nurse Objects, and Artificial Shelters

Seedlings survive better under protected conditions in several different conifer species (Strand et al., 2006; Rodriguez-Garcia et al. 2011; Ruano et al. 2009; Castro et al., 2004; Ronco, 1970a; Jacobs and Steinbeck, 2001; Germino and Smith, 1999), including the shade-intolerant maritime pine (Rodriguez-Garcia et al. 2011).

Engelmann spruce and subalpine fir in the alpine-treeline ecotone had higher survival rates in tree islands or in other areas with overhead cover, which decreased solar radiation intensity, increased soil moisture, increased depth of snow pack, and moderated temperature extremes (Germino et al., 2002). In the Mediterranean mountains, Scots pine regeneration was strongly influenced by tree and shrub overstory, with moderate shade (based on a continuous measurement of shade from low to high) promoting regeneration (Barbeito et al., 2009).

Germino et al.'s (2002) found that tree islands facilitated seedling growth; this goes against the generalization that adult trees suppress seedlings by shading them. However, several studies in the sub-alpine zone support the tree island protection hypothesis. Maher et al.'s (2005) findings agree with Germino et al.'s (2002) in a whitebark pine, subalpine fir, Engleman spruce forest. They hypothesized that along with protecting seedlings from temperature fluctuations, the tree islands enhance mycrorhyzal infection and may decrease seedling water loss.

Counter to the implication of the "shade-intolerant" classification of maritime pine, Rodriguez-Garcia et al. (2011) found that canopy cover was an important factor in the survival of early regeneration. Naturally germinating seeds and emerging seedlings had higher levels of survival over the two-year study (Rodriguez-Garcia, 2011). Additionally, there was an inverse relationship between solar radiation and seedling survival (Rodriguez-Garcia, 2011). Ruano et al. 2009 performed a similar study with maritime pine, in which they had higher seedling survival under greater basal area. This response may initially seem counter to Strand et al.'s (2006) study that found a positive relationship between growth and light availability in both naturally regenerating and planted seedlings, particularly in shade-intolerant species. However, Strand et al (2006) focused on tree growth rates, not survival. In addition, they observed less abiotic damage on trees in shelterwoods, and abiotic damages are likely to be correlated with tree survival. Furthermore, Rodriguez-Garcia et al. (2011) acknowledge that soil conditions may be more favorable under canopy cover, even though they did not observe significantly differences in the soil chemistry of their sites. If canopy is simply a proxy for other factors that increase survival, then environmental conditions and species composition of a habitat may influence the impact of canopy density, even if these factors are not individually statistically significant (Rodriguez-Garcia et al., 2011).

. The combined results from the above studies provide evidence that overstory canopy may be an important measure to predict initial establishment of regeneration in habitats where drought-stress is a major cause of mortality (Rodriguez-Garcia, 2011; Ruano et al., 2009).

Shelter from nearby vegetation or objects, also known as nurse objects, is important for seedling survival, even in situations where the vegetation would usually be considered a competitor (Castro et al., 2004; Tomback et al., 2005). In a study of Engelmann spruce in Wyoming, seedlings had higher survival rates on the north side of tree islands, or under branch or grass cover (Germino et al., 2002). In a study of Scots pine and European black pine in the Mediterranean mountains (Castro et al., 2004), shrub presence enhanced seedling establishment at the end of a four year study, even though shrubs are usually considered competitors of planted seedlings. Castro et al., (2004) found higher survival in both tree species under shrub canopy than on bare soil, even though planting conditions were more favorable in open areas. When analyzed by cardinal direction, both Castro et al. (2004) and Germino et al (2002), found higher rates of seedling presence or survival on the north side of objects, providing further evidence that objects provide protection from solar insolation and the resulting water stress.

Seedling protection from abiotic conditions by nurse objects is important specifically in limber pine. Coop and Schoettle (2009) found that nurse objects were predictors of seedling presence in the Front Range of the Rocky Mountains. While object type was not important in Coop and Schoettle's (2009) study, they did not find evidence for facilitation by neighboring plants. Conversely, Tomback et al., (2005) found that the presences of nurse plants were positively correlated with limber pine seedling presence in the low elevation, eastern edge of limber pine's range.

Since Coop and Schoettle's (2009) study looked at both limber pine (a bird-dispersed species), and Rocky Mountain bristlecone pine (a wind-dispersed species), and found the same facultative relationship with nurse objects, their findings indicate that the relationship between seedlings and objects is not just an artifact of Clark's Nutcrackers preferentially caching seeds near objects. The presence of these objects not only provides protection, but may also increases soil moisture, which could be vital to germination and early seedling survival, due to the xeric nature of limber pine habitat (Coop and Schoettle, 2009). Nurse objects may also increase snowpack, which protects seedlings from cold temperatures (Germino et al., 2002). This is important because the combination of low temperature and high sun found in high elevation sites causes low temperature photoinhibitation in some species, leading to low carbon gain (Germino et al., 2002). Nurse objects may also enhance mycorrhizal infection and decrease seedling water loss (Callaway and Walker, 1997).

The use of tree shelters is a technique that has been used to improve planted seedling survival. If they are in place year-round, shelters provide a more constant level of shading; if the shading is provided by trees with deciduous foliage, or if shelters are removed overwinter, seedlings may be injured by high levels of light intensity early in the spring (Jacobs and Steinbeck, 2001).

Ronco (1961, 1967, and 1970a) performed several experiments using wood shingles to shelter Engelmann spruce and lodgepole pine seedlings in the sub-alpine zone of the White River Plateau in Western Colorado. In studies analyzing the survival of three-year-old outplanted seedlings, wooden shingles placed on the south side of Engelmann spruce increased survival. However, shade provided by a shingle on the south side of a lodgepole pine seedling did not increase survival, which had much lower mortality rates than Engelmann spruce overall (1961, 1967). In all of Ronco's studies (1961, 1967, 1970a), the highest mortality was due to photoinhibition, but occasionally gophers and frost heaving caused high rates of mortality as well. He found mortality associated with herbivore browsing, and most tree mortality occurred in the first year after planting (Ronco, 1970a).

In a more recent study of Engelmann spruce seedlings, Jacobs and Steinbeck (2001) studied the use of tubular plastic shelters in the high elevation spruce-fir zones in the central and southern Rocky Mountains. The shelters they tested were plastic cylinders of different opacities and were placed around the seedlings. The shelters held up well for two winters, and almost all of them were functional for the following growing season with minimal maintenance. Jacobs and Steinbeck (2001) projected that most shelters will remain functional for five growing seasons, after which they are no longer necessary. The tree shelters protected seedlings from heavy winter snowpack by decreasing the number of leaning seedlings. Overall, seedlings in all but the darkest shelter treatment grew faster than the control treatment (Jacobs and Steinbeck, 2001). Since Engelmann spruce grow very slowly, taking 20-40 years to reach breast height, even slight improvements in growth rates may help improve survival. Plastic shelters increase temperatures and carbon dioxide levels around the seedlings, stimulating growth. Additionally, the warmer temperatures in the shelters caused budburst to occur earlier, and provide protection from late season frosts.

Commercial shelters allowing 21-24% of PAR (photosynthetically active radiation) to reach the seedlings resulted in the best growth results and the trees were shorter and stockier, and therefore, less vulnerable to gopher browsing and snow damage (Jacobs and Steinbeck, 2001). Darker shelters stimulated height growth over length growth, which is also seen when Engelmann spruce grow under heavy vegetative competition.

While there is no direct research on limber pine seedlings grown in artificial shelters, the results of Ronco's (1961, 1967, and 1970a) and Jacobs and Steinbeck's (2001) work on Engelmann spruce, in combination with the importance of nurse objects for limber pine seedling survival (Coop and Schoettle, 2009), indicate that artificial shelters may be an option for providing appropriate microsite conditions for planted limber pine seedlings.

Other White Pine Planting Techniques

Whitebark pine seedling survival, and therefore possibly limber pine seedlings survival, is affected by desiccation, wind, predation, and overstory density and species (Scott and McCaughey, 2006). As desiccation is a major challenge to seedling survival in xeric sites (Coop and Schoettle, 2009), planting techniques that ameliorate water stress may be important to seedling survival.

A planting study of whitebark pine in Gallatin National Forest in Montana found that planted seedlings had the highest survival rate after 11 years on ridges (47%) and benches (39%) and the lowest survival on swales (2%) and on a 15% slope (20%) (Scott and McCaughey, 2006). Additionally, these researchers found higher survival rates after 9 years on dry sites (86%) compared to moist sites (50%) in planting sites near Cooke City, Montana.

Based on Scott and McCaughey's (2006) planting trials and their assessment of other planting studies and literature, they recommend preparing whitebark pine planting sites in the following manner: reduce overstory competition; reduce understory vegetation except for grouse whortleberry; consider the overall topography of the planting area, avoiding swales and frost pockets; plant near stumps or provide shade in some manner; plant where there is protection from heavy snow and drifts, stumps, rocks, and large logs provide recommended microsites; avoid overcrowding to prevent competition between planted trees; plant where there is sufficient soil moisture; and plant large seedlings with good root development. Scott and McCaughey's (2006) whitebark pine guidelines provide foundation for developing limber pine planting guidelines.

Scott and McCaughey's (2006) whitebark pine guidelines, along with the expanded management guide to whitebark pine (Shoal et al., 2008) provide foundation for developing limber pine planting guidelines. Additionally, Mahalovich et al. (2006) provides information in selecting appropriate seed stock for outplanting projects.

While whitebark pine is similar to limber pine, both in physiology and in range, for some tree species, including the shade-intolerant maritime pine, the benefit provided by protection outweighs the cost of competition (Castro et al., 2004; Maher et al., 2005, Rodriguez-Garcia et al., 2011). Even though the planting guidelines for whitebark pine recommend removal of overstory canopy (McCaughey et al., 2009), studies are necessary to understand the impact of canopy on survival, growth, and maturation of planted limber pine seedlings. Because planting WPBR resistant limber pine in areas where they are likely to become extirpated has been identified as an important management strategy (Schoettle and Sniezko, 2007), information about how seedlings survive in areas with partial canopy versus in more exposed areas is important for planting in locations where the overstory may be killed by MPB and WPBR.

Chapter 2: Manuscript: Restoration Planting Options for *Pinus flexilis* James in the Southern Rocky Mountains

Summary

CASPER, A.M. (Graduate Degree Program in Ecology, Department of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, CO 80523), JACOBI, W.R. (Department of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, CO 80523), SCHOETTLE, A.W. (USDA, For. Service, RM Research Station, Fort Collins, CO 80526). BURNS, K.S. (USDA, For. Service, FHP, Lakewood Service Center, Golden, CO 80401).

Restoration planting options for *Pinus flexilis* James in the southern Rocky Mountains. J. Torrey Bot. Soc. in preparation. *Pinus flexilis* James populations in the southern Rocky Mountains are severely threatened by the combined impacts of mountain pine beetles and white pine blister rust. *P. flexilis'* critical role in high elevation ecosystems heightens the importance of mitigating threats to *P. flexilis* survival. To develop forest-scale planting methods, *six P. flexilis* seedling planting trial sites were installed, extending from the Medicine Bow National Forest in southern Wyoming to the Great Sand Dunes National Park and Preserve in southern Colorado. Six plots were established at each site, with three plots under areas of high density canopy, and three plots in areas of low density canopy. The following experimental treatments were implemented at each of the six plots: presence/absence of a nurse object and presence/absence of hydrogel. There were six replicates of each treatment combination, with 432 seedlings planted at each of four sites with hydrogel treatment and 216 seedlings planted at each of the two sites without hydrogel treatment, totaling 2,160 seedlings. To determine *P. flexilis* natural

regeneration periodicity and site requirements in surrounding *P. flexilis* stands, three random plots were installed with five, 4 x 25 m subplots each. In the seedling planting plots, 76% of all planted *P. flexilis* seedlings were alive three growing seasons after planting. Therefore, data were analyzed comparing healthy trees to those with some degree of foliar damage. When analyzed by orientation to nurse object, there was a higher percentage of healthy trees on the north (77%) and west (78%) side of the nurse object than on the east side (68%) or without an object (63%) ($p<0.05$). Denser canopy cover was positively correlated with healthier planted seedlings. There was no hydrogel effect for any of the parameters measured. Terminal growth length and needle length were positively correlated with healthy trees. Pith dates from trees in transects within established *P. flexilis* stands indicate regular recruitment in most decades in the last century. Neither natural regeneration presence nor age was correlated with site characteristics. Density of naturally regenerating seedlings was positively correlated with increasing *P. flexilis* basal area in the surrounding stand and percent groundcover of trees in the microsite. Presence of natural *P. flexilis* regeneration in transects was not correlated with planted seedling health. In conclusion, for best growth and survival in the first three yeas after planting, *P. flexilis* seedlings should be planted on the north or west side of a nurse object under canopy. However, further data on the effect of canopy cover and object presence on *P. flexilis* growth is necessary to determine if the impact of canopy and object presence have a negative impact on *P. flexilis* maturation. Natural regeneration in established *P. flexilis* stands occurred regularly, and not in infrequent bursts relying on large disturbances.

Key Words: mountain pine beetle, *Dendroctonus ponderosae, Cronartium ribicola,* white pine blister rust, tree planting, five needle pine, exotic disease

Introduction

Pinus flexilis James, hereafter referred to as limber pine, is a five-needle pine that grows at a wide range of elevations in xeric sites (Steele, 1990). Its range extends from northern New Mexico north to Alberta, and west to California, Oregon, and British Columbia, in North America (Tomback and Achuff, 2010). Limber pine grows in similar habitat and overlaps range with *Pinus albicaulis* Engelm., hereafter referred to as whitebark pine, in the northern part of its range and *Pinus aristata* Engelm., hereafter referred to as Rocky Mountain bristlecone pine, in the southern part of its range (Steele, 1990).

Limber pine has wingless or near-wingless seeds that are bird dispersed, allowing for long distance dispersal across elevation gradients (Tomback and Linhart, 1990). Both limber pine and whitebark pine seeds are dispersed primarily by *Nucifraga columbiana* Wilson, hereafter referred to as Clark's Nutcracker, and both the pine and bird species have characteristics which indicate co-evolution (Tomback and Linhart, 1990).

Due to bird dispersal, limber pine colonizes disturbed areas rapidly. In harsh sites, limber pine grows in monocultures and performs many important ecosystem functions, including decreasing erosion, facilitating other plant growth, and stabilizing snowpack. Its large seeds are an essential food for *Ursus americanus* Pallas, (McCutchen, 1996), corvids, and small mammals (Lanner and Vander Wall, 1980; Tomback, 1982).

In mesic sites, limber pine may facilitate the establishment of *Abies lasiocarpa* Hooker, hereafter referred to as subalpine fir, and *Picea engelmannii* Parry ex Engelm., hereafter referred to as Engelmann spruce, post-disturbance by ameliorating site conditions (Shankman and Daly, 1988). In these more mesic sites, limber pine grows mixed with other sub-alpine to lower tree line species, such as subalpine fir, Engelmann spruce*,* *Pinus ponderosa* Douglas ex Lawson, hereafter referred to as ponderosa pine, *Populus tremuloides* Michx., hereafter referred to as aspen, *and Pinus contorta* Douglas ex. Loudon, hereafter referred to as lodgepole pine. As limber pine is a poor competitor, in more moderate sites it is often outcompeted by these species over time (Donnegan and Rebertus, 1999; Schoettle, 2004).

In the Rocky Mountains, all *Pinus* species are facing extensive mortality by *Dendroctonus ponderosae* Hopkins, hereafter referred to as mountain pine beetle (MPB), in an outbreak that is historically unprecedented (Schwant et al., 2010.) Additionally, all members of the subgenus *Strobus*, which includes limber pine, are susceptible by inoculation tests to the introduced fungus *Cronartium ribicola* J.A. Fisch., the pathogen that causes white pine blister rust (WPBR) (Hoff et al., 1980). These factors, combined with climate change, are major threats to the survival of limber pine. While genetic resistance to WPBR naturally occurs in some limber pines, these resistant trees are threatened by MPB (Schoettle et al., 2011). As climate change shifts the lifecycle of these beetles, this impact may only intensify (Schwandt et al., 2010).

The additional threats of WPBR and climate change may combine to prevent limber pine from rebounding from MPB outbreaks, as it has in the past (Schoettle et al., 2011). Since MPB kills mature, WPBR-resistant limber pines, and WPBR tends to kill younger/smaller trees faster than large trees, natural regeneration in severely impacted areas may cease, leading to species extirpation (Resler and Tomback, 2008).

With climate change shifting suitable habitat, seedlings may be unable to establish in existing limber pine stands, even if mature trees survive (Hogg and Schwartz, 1997). However, there is evidence that climate has shifted enough that artificially planted *Picea mariana*, hereafter referred to as black spruce, seeds can germinate and survive above existing tree line and krummholtz zones (Munier et al., 2010). Therefore, other tree species, including limber pine, may also be able to germinate and survive above their existing tree line.

Current research is finding trees genetically resistant to WPBR that can eventually provide a means of restoring limber pine in some sites or establishing limber pines in newly suitable habitat (Schwandt et al., 2010). Utilizing resistant planting stock will be an important management strategy to sustain the species (Schoettle and Sniezko, 2007; Burns et al., 2008). To plant resistant trees, limber pine seedlings will need to be propagated in a nursery and outplanted into areas of particular concern (Coop and Schoettle, 2009). Proscribed planting methods and techniques that optimize survival exist for *Pinus monticola* Dogl., and whitebark pine, but have not yet been developed for limber pine (Schwandt et al., 2010). Thus, the objectives of this study focus on developing planting methodology for limber pine seedlings for use by land managers in the southern Rocky Mountains.

Whitebark pine seedling survival, and therefore possibly limber pine seedlings survival, is affected by desiccation, wind, predation, and overstory density and species (Scott and McCaughey, 2006). As desiccation is a major challenge to seedling survival in xeric sites (Coop and Schoettle, 2009), planting techniques that ameliorate water stress may be important to seedling survival.

Hydrogels, or polyacrylimide gels, can improve tree survival and growth in some arid environments and in horticulture (Rowe et al., 2005; Agaba et al., 2010). However, the literature does not indicate a universal positive effect. Soil type and nutrient availability may be important factors influencing the effect of hydrogels (Rowe et al., 2005; Agaba et al., 2010).

Nurse objects are associated with limber pine and Rocky Mountain bristlecone pine establishment in xeric environments (Coop and Schoettle, 2009). These objects provide protection from the sun; increase snowpack, which protects seedlings from cold temperatures; increase soil moisture; and may enhance mycorrhizal infection (Callaway and Walker, 1997; Germino et al., 2002).

While nurse objects are sometimes assumed to provide protection from herbivory, a study of four tree species (*Acer opalus* ssp. *granatense*, *Quercus ilex*, *Pinus nigra* ssp. *salzmanii* and *P. sylvestris* var. *nevadensis*) in the Mediterranean mountains found that nurse objects had no effect on herbivory in any of their study species (Gomez-Aparicio, et al., 2008). In other high-elevation tree species, the presence of shrubs or other plants are associated with an increase in natural seedling survival (Benedict, 1984; Castro et al., 2004). Coop and Schoettle's (2009) limber pine study in the Front Range of the Rocky Mountains did not find living plants to benefit from dead or non-living objects, while Tomback et al's (2005) study, in the low elevation, eastern edge of limber pines' range, found a positive correlation between the occurrence of plants as nurse objects and the presence of limber pine seedlings

Since limber pines are poor competitors, but establish well post-disturbance (Rebertus et al., 1991; Coop and Schoettle, 2009), studies often focus on disturbance establishment. However, stand dynamics and establishment patterns post-disturbance are not inherently the same as continued establishment in an existing stand (Johnson et al., 1994). Because nurse objects and tree cover are important in seedling establishment and survival in harsh sites (Coop and Schoettle, 2009; Germino et al., 2002), canopy cover may provide protection for limber pine seedlings during initial establishment.

A planting study of whitebark pine in Gallatin National Forest in Montana found that planted seedlings had the highest survival rate after 11 years on ridges (47%) and benches (39%) (and lowest survival on swales (2%) and on a 15% slope (20%) Scott and McCaughey, 2006). Additionally, Scott and McCaughey (2006) found higher survival rates after 9 years on dry sites (86%) compared to moist sites (50%) in planting sites near Cooke City, Montana.

Based on Scott and McCaughey's (2006) planting trials and their assessment of other planting studies and literature, they recommend preparing whitebark pine planting sites in the following manner: reduce overstory competition; reduce understory vegetation except for grouse whortleberry; consider the overall topography of the planting area, avoiding swales and frost pockets; plant near stumps or provide shade in some manner; plant where there is protection from heavy snow and drifts, stumps, rocks, and large logs provide recommended microsites; avoid overcrowding to prevent competition between planted trees; plant where there is sufficient soil moisture; and plant large seedlings with good root development. Scott and McCaughey's (2006) whitebark pine guidelines provide foundation for developing limber pine planting guidelines.

While whitebark pine is similar to limber pine, both in physiology and in range, for some tree species, including the shade-intolerant *P. pinaster* Ait., hereafter referred to as maritime pine, the benefit provided by protection outweighs the cost of competition (Castro et al., 2004; Maher et al., 2005, Rodriguez-Garcia et al., 2011). Even though the planting guidelines for whitebark pine recommend removal of overstory canopy (McCaughey et al., 2009), studies are necessary to understand the impact of canopy on survival, growth, and maturation of planted limber pine seedlings. Because planting WPBR resistant limber pine in areas where they are likely to become extirpated has been identified as an important management strategy (Schoettle and Sniezko, 2007), information about how seedlings survive in areas with partial canopy versus in more exposed areas is important for planting in locations where the overstory may be killed by MPB and WPBR.

To help determine planting protocols for *P. flexilis,* we established a study to address the following questions: (1) do seedlings planted next to a nurse object have higher survival rates than seedlings planting in the open; (2) will hydrogels improve seedling survival; (3) does canopy density impact seedling survival; (4) is natural regeneration in existing limber pine stands infrequent; (5) does natural regeneration occur only in microsites with suitable ground cover and nurse object protection; and (6) is there a relationship between presence of natural limber pine regeneration in nearby forests and planted limber pine seedling survival?

Methods

In the spring and early summer of 2009, we planted 2,160 limber pine seedlings at five study sites in Colorado and one site in Wyoming. The six study sites were located in the following locations: Trout Creek in the Salida District, San Isabel National Forest

(NF); Buffalo Peak in the South Park District, Pike NF; Columbine in the Boulder District, Arapaho NF; Killpecker in the Canyon Lakes District, Roosevelt NF; Pilot Hill in the Laramie District, Medicine Bow NF; and Mosca Pass in the Great Sand Dunes National Park and Preserve (Table 1).

The seedlings were grown at the Colorado State Forest Service Nursery, Fort Collins, Colorado, from seeds collected from natural stands of limber pine growing at 2,895 m near Rollinsville, Colorado. Seeds were sown in June 2006 in a greenhouse. Seeds were placed within 2.5 x 17.8 cm cone-tainers and filled with a 50/50 peat and vermiculite soil-less mix. Seedlings were watered and fertilized with 100, 100, 150 ppm NPK mix twice a week until January through March, when they were hardened off. During hardening off seedlings were fertilized with a hardening off solution of 500 100 150 ppm NPK and watered as needed (approximately once a week). In March 2007, seedlings were moved into a shadehouse, until planted in the field. They were fertilized with 100 100 150 ppm NPK twice a week in June and July and with hardening off solutions in August and September during 2007-2008.

Planting Treatments:

Each planting site was systematically blocked into sections with high and low density canopy cover. Crown density varied from open canopy (clear felled areas or old wild fire areas) to thinned and unthinned forests with higher density canopy cover. Crown density treatments were relative for each study area, since there was variability in canopy cover between sites. At each site, we planted three plots (repetitions) in each of the high and low canopy cover areas. Crown density was measured by convex densitometer readings at the four cardinal directions, 0.1 m from each group of two to four planted seedlings, for a total of 48 readings at sites without hydrogel, and 96 readings at hydrogel sites..

Within each plot, we had a nurse object, or stump, versus a control treatment, without an object. To create stumps, we used stem sections (approximately 20 cm dia and 50 cm tall) of dead conifer trees. Each stump was buried in the ground 5-15 cm to provide stability. A stake marked the location for the site with no object. Four seedlings were planted as close as possible to the stump at the cardinal directions. For sites with no object treatments, two seedlings were planted 40 cm apart on the east and west sides of the stake. In areas without hydrogel treatment, nurse and no object treatments were randomly located in a 2 x 6 grid at 2 m apart (Mosca Pass and Trout Creek). In areas with hydrogel treatment, a 4 x 6 grid was used (Buffalo Peak, Columbine, Killpecker, and Pilot Hill. When necessary, we modified the grid to fit the planting location, maintaining randomization and at least a 2 m spacing.

At the two sites without hydrogel treatments (Mosca Pass and Trout Creek), we had a total of six stumps and six no object treatments per plot, for a total of 216 planted seedlings per site. At the four sites with the hydrogel treatment (Buffalo Peak, Columbine, Killpecker, and Pilot Hill), we had a total of 12 stumps and 12 no object treatments per plot, with half of these receiving the hydrogel treatment, for a total of 432 planted seedlings per site.

Hydrogel treatment used the following procedure: seedling roots were dipped in a slurry of Terra-sorb hydrogel (Plant Health Care Inc., Pittsburg, PA), mixed to the manufacturer’s specifications (142 g per 19 L), prior to planting. All seedlings that were not treated with hydrogel (in both the mixed sites and in the entirely hyrodgel-free sites) had their roots dipped in water prior to planting. These seedlings were only watered once, at the time they were planted, and received either 0.5 or 1 liter of water, as determined by site soil moisture at the time.

Meterological Data:

We installed an automated HOBO weather station (Onset Computer Corporation, Pocasset, MA), to measure temperature, relative humidity, and precipitation, near one of the plots with low canopy density at each site. Temperature and relative humidity measurements were recorded every 30 minutes from planting in spring 2009 until late fall 2010, while precipitation was recorded by HOBO tipping grain gauges (Onset Computer Corporation, Pocasset, MA) from May/June to October/November of 2009 and 2010.

Soil Data:

Soil samples were taken to determine nutrient content at planting, and samples were collected for soil moisture analysis each time seedlings were assessed in the summers of 2009 and 2010. For all samples, we collected three 18 cm deep soil samples in each plot by removing the duff layer and sieving the soil through 0.64 cm wire mesh. The three soil samples were located equal distances across the middle of each plot. Soil moisture samples were collected in metal soil sample cans and nutrient samples were collected in sample bags.

In the lab, soil moisture samples were weighed and then placed in a drying oven at 140°C for a minimum of 72 hours, and then re-weighed to obtain the dry weight. Nutrient analysis samples were dried and sieved through a 1 mm wire mesh. The three soil nutrient samples from each plot were then combined and sent to AgSource Laboratories – Harris, Lincoln, NE, for analysis of soil pH, buffer pH, sodium, soluble salt, nitrate, phosphorus, potassium, magnesium, calcium, estimated CEC, percent base saturation, organic matter, and particle size.

Seedling Assessment:

After they were planted in 2009, seedlings were assessed once every four weeks throughout the summer. At each assessment, seedling heights, length of 2009 terminal leader growth, and length of 2009 needles, were recorded to the nearest 1 mm. Presence of damage, insects, or pathogens was noted. In the fall, seedlings were assessed for overall health status by rating them on a standard 1 to 4 scale, using the following classifications: 1) tree was healthy with less than 5% dead needles or branches; 2) tree had 5-50% dead needles and/or branches; 3) tree had 50-99% dead needles and/or branches; 4) tree recently died (within the last five years), and still had needles and fine branches intact. In 2010, seedling health status was assessed at the beginning of the summer and at the end of the summer, along with 2010 terminal growth and needle length, and basal diameter. In the fall of 2010, seedlings were assessed for overall health by rating them on the standard 1 to 4 scale. In 2011, seedling health stats was assessed in August by rating them on the standard 1 to 4 scale.

To assess stand structure in the seedling planting sites, we installed one natural regeneration plot (4 m x 50 m) directly on each of the seedling planting subplots. The middle of the natural regeneration subplot was placed at the middle of the seedling planting plot and the long axis (50 m) of the plot followed the contour lines.

Natural Regeneration Survey:

To assess natural limber pine regeneration periodicity and related stand structure in existing limber pine stands, we installed random plots-transects in stands near the seedling planting sites. We used limber pine cover type maps from the U.S. Forest Service to find target areas. After locating at least five suitable areas, we randomly selected three areas. Each transect was made up of five, 4 x 50 m subplots. Each subplot was at least 25 m away from the other subplots and from the road.

In each natural regeneration subplot we used a Biltmore stick to measure diameter breast height (dbh) to nearest 1.0 cm at 1.35 m for each tree, and a measuring stick to measure heights of seedlings less than 135 cm tall. We also recorded tree and seedling health status and species using same health status rating used for the planted limber pine seedlings. In addition to the health classification variables mentioned in the planted seedling section, we used a fifth classification for trees that had been dead for more than five years, and no longer had needles or fine branches.

At the end of each subplot, we recorded percent slope, elevation (m) from a Garmin eTrex GPS unit (Garmin International, Inc., Olathe, KS), presence of any major damage agent on the trees within the subplot, including presence of MPB, WPBR, or dwarf mistletoe (*Arceuthobium cyanocarpum* A. Nelson ex Rydberg), and signs of historical fire. We marked the start and end of each subplot by tagging a tree at ground level and recording the GPS location.

To determine the age of limber pines in the stand, we cored a maximum of 12 limber pine trees per subplot at 0.20 m above the ground. These cored trees were distributed across the following dbh size classes: 4-10, 10.1-20, 20.1-40, and >40 cm.

For cored trees, we also measured tree height to nearest 0.1 m using either a inclinometer or a laser rangefinder (OPTi-LOGIC, Tullahoma, TN), and used a dbh tape to measure dbh to the nearest 0.1 cm. In each subplot, we also destructively sampled one natural regeneration seedling between 0.3-0.54 and 0.55-1.34 m tall, including the root ball, which ensured we had the root/shoot boundary for accurate aging. We did not sample regeneration that was shorter than 0.3 m because they were not considered to be established.

To compare microsite conditions surrounding natural limber pine seedlings with random locations without regeneration, we followed a modified version of the technique developed by Bonnet et. al. (2005) and also followed by Coop and Schoettle (2009). We sampled three square 1 x 1 m^2 microsite plots at 10, 25, and 40 m along the plot length and one plot at every naturally regenerating seedling (30 - 135 cm tall). At each microsite plot, we estimated percent ground cover of shrubs, gravel, rocks, grass, forbs, litter, logs, and bare ground. Using a convex densitometer, we took four readings of canopy cover 0.1 m from the seedling at each of the cardinal directions. We identified the three closest potential nurse objects (tree, log, stump, shrub, rock, or other) that had a diameter and height greater than 10 cm, and measured the distance to each object.

Processing Tree Cores and Sections:

Tree cores were stored in paper straws and allowed to air dry for a week before they were mounted in wooden core mounts. Tree ages were determined by counting rings using a dissecting microscope between 25-40x power, and cross dating when possible. Due to the young age of the trees and complacency (lack of sensitivity to environmental change) of limber pines, cross dating was not always possible. Seedlings were collected in paper bags and dried in an oven at 40ºC for three days. Seedlings sections were cut at the root-shoot boundary on a band saw. To find the root-shoot boundary, we looked at the change in bark texture, location of roots and branches, and the dark pith indicating shoot growth. However, due to discoloration of central heartwood and pith, pith color was not always a reliable indicator of root-shoot boundary on its own (see Appendix I). These sections were sanded and aged using the same protocol as the cores. Sixty-seven percent (390 of 586) of the tree cores and 100% (all 89) of the seedling cross sections sampled resulted in usable age data. Histograms of pith dates for both trees and seedlings were generated for analysis.

Data Analysis:

All data analysis was done using SAS version 9.2 for Windows XP Professional (SAS Institute 2002-2008). Temperature data was averaged by week and month for July and January temperatures (Table 2), while precipitation data was summed by growing season (June to October 2009, July to October 2010) (Table 2). Due to a malfunction of the data logger at Columbine, the 2010 precipitation data was not included in modeling efforts. Thirty-year average precipitation data for each site was obtained from Rehfeldt / USFS climate models (U.S. Department of Agriculture, Forest Service, 2001) (See Appendix II, Figure 4). Canopy cover was used as a continuous variable in models since the open and dense canopy blockings were relative within a site and highly variable across sites, with open canopy ranging from 8% to 42% and dense canopy ranging from 56% to 93% (Table 3).

Prior to modeling some data manipulations were performed. Microsite ground cover percentages were square-root transformed for use in modeling due to the high number of percentages below 20%. Densiometer readings for each object location (i.e., stump or stake) were averaged to get a value for the object, then averaged across the plot for a plot average. Since the effect of hydrogel was not significant in any model, all other analyses were performed averaging over this treatment. For seedling health analysis, averages for each plot were created, e.g. average health for trees on the west side of a stump in plot one at Pilot Hill. Additionally, due to the high number of healthy seedlings and the high frequency of seedlings dying following a classification of 2 or 3, all health analysis was performed comparing trees classified in category 1 versus trees classified as 2-4.

Site and stand variable means, planted seedling health data, and microsite data presented in Tables 1, 2, 3, 4, 5, 6, 10, 11, 12, 13, and 14 are all arithmetic means or percentages (PROC MEANS). Least-square means for percent gravimetric soil moisture were calculated using PROC GLIMMIX and took into account site, transect, and plot (Table 2). To protect for multiple comparison errors we used Fisher's protected t-test/lsd.

Backwards stepwise regressions were used for modeling (PROC GLIMMIX). Because this procedure does not provide a goodness of fit, we used PROC GLM to determine r^2 values for the models. We ran PROC GLM both with ($r^2{}_2$) and without ($r^2{}_1$) factors in the random statement and used the formula $r^2{}_2/(1-(r^2{}_1-r^2{}_2))$ to determine the appropriate r^2 for the PROC GLIMMIX models. Following the backwards stepwise regression process, we analyzed the AIC and generalized chi-square/df values to test goodness of fit. We tested the AIC of models by individually dropping variables to find the model with the lowest AIC. From these analyses, we presented models with generalized chi-square/df values between 0.16 and 2.30. Models with chi-square/df values greater than 12 indicated poor fit and are not presented in detail.

Models of planted seedling health, 2010 terminal growth length, and 2010 needle length (Figure 1, and Tables 7, 8, and 9) all used PROC GLIMMIX models starting with the following variables: seedling height at planting, mean soil moisture, mean canopy cover, elevation, percent slope, aspect, percent clay, mean dbh of surrounding trees, mean basal area of surrounding trees, total summer 2009 precipitation, mean July 2009 and 2010 temperature, mean January 2010 temperature, and orientation to object. We did not use basal area by species because several of the species were specific to one or two sites, and the tree species that were common across sites were correlated with overall basal area, which prevented the model from converging. Live basal area was also not included in the initial model because it caused similar problems in the model due to its correlation with basal area. Site and plot nested within site were in the random statement of the model. Variables were left in the model when they had a significance of $p<0.05$.

Models of natural regeneration seedling density and age of natural regeneration seedlings all used PROC GLIMMIX models starting with the variables: microsite ground cover, distance of natural regeneration seedling to three nearest objects, type of three nearest objects, basal area of surrounding trees by species, percent live trees of stand, percent slope, aspect, elevation, and mean canopy cover (Table 15). The results of the model using the above site and microsite characteristics to predict natural regeneration seedling age are not presented because the generalized chi-squared/df value of 12.5 indicates that the variables do not sufficiently fit the model.

Mature tree age and height were also modeled using PROC GLIMMIX and the following site and tree variables: dbh of tree, basal area of surrounding trees by species, percent slope, aspect, elevation, percent live trees of stand, presence or absence of WPBR in the stand, and mean canopy cover. The covariate of height was included in the age model, and the covariate of age was included in the height model. The height model had a generalized chi-squared/df value of 462, which indicates that the variables do not sufficiently fit the model. The height model was reduced down to positively correlating mature tree height with dbh, and is not included.

To test for a relationship between the number of natural regeneration limber pine trees and the number of healthy planted seedlings, we performed a Spearman correlation of coefficients test (PROC CORR). For this correlation we compared the total number of natural regeneration seedlings in all three transects to the number of healthy planted seedlings in August 2011 at the nearby planting site.

Results

Seedling Planting Site Characteristics:

Site characteristics varied; however, most were similar across planting study sites (Table 1). Elevations ranged from 2680 m at Pilot Hill to 3196 m at Buffalo Peak, and slopes were gradual (0-12%) at all sites (Table 1). While the soil pH (6.3 to 7.5), texture (sandy loam to clay loam), percent organic matter (1.6 to 5.6%), nitrate (1.0 to 6.5 ppm), P (11.6 to 57.0 ppm), and K (78 to 228 ppm) characteristics varied between sites, the variation within sites was minimal (see Appendix II, Table 16).

Overall, mean January 2010 and July 2009 and 2010 temperatures fluctuated similarly across sites and only varied by a few degrees between (Table 2). Excluding Buffalo Peak's missing data, July 2009 average temperatures were similar across all sites and ranged from 13ºC at Columbine and Killpecker, to 15ºC at Trout Creek. July 2010 mean temperatures were similar, ranging from 13ºC at Buffalo Peak to 16ºC at Trout Creek. January 2010 temperatures were also similar, and ranged from -9ºC at Killpecker to -6ºC at Columbine.

Precipitation in 2009 and 2010 varied from less than 2 cm to greater than 6 cm across sites (Table 2, see Appendix II Figure 4). For the 17 weeks from June 7th to October 3rd, total precipitation in 2009 ranged from 3.1 cm at Killpecker to 31.7 cm at Columbine. Total precipitation from July to October 2010 ranged from 4.4 cm at Killpecker to 17.6 cm at Mosca Pass. Total monthly precipitation in 2009 and 2010 was similar or lower than 30-year averages (see Appendix II, Figure 4). Least-square-mean soil moisture from six readings, four in 2009 and two in 2010, ranged from 8.1 to 15.6% across all sites (Table 2). Relative soil moisture across sites did not vary in the same manner as relative precipitation across sites (Table 2).

Forest structure varied between sites and between open and closed canopy at each study site (Tables 3 and 4). The total basal area of all species in the open canopy plots ranged from 26 to 248 m^2/hectare, while in the dense canopy plots basal area ranged from 179 to 614 m^2/hectare (Table 3). The mean dbh of all species ranged from 2.0 to 32.6 cm across all plots. In the open canopy sites the density of stems of all species taller than 135 cm ranged from 283 to 3033 stems/ha. In the dense canopy sites the density of stems of all species taller than 135 cm ranged from 967 to 3783 stems/ha (Table 3).

The canopy in planting sites was composed of conifer species, most commonly spruce, lodgepole pine, fir, ponderosa pine, and Douglas-fir, with aspen as the only hardwood (Table 4). Pilot Hill had the least variation in species, with most of the basal area comprised of limber pine. The northern sites had more homogenous forests, whereas the sites farther south had more heterogeneous forests, incorporating up to six species at a site.

Planted Seedling Survival and Growth:

Seventy-six percent of planted seedlings were alive after three growing seasons, and 65% were classified as health status 1, "healthy" (Table 5). The percentage of live planted seedlings by site ranged from 56% to 84%, and the percentage of healthy planted seedlings ranged from 40% to 78% (Table 5). Due to these high levels of health and survival, and since classification as live but not healthy (2 or 3) was a good predictor for seedlings death (86% of planted seedlings classified as a 2 or 3 in fall 2009 to August 2010 were dead by August 2011), all treatment effect analysis was performed comparing seedlings classified as healthy to all others (status rating 1 vs. 2 to 4).

Overall, seedling health and survival both gradually declined each growing season (Table 5). Each site had approximately 5-10% mortality each growing season, except Pilot Hill, which had a 33% decline in live seedlings between the end of the second growing season to the end of the third (Table 5).

The percent of healthy seedlings declined in a similar pattern, with declines of approximately 5-15% of healthy seedlings per growing season (Table 5). The seedlings at Pilot Hill showed a large decline in healthy seedlings over growing seasons two and three, with a 22% decline between seasons one and two, and a 36% decline between seasons two and three.

At the last revisit of the seedlings, in August 2011, the percent of healthy seedlings without an object (14-81% open, 56-59% dense) was lower than the overall percent of healthy trees at all sites under both open (18-82%) and dense (63-91%) canopy cover (Table 6). The percent of live seedlings did not follow this clear pattern (Table 6).

At the end of the third year, the highest percent of healthy trees at a site was located either on the north or west side of an object both canopy densities, with 5 to 15% more healthy trees than any other orientation under dense canopy and 3 to 14% more healthy trees under open canopy. The one exception was the seedlings under dense canopy at Mosca Pass, where the highest percent of healthy seedlings was on the east side of an object (6% higher than any other orientation) (Table 6). The highest percent of live trees followed the same orientation to object pattern, except for under dense canopy at Columbine, where the highest percent of live seedlings were planted without an object (Table 6).

Modeling Planted Seedling Health:

The regression model predicting the percent of healthy planted seedlings included mean canopy cover (%), elevation (m), 2009 growing season precipitation (cm), and orientation to object (Table 7, R^2=0.70). Site effect was not significant. All variables were positively correlated with planted seedling health: for each 10% increase in canopy cover, there was a 22% increase in the percent of healthy seedlings; for each 100 m increase in elevation, there was a 15% increase in the percent of healthy seedlings; for each 1 cm increase in precipitation in 2009, there was a 36% increase in the percent of healthy seedlings; and for each 1 cm of seedling height at planting, there was a 9% increase in the percent of healthy seedlings.

Modeling Planted Seedling Growth:

Planted seedling growth in the 2010 growing season varied greatly; at the end of the growing season, terminal growth ranged from 0 to 22 cm and needle length ranged from 0 to 9 cm. The only significant variable predicting 2010 terminal growth was mean percent canopy cover (Table 8, R^2=0.61). With a 25% increase in canopy cover, terminal growth length increased by 0.6 cm. The model predicting 2010 needle length included mean percent canopy cover and seedling height at planting (cm) (Table 9, R^2=0.70). Needle length was 0.4 cm longer with a 25% increase mean canopy cover and 0.2 cm shorter with each 5 cm increase in initial planting height. Site was not significant in either model.

Treatments:

Hydrogel, canopy cover and nurse object treatments differed in their impact on seedling health. Hydrogel treatment did not have a significant effect in any model, therefore variables were averaged over this treatment. Canopy cover was a significant variable in models predicting tree health, 2010 terminal growth length, and 2010 needle length (Tables 7, 8, and 9). Even when used as a categorical variable by dense versus open canopy, the percent of healthy seedlings was higher in the dense (73%) versus the open (53%) plots (Table 9), and the least square means of needle length and 2010 terminal leader length were both longer under denser canopy (p=0.05) (Tables 8 and 9) .

The least-square-mean percentage of healthy planted seedlings in August 2011 that were planted next to a nurse object was 73%, compared to 63% planted without a nurse object (p<0.0001) (Table 7). When analysis was performed using orientation to a nurse object rather than presence/absence of an object, orientation to the object remained significant, and there was no interaction with site or correlation between canopy cover and orientation to an object. The percentage of healthy planted seedlings was greater on the north side (77%) than on the east (68%), or for trees planted without an object (63%) (p<.05) (Table 7, Figure 1). Difference between the percent of healthy planted seedlings on the north side (77%) and on the south side (69%) was significant, at p=0.056. The percentage of planted healthy seedlings on the west side (77%) was greater than the percent for planted seedlings on the east side, south side, or without an object (p<0.05).

Planted Seedlings Damages:

We did not observe any WPBR infection on the planted seedlings, and evidence of damage due to other diseases, insects, herbivory, or snow pack was rare. WPBR was present in the surrounding stands at Mosca Pass and Pilot Hill. There were no seedlings with insects, disease, or herbivory as a direct cause of death. Only 1% of seedlings had damage that may have been caused by herbivory. Most insects noted were sucking insects, and there was only one seedling in August 2011 that appeared to be damaged directly due to sucking insects.

The Pilot Hill site had high levels of *Thomomys talpoides* Richardson, hereafter referred to as pocket gopher, with gopher mounds present directly in 100% plots in the site. However, we did not observe any clear evidence that any dead planted seedlings were killed by pocket gopher activity. Dead seedlings did not pull out of the ground easily. If they had, this could have indicated pocket gopher root damage. Pocket gopher activity was also observed at Columbine, but only near one planting plot, not directly next to the planted seedlings. At the two furthest north sites (Pilot Hill and Killpecker), there were a few planted seedlings (< 0.5% of all seedlings) that were flattened and killed by snow pack.

Natural Regeneration Transects:

Our transects within existing limber pine stands captured a wide range of site and stand conditions (Tables 10 and 11). Mean elevation ranged from 2,652 m to 3,385 m; mean aspect ranged from 170 – 244 degrees; and mean slope ranged from 5 – 39 percent (Table 10). Mean canopy cover, from the random microsite data, ranged from 26% to 63%. Mean dbh ranged from 7.2 to 15.5 cm for all tree species, and 6.3 to 18.1 cm for limber pines (Table 11). Basal area ranged from 65.7 to 249.1 m^2/hectare for all species and 20.5 to 219.6 m^2/ha for limber pines (Table 11). Limber pine stems accounted for 18 to 75% of the living mature trees (Table 11). Seedling density for all species ranged from 42 to 178 stems/ha, and for limber pines, seedling density ranged from 13 to 300 stems/ha (Table 12).

The distance to nurse objects, percent canopy cover, and type of ground cover all varied greatly within microsite type (naturally regenerated limber pine seedling microsite versus random microsites) compared to variation across microsite type (Tables 13 and 14). Canopy cover was 43.8% for natural limber pine seedling microsites and 42.1% for the random plots, with standard deviations of 33.7% and 32.4%, respectively (Table 13). Mean distances to the three nearest objects for naturally occurring limber pine were 40.9 +/- 49.6, 52.0 +/- 50.6, and 67.1 +/- 62.5 cm, compared to 95.0 +/- 152.4, 120.4 +/- 148.2, and 142.3 +/- 182.1 cm for the random microsites (Table 14). The high variance in distance to the three nearest objects, indicated by the standard deviations presented, prevented this data from having predictive power. Thus, models predicting natural limber pine seedling occurrence and age by microsite or site characteristics had very poor fit (see methods for models attempted and variables used).

The model using site and microsite variables to predict natural limber pine seedling regeneration passed our goodness of fit criterion, but had a low ability to explain variation between treatments, R^2 =0.39 (Table 15). In this model, percent ground cover that was a tree in the microsite and the basal area of limber pine in the surrounding stand were both positively correlated with higher densities of naturally regenerating limber pine seedlings. Presence of WPBR in the plot was not a significant in this model. For each 1% increase in percent groundcover of tree in the microsite (i.e. the percent of the microsite covered by tree or seedling stem), the density of naturally regenerating limber pine seedlings increased by 92.2 stems/ha. For each 25% increase in basal area of limber pine in the surrounding stand, the density of naturally regenerating limber pine seedlings increased by 11 stems/hectare.

Tree age distributions from tree cores in the natural regeneration surveys were similar to tree age distributions from stand surveys directly in the planting plots (Figures 2 and 3). When grouped by decade, trees and seedlings were found, originating in most decades, and dating back to the late 1800s or early 1900s in all transects. Pilot Hill, Buffalo Peak, and Trout Creek sites had trees that dated back to the 1700s. The oldest tree in our survey was found at Killpecker, and dated to 1278.

We only observed evidence of historical fire related disturbance but did not observe evidence of recent major disturbance in plots. There were biotic disturbances present in many of the natural regeneration transects; however, none of these were causing high levels of visible tree mortality. Since dead young seedlings decompose rapidly, we cannot made conclusions about overall regeneration mortality. From a total of 18 transects, 11 had trees with insect damage, 8 had trees with WPBR, 5 had limber pine dwarf mistletoe, and 5 had signs of pocket gophers.

When the percent of healthy planted limber pine seedlings was compared with the density number of naturally regenerating limber pine seedlings in the nearby natural regeneration transects, there was no relationship (R^2=0.16).

Discussion

We had unexpectedly high levels of survival in our planting study. Three growing seasons after planting, 76% of our seedlings were still alive and 65% were classified as healthy (Table 5). We did find that seedling health was affected by the presence of a nurse object and canopy density. While further studies and longer term results are necessary for generalized conclusions, the results after three growing seasons indicate that planting limber pine seedlings is a viable way to establish limber pine regeneration cohorts.

Our plots ranged across a relatively small elevation gradient, 2680 – 3196 m, with a comparatively wide latitudinal gradient. The planting sites also were in different forest types. Therefore, while our highest proportion of healthy planted seedlings was at our highest elevation site at a mid-range latitude for our study, and the lowest was at the lowest elevation site furthest north, it is likely that elevation and latitude are confounded, preventing us from making clear conclusions about them for planting guidelines. Because the elevation gradient of our plots is only a small portion of limber pine's elevation range, (Schoettle and Rochelle, 2000) our planting protocols should be applied to different elevations with caution.

We used seedling health, terminal length growth, and needle length parameters to determine which site characteristics are important for seedling survival so we could develop planting protocols. These protocols will be useful for land managers. Due to the unexpectedly high numbers of healthy and surviving seedlings, and a high correlation of seedlings classified with a health status of 2 and 3 during early assessments that died (health status 4), health data analysis compared seedlings classified as healthy (status rating 1) to all others (2 to 4).

Planting Treatments:

Canopy

Our results indicate that dense canopy provides protection for planted seedlings (Tables 7, 8, and 9). While percent canopy cover was not significant in the model predicting natural limber pine seedling density, increasing basal area of limber pine was positively correlated with higher numbers of naturally regenerating limber pine seedlings (Table 15). However, this correlation may simply be indicating higher seedling density closer to a seed source.

Based on our planted seedling results as well as Rodriguez-Garcia et al.'s (2011) research on the shade-intolerant maritime pine, as well as Germino and Smith's (1999) research on photoinhibition in seedlings without canopy cover, we recommend planting seedlings under canopy cover. In our plots, mean dense canopy cover ranged from 55.8% (s.d. 12.6%) to 92.1% (s.d. 6.0%), and mean open canopy ranged from 8.1% (s.d. 6.5%) to 34.0% (s.d. 8.6%). Based on our model, which analyzed canopy as a continuous variable, a 10% increase in canopy cover was associated with 22% increase in the percent of seedling health.

Because our study only covers the first three growing seasons after planting, we cannot make long term recommendations. It is possible that canopy cover may slow limber pine maturation, or impact tree reproduction. Therefore, further studies are necessary to understand the long term impact of canopy cover on planted limber pine seedling survival, maturation and reproduction.

The positive correlation between the proportion of healthy planted seedling and canopy density runs counter to the prevailing thought that limber pines require openings to establish, as well as the guidelines for planting whitebark pine (McCaughey et al., 2009) (Table 7). However, it agrees with Rodriguez-Garcia's (2011) study of maritime pine, a shade-intolerant conifer that grows in the drought-stressed habitat of the Mediterranean mountains of Spain. Since our study only covers the first three growing seasons post-planting, we cannot make long term conclusions about the shade tollerance of limber pine. Rebertus et al. (1991) found that some seedling-sized limber pines were suppressed older trees, and also found evidence that these suppressed small trees die. However, Rebertus et al. (1991) did not age the smallest seedlings at their sites, possibly causing them to miss new waves of recruitment.

The age of sampled seedlings taller than 30 cm on the stand structure surveys indicate regeneration originating between 1968 to 1997 (n = 23 across three sites with limber pine), except one seedling that dated to 1937 (Figure 3). This range of pith dates at the root/shoot boundary indicates that seedling establishment is ongoing in these stands, and that the seedlings are not simply repressed, older trees.

The differences in planted seedling response to canopy at these different sites indicate that the influence of canopy cover is not straightforward. Despite this complexity, mean canopy cover was a positively correlated significant factor in all models of planted seedling health and growth (Tables 7, 8, and 9).

It is possible that conditions required for natural establishment of seedlings may be different than those required for planted seedlings, which may be related to our canopy results. The seedlings we planted ranged from 9 to 28 cm tall, which allows them to be stronger competitors than newly emergent seedlings. Therefore, the benefit of canopy cover may outweigh the negative effect of competition from other trees that provide the canopy cover. Additionally, Clark's Nutcracker may preferentially cache seeds in burns, skewing the presence of limber pine in open areas by seeding them with higher densities of seeds (Tomback, 2001).

Nurse Object

The percentage of healthy trees with a nurse object (73%) versus without a nurse object (63%) supported our answer to the question if nurse objects can help improve seedling survival and health (Table 7, $p<0.0001$) (Colack, et al., 2003; Coop and Schoettle, 2009; Barbeito et al., 2009). The higher percentage of healthy planted seedlings on the north side of the nurse object supports concurs with the findings of previous studies that nurse objects protect seedlings from intense solar radiation, which is believed to cause photoinhibition and reduce soil moisture, leading to desiccation (Figure 1) (Castro et al., 2004; Germino and Smith, 1999). The higher number of healthy trees on the west side of an object may be a result of protection from high intensity sunlight early in the day when needles are cold. High intensity sunlight during cold temperatures causes photoinhibition in Engelmann spruce and sub-alpine fir (Germino and Smith, 1999; Ronco 1970b). However, since we did not have an interaction between canopy and nurse object effect, it is possible that the nurse objects are offering additional protection beyond shade.

Due to our use of artificial nurse objects, we cannot make any recommendations about type of naturally occurring object to plant next to. However, digging holes next to objects is difficult, and one cannot plant directly next to stumps or living trees due to root flares. Large rocks on the landscape may indicate rocky areas with shallow rocky soil that prevents digging and may negatively impact seedling survival. Jacobs and Steinbeck's (2001) success with artificial shelters and Ronco's (1970a) success with shingles to protect Engelmann spruce seedlings in the central and southern Rocky Mountains indicate that shelters or shingles may be a viable option for use in protecting planted limber pine seedlings as well. While these shelters have the possible drawback of introducing artificial materials into the landscape, they solve the problems caused by planting near naturally occurring objects. Wooden shingles do not require introducing artificial materials into the environment, but require removal overwinter so they do not damage the seedlings by flattening under snowpack (Ronco, 1970a).

Hydrogels

While further research on hydrogels under different soil and moisture conditions may provide alternative results, we currently do not recommend the use of hydrogel in limber pine plantings because it had no effect in any of the parameters we measured. The placement of compounds that have an unknown half life and long term effects in natural ecosystems is another concern for the use of hydrogels in planting projects.

Even though the precipitation planted seedlings experienced was not higher than average (see Appendix II, Figure 4) (U.S. Department of Agriculture, Forest Service, 2011), it is possible that the type of seedlings we used for planting influenced the effectiveness of our hydrogel treatment. The lack of any positive effect of the hydrogels in our study may have been because the seedlings were well prepared for outplanting. Additionally, the seedlings were cone-tainer grown, and not bare root, therefore the hydrogels were not in direct contact with all the roots of the seedlings.

Other research on hydrogels indicates that hydrogel is most effective in sandy soils; therefore, it is possible that hydrogels could have a beneficial effect in areas with sandier soils than our study sites, which ranged from sandy loams to clay loams (see Appendix II, Table 16) (Agaba et al, 2010). Hydrogel effect is also linked to tree species and brand of hydrogel (Agaba et al., 2010). Since there have been no limber pine-specific hydrogel studies and hydrogel ingredients are proprietary, we cannot make direct comparisons between our results and other studies for either of these two factors.

Growth Parameters:

Terminal leader and needle growth are good measures of establishment in a research test such as ours. In the planted seedlings, greater growth in terminal leaders and needles was positively correlated with tree health. However, the measurements are not an efficient way to measure the probability of seedling survival since health status rating are faster and can predict survival. In addition to positively impacting planted seedling health, mean percent canopy cover was positively correlated with terminal leader growth in 2010, and 2010 needle length (Tables 7, 8, and 9). Mean percent canopy cover was the only significant variable in the model of terminal leader growth in 2010

(Table 8), and one of two significant variables in the model of needle length (Table 9). The importance of canopy cover in seedling growth is supported by the literature, since growth and survival are correlated, and others have found canopy cover is important for seedling survival, even in shade-intolerant conifer species (Rodriguez-Garcia et al., 2011; Germino and Smith, 1999).

The relationships between seedling health and growth with precipitation, seedling height at planting, and elevation in the models probably indicate support of basic physiological needs of the planted seedlings. Precipitation is positively correlated with healthy planted seedlings, as water stress is a frequent cause of seedling death (Bernier, 1993; Grosnickle, 2005) (Table 7). The positive correlation with planted seedling health and height at planting indicates that the taller seedlings had more stored resources to help them survive the initial growth check and root establishment experienced by seedlings when they are outplanted (Table 7) (Grossnickle, 2005). However, the relationship between seedling survival and height is probably not simply linear; taller seedlings also require more resources for survival, therefore height can become limiting. The positive correlation between elevation and healthy seedlings may indicate that elevation is a proxy for some other variable, such as depth and persistence of snow pack, since limber pines naturally occur at a wide range of elevations (Table 7) (Schoettle and Rochelle, 2000).

The negative correlation between initial planting height and needle length in 2010 may indicate that larger seedlings had enough needles to support their carbon needs through photosynthesis (Table 9) (Grossnickle, 2005). Instead of needle growth, taller seedlings may have been able to allocate more resources into root growth to overcome water stress (Grossnickle, 2005). Conversely, taller seedlings may have been under more stress from planting than smaller seedlings, and thereby resulted in shorter needles. The shorter 2010 needles in the taller seedlings is a further indication that taller seedlings are not simply better for outplanting.

Herbivory, Insects, Disease, and Abiotic Damages:

We observed little evidence of seedling damages from animals, insect or snow creep. When insects were observed, they were not directly damaging the trees. In a few cases, herbivory by large ungulates was suspected because a few trees had several centimeters of terminal growth removed between site visits. The low impact of herbivory in our study is similar to Ronco's (1970a) low levels of herbivory in his Englemann spruce planting experiments. The combination of Ronco’s and our results indicate that in the Colorado Rocky Mountains herbivore exclusion from seedling planting sites may be unnecessary. However, our planting sites were a small presence in the forests, and if the plantings were larger they may have attracted more herbivores.

We did not have a problem with snow creep uprooting trees or knocking over artificial stumps. Only one stump in the entire study was leaning following the first winter, and it has remained stable at its angle since. Since all of our sites had mild slopes, ranging from 0-12 percent, we do not know how snow creep would influence seedlings or artificial nurse objects on steeper slopes. However, in steep mountain terrain, the protection objects provide may include protection from snow creep (Scott and McCaughey, 2006).

Natural Regeneration Survey:

Among the natural regeneration survey transects and the stand surveys of natural regeneration directly within the planting plots, there were trees or seedlings with pith dates in most decades since 1819 at Plot Hill, 1870 at Killpecker, 1910 at Columbine, 1900 at Buffalo Peak, 1910 at Trout Creek and 1860 at Mosca Pass, (Figures 2 and 3). This indicates that in these areas limber pine establishment has not been a rare event in the last century. While we do not have disturbance histories for these areas, we did not observe signs of recent major disturbance. Thus, the presence of at least some regeneration dating to most decades in the last century in the surveyed stands, suggests that regular major disturbance events are unlikely. Additionally, plots had a wide range of tree sizes and ages, indicating a mixed stand that would not be present if large, recent disturbance, such as stand replacing wild fire, had occurred. Due to differences in size classification of seedlings and trees, we cannot compare our densities of trees to those in Coop and Schoettle's (2009) study of post-fire regeneration. While our results are counter to the common paradigm focusing only on limber pine establishing post disturbance (Rebertus et al., 1991), these results do agree with Knowles and Grant's (1983) findings that limber pine establishment in mixed conifer forests regularly occurs at low levels.

Further research is necessary to make more refined conclusions about the periodicity of regeneration. A larger sample size at each sampling location is necessary for more refined conclusions. Sampling of dead and downed trees, as well as the inclusion of data indicating the presence of uncorable trees would build a stronger data set for conclusions of smaller years. Due to our small sample size, the lack of analysis of dead and downed trees, and the high rate of decay for dead seedlings, we cannot make any conclusions about missing data in our data set.

Due to the lack of fit of our models relating to natural seedling presence and relationships between seedling or tree age and height, we can make few conclusions about factors that influence natural limber pine establishment or persistence. Since most of our models did not meet our goodness of fit criteria, we can conclude that factors promoting limber pine survival and growth may be highly inter-correlated with a large number of variables.

Since our model predicting the location of natural regeneration using microsites did not meet our fitness criteria, we cannot compare our data to Coop and Schoettle's (2009) findings regarding important microsite conditions for natural limber pine seedling establishment in burned and nearby forested sites. Their ability to fit quality models to their data could be attributed to the fact that they had smaller microsites (0.25 m x 0.25 m) and a much larger sample size (740 microsite plots versus our 270 microsite plots).

The one model using our natural seedling regeneration data that did have a reasonable goodness of fit used microsite and site characteristics to predict naturally regenerating seedling density (Table 15). In this model, percent ground cover of trees in the microsite and limber pine basal area in the surrounding forest were both positively correlated with higher densities of natural seedling regeneration. These factors support results from the planted seedling study as well as outside literature. Tree presence in the microsite may be acting as a nurse object (Germino et al., 2002, Tomback et al., 2005). Tomback et al. (2005) found a correlation between plants and natural limber pine seedling presence in the isolated limber pine stands of eastern Colorado, furthering evidence that naturally occurring limber pine seedlings may be facilitated by plants, much like nurse objects. Higher basal area is often associated with higher canopy cover, which was significant in our planting study. Canopy cover was also a significant factor in a study of natural regeneration of maritime pine, which is another shade-intolerant conifer species (Rodriguez-Garcia et al., 2011). Our findings support Rodriguez-Garcia et al.'s (2011) conclusion that even shade intolerant species may benefit from canopy cover to facilitate natural seedling establishment. But, the shaded environment may not support long-term survival and maturation.

We did not find a relationship between density of naturally occurring limber pine seedling regeneration and health of our planted seedlings. Our results are contrary to the findings of Elman and Peterson (2003), who studied *Abies amabilis* Dougl. ex Forbes and *Abies procera* Rehd., and found a positive relationship between survival of planted seedlings and the density of natural regeneration. It is possible that planted limber pine seedlings require different conditions than naturally occurring regeneration. Artificially planted seedlings are not dependent on the locations Clark's Nutcrackers select for seed caching, they are not subject to the pressures of seed herbivory, and they are already taller than some of the surrounding vegetation that may provide competition. Additionally, the differences in forest composition between our planting sites (Table 4) and our natural regeneration survey sites (Table 11) indicate that the different sites may not be comparable.

Future Research

Future studies are necessary to understand the important factors for successful natural and artificial regeneration of limber pines. To understand natural regeneration, studies comparing regeneration periodicity to climate modeling may provide further information on what meteorological factors contribute to successful natural regeneration.

Our data show that there are higher rates of healthy planted limber pine seedlings under canopy, near a nurse object, at higher elevations, with more precipitation, and that seedling height is an important survival and growth factor, three growing seasons after planting. We do not know how the factors in our study will impact long term survival and maturation, nor do we know how loss of canopy cover over time due to MPB or WPBR would impact survival and maturation. Studies with larger sample sizes; greater elevation ranges; greater diversity in canopy densities, including clearcuts; and various disturbances are necessary to understand the microsite characteristics and nurse object factors that are important for natural limber pine regeneration, continuing to expand on the work of Coop and Schoettle (2009).

Further planting studies are necessary to understand important microsite characteristics, as well as the long-term influence of canopy cover on seedling maturation and survival. Because our techniques of installing nurse objects are not practical for large scale planting, studies using alternative nurse objects or methods of seedling protection are necessary, possibly following up on the work with shingles or tree shelters that has been done with Engelmann spruce (Ronco, 1970a; Jacobs and Steinbeck, 2001). While stumps of MPB killed trees may be an option, planting seedlings would probably need to be 0.5 m from the stump to avoid roots. Planting studies that include disturbance and planting in MPB and WPBR disturbed stands will further our knowledge of any factors important to planting success in these disturbed areas that are targets for limber pine outplanting.

Conclusions

In conclusion, for optimum survival and growth in the first three years, we recommend planting limber pine seedlings next to a nurse object, and preferably on the north or west side of a nurse object. Additionally, we recommend planting in areas with higher precipitation. Higher elevations may also be beneficial, although we suspect elevation is a proxy for other environmental variables. Seedling height at planting was positively correlated with seedling health, but negatively correlated with 2010 needle length, indicating a complex relationship between seedling height at planting and seedling survival and growth.

While the survival of well planted seedlings without a nurse object was acceptable (63%) three growing seasons after planting, further study of seedling health is vital to determine the future survival. We have observed approximately a 10% death rate of seedlings during each growing season, and approximately a 10% decrease in healthy seedlings in each of the first two growing seasons, with nearly a 20% decrease in healthy seedlings between the second and third growing seasons (Table 5). Therefore, seedling survival and health results may be more drastically different five or ten years after planting. Future monitoring of the planted seedlings will provide answers to some of these questions.

Tables and Figures

Table 1: Limber pine planting sites along eastern side of Colorado Front Range and southern WY, 2009

Site[1]	UTM Coordinates[2]		Elevation[4] (m)		Slope[5] (%)		Aspect[6] (degrees)	
			mean[3]	std	mean[3]	std	mean[3]	std
Pilot Hill Medicine Bow NF	13 T 0462953	4568390	2680	54	2	1	138	9
Killpecker Arapaho-Roosevelt NF	13 T 0442451	4516571	2998	17	8	4	234	161
Columbine Arapaho-Roosevelt NF	13 S 0452012	4407606	2843	17	1	1	30	14
Buffalo Peak Pike NF	13 S 0406271	4320156	3196	9	4	2	175	186
Trout Creek San Isabel NF	13 S 0415082	4306549	2952	39	4	2	15	7
Mosca Pass Great Sand Dunes NP&P	13 S 0459417	4176079	2948	30	3	1	260	23

1: Each site consists of six plots.

2. UTM coordinate for the first plot in each category was used and is in NAD 83 projection.

3. Arithmetic means

4: Elevation was recorded from a Garmin eTrek GPS

5: Slope was estimated visually

6: Aspect was recorded using a compass.

Table 2: July and January mean temperatures, total summer precipitation, and soil moisture for six limber pine planting sites, 2009 and 2010

	Temperature (ºC)[1]						Total Summer[2] Precipitation (cm)		Soil Moisture[3]	
	July 2009		January 2010		July 2010					
Site	mean	std	mean	std	mean	std	2009	2010	least-square-mean (%)	s.e.
Pilot Hill	13.57	12.34	-7.50	12.59	14.84	12.17	1.67	1.23	11.9	0.64
Killpecker	12.63	12.51	-9.40	14.76	13.77	12.36	1.21	1.1	10.4	0.64
Columbine	12.74	11.36	-6.12	12.61	13.83	11.45	5.41	-[4]	10.4	0.64
Buffalo Peak	-[4]	-	-8.39	13.55	12.64	11.79	1.35	1.74	15.6	0.64
Trout Creek	15.05	9.82	-7.81	15.75	15.88	10.24	2.94	3.97	11.9	0.64
Mosca Pass	14.23	10.73	-6.70	13.52	15.03	11.19	3.49	6.02	8.1	0.64

1: For each month's temperature data n=1488.

2: June 7th – October 3rd in 2009, July – October 2010.

3: Soil moisture was collected four times in 2009 and twice in 2010, numbers are least square means of these six data points

4: Data logger malfunction caused the loss of July 2009 temperature data at Buffalo Peak and 2010 precipitation data at Columbine.

Table 3: Stand characteristics of limber pine planting sites.

Site	Canopy	Basal Area (m^2/hectare)[1]	Mean DBH[2]			Mean Percent Canopy Cover[3]			Stems per hectare[4] taller than 135 cm
			n (trees)	mean	std	n (plots)	mean	std	
Pilot Hill	Dense	283	159	7.4	9.0	72	93.1	6.0	2650
	Open	195	105	6.5	10.0	71	29.4	13.5	1750
Killpecker	Dense	614	86	19.6	12.7	72	72.3	7.8	1433
	Open	43	30	9.7	3.7	72	11.5	8.7	500
Columbine	Dense	273	108	9.0	10.6	72	77.5	6.1	1800
	Open	181	19	32.6	15.6	72	34.0	8.6	283
Buffalo Peak	Dense	179	99	7.0	9.5	72	55.8	12.6	1650
	Open	26	47	2.2	6.2	70	8.1	5.4	783
Trout Creek	Dense	350	58	18.3	11.4	36	81.1	6.5	967
	Open	246	42	18.7	10.0	36	41.5	6.2	700
Mosca Pass	Dense	468	227	10.0	7.6	36	81.1	8.7	3783
	Open	248	182	7.4	7.1	36	27.3	9.3	3033

1: Basal area is from the 4x50 m transects centered in the center of the planting plots and includes all trees of all species taller than 135 cm.

2: DBH (diameter at breast height, 135 cm) is from all trees of all species in the 4x50 m plots centered in the center of the planting plot and was measured in cm. Trees less than 135 cm in height were not included in this measurement.

3: A canopy measurement using a convex densitometer at the four cardinal direction was taken over each stump or stake, then averaged over all replication within a low or high canopy treatment

4: Stems/ha includes all species at least 135 cm tall from the 4x50 m plot centered in the center of the planting plot.

Table 4: Stand composition by species of six limber pine planting sites planted along the eastern side of the Colorado Front Range and southern WY, 2010

Site	Canopy	Species	n Trees	Stems/ha[1]	% Live Stem	Basal area[2] m^2/ha
Pilot Hill	dense	limber pine	72	1200	96	231
		aspen	87	1450	98	52
		total	159	2650	97	283
	open	limber pine	43	717	100	139
		aspen	62	1033	97	56
		total	105	1750	98	195
Killpecker	dense	fir	29	483	100	86
		lodgepole pine	45	750	93	406
		spruce	12	200	100	121
		total	86	1433	97	614
	open	Fir	2	33	100	7
		Lodgepole Pine	28	467	100	36
		Total	30	500	100	43
Columbine	dense	lodgepole pine	50	833	92	181
		aspen	58	967	72	92
		total	108	1800	81	273
	open	lodgepole pine	16	267	94	167
		aspen	1	17	100	14
		total	17	283	94	181
Buffalo Peak	dense	lodgepole pine	14	233	100	90
		spruce	1	17	100	0
		aspen	84	1400	80	89
		total	99	1650	83	179
	open	lodgepole pine	3	50	100	21
		spruce	1	17	100	2
		aspen	43	717	95	4
		total	47	783	96	26

Table 4 continued:

Site	Canopy	Species	n Trees	Stems/ha[1]	% Live Stem	Basal area[2] m2/ha
Trout Creek	dense	fir	6	100	100	29
		lodgepole pine	1	17	100	13
		ponderosa pine	1	17	100	8
		spruce	17	283	100	106
		aspen	6	100	67	29
		Douglas-fir	18	300	89	142
		unknown	9	150	0	23
		total	58	967	78	350
	open	picea	4	67	100	16
		Douglas-fir	34	567	68	200
		unknown	4	67	0	29
		total	42	700	64	246
Mosca Pass	dense	fir	10	167	100	57
		cedar	1	17	100	0
		limber pine	70	1167	100	121
		ponderosa pine	3	50	100	4
		aspen	138	2300	53	282
		unknown	5	83	20	4
		total	227	3783	70	468
	open	fir	3	50	100	11
		Rocky Mountain bristlecone pine	1	17	100	0
		limber pine	44	733	95	69
		ponderosa pine	6	100	100	54
		pinyon pine	2	33	100	0
		aspen	123	2050	50	114
		unknown	3	50	0	0
		total	182	3033	63	248

1: Stems/ha includes all species at least 135 cm tall from the 4x50 m plot centered in the center of the planting plot.

2: Basal area is from the 4x50 m transects centered in the center of the planting plot and includes all trees of all species taller than 135 cm.

Table 5: Percent of healthy and live limber pine seedlings planted in 2009 and assessed in fall[1] 2009, August 2010, and August 2011, for all trees and by site

		Healthy[2]			Live		
		Fall	Aug	Aug	Fall	Aug	Aug
Site	n	09	10	11	09	10	11
All	2160	92	82	65	95	87	76
Pilot Hill	432	98	76	40	99	89	56
Killpecker	432	88	81	69	94	86	79
Columbine	432	84	79	65	89	81	77
Buffalo Peak	432	95	89	78	97	91	84
Trout Creek	216	94	81	74	96	84	78
Mosca Pass	216	93	88	74	96	90	83

1: Fall 2009 assessment occurred in October and November.

2: Seedlings were assessed for overall health status by rating them on a standard 1 to 4 scale, using the following classifications: 1) tree was healthy with less than 5% dead needles or branches; 2) tree had 5-50% dead needles and/or branches; 3) tree had 50-99% dead needles and/or branches; 4) tree recently died (within the last five years), and still had needles and fine branches intact.

Table 6: Percent of healthy and live limber pine seedlings planted in spring 2009 and assessed in fall[1] 2009, August 2010, and August 2011, by site, canopy cover, and nurse object orientation.

				Healthy[2]			Live		
Site	Canopy	Side	n	Fall 09	Aug 10	Aug 11	Fall 09	Aug 10	Aug 11
Pilot Hill	dense	All	216	99	95	63	99	97	82
		N	36	100	100	61	100	100	89
		E	36	97	94	58	97	94	86
		S	36	97	94	67	97	97	86
		W	36	100	97	72	100	100	89
		K[3]	72	100	92	58	100	96	72
	open	All	216	98	56	18	99	80	30
		N	36	97	75	25	100	92	42
		E	36	100	58	19	100	86	31
		S	36	97	61	17	100	89	28
		W	36	97	61	17	100	89	31
		K	72	97	42	14	97	63	25

Table 6 continued:

				Healthy[2]			Live		
Site	Canopy	Side	n	Fall 09	Aug 10	Aug 11	Fall 09	Aug 10	Aug 11
Killpecker	dense	All	216	91	82	73	98	89	81
		N	35	97	89	86	100	94	89
		E	35	86	77	71	97	83	74
		S	35	94	80	71	94	94	77
		W	35	89	80	66	100	83	80
		K	76	91	84	71	97	89	83
	open	All	216	85	80	65	90	84	78
		N	36	83	75	72	86	78	75
		E	36	83	83	67	83	83	78
		S	36	83	81	64	94	89	72
		W	36	86	86	81	89	86	86
		K	72	86	78	53	93	83	78

Table 6 continued:

				Healthy[2]			Live		
Site	Canopy	Side	n	Fall 09	Aug 10	Aug 11	Fall 09	Aug 10	Aug 11
Columbine	dense	All	216	89	82	68	94	85	80
		N	36	86	83	72	94	83	78
		E	36	92	81	64	97	83	78
		S	36	83	72	67	86	75	72
		W	36	86	83	78	92	86	81
		K[3]	72	93	86	64	96	90	86
	open	All	216	79	75	61	85	76	74
		N	36	89	81	72	92	81	78
		E	36	89	78	69	94	83	75
		S	36	67	67	58	72	67	67
		W	36	78	78	67	86	78	75
		K	72	76	75	49	83	75	75

Table 6 continued:

				Healthy[2]			Live		
Site	Canopy	Side	n	Fall 09	Aug 10	Aug 11	Fall 09	Aug 10	Aug 11
Buffalo Peak	dense	All	216	95	88	83	97	90	86
		N	36	100	97	94	100	97	94
		E	36	92	78	78	94	78	78
		S	36	97	97	89	97	97	92
		W	36	100	94	92	100	94	94
		K	72	92	81	74	96	86	78
	open	All	216	94	89	73	96	92	83
		N	36	94	86	83	94	92	86
		E	36	91	86	71	94	89	81
		S	36	94	92	72	94	92	83
		W	36	94	92	86	97	94	89
		K	72	96	90	62	97	92	79

Table 6 continued:

				Healthy[2]			Live		
Site	Canopy	Side	n	Fall 09	Aug 10	Aug 11	Fall 09	Aug 10	Aug 11
Trout Creek	dense	All	108	94	72	65	95	77	71
		N	18	89	72	56	89	78	67
		E	18	100	78	72	100	83	83
		S	18	100	67	67	100	72	67
		W	18	94	89	83	94	89	89
		K[3]	36	89	64	56	94	69	61
	open	All	108	95	91	82	96	92	85
		N	18	94	94	89	100	94	89
		E	18	100	89	67	100	94	72
		S	18	94	89	83	94	89	89
		W	18	100	100	94	100	100	94
		K	36	92	86	81	92	86	83

Table 6 continued:

				Healthy[2]			Live		
Site	Canopy	Side	n	Fall 09	Aug 10	Aug 11	Fall 09	Aug 10	Aug 11
Mosca Pass	dense	All	108	98	93	91	100	96	92
		N	18	100	89	89	100	100	89
		E	18	100	100	100	100	100	100
		S	18	94	94	94	100	94	94
		W	18	94	89	83	100	89	83
		K	36	100	92	89	100	97	92
	open	All	108	88	82	57	93	84	75
		N	18	94	94	67	100	94	89
		E	18	94	78	61	94	78	72
		S	18	89	83	56	94	89	78
		W	18	94	94	72	94	94	89
		K	36	78	72	44	86	75	61

1: Fall 2009 measurements were taken from October to November 2009.

2: Seedlings were assessed for overall health status by rating them on a standard 1 to 4 scale, using the following classifications: 1) tree was healthy with less than 5% dead needles or branches; 2) tree had 5-50% dead needles and/or branches; 3) tree had 50-99% dead needles and/or branches; 4) tree recently died (within the last five years), and still had needles and fine branches intact.

3: K indicates seedlings planted without a nurse object.

Table 7: Linear multiple regression model parameters for predicting proportion of healthy[1] planted limber pine seedlings planted in 2009. These parameters are from a backwards stepwise regression modeling 2010 tree health, based on site characteristics. Positive estimates indicate a positive relationship with healthy trees. R^2=0.70. Generalized Chi-Square/df = 2.3.

Parameter	Estimate	s.e.	d.f.	p-value
Intercept	-17.58	3.53	29.34	<0.0001
Mean Canopy Cover (%)	0.02175	0.00596	28.25	0.0011
Elevation (m)	0.001523	0.00034	28.27	0.0001
2009 Precipitation (cm)	0.3604	0.1183	30.55	0.0048
Planting Height (cm)	0.09455	0.03066	171	0.0024

Orientation to Object[2]	least-squares mean[3]	se	df	p-value
East	0.6848	0.0363	32.34	<0.0001
West	0.781	0.0349	72.01	<0.0001
North	0.7677	0.0362	70.02	<0.0001
South	0.7024	0.0416	64.84	<0.0001
None[4]	0.6303	0.0414	41.47	0.0045

1: Seedlings were assessed for overall health status by rating them on a standard 1 to 4 scale, using the following classifications: 1) tree was healthy with less than 5% dead needles or branches; 2) tree had 5-50% dead needles and/or branches; 3) tree had 50-99% dead needles and/or branches; 4) tree recently died (within the last five years), and still had needles and fine branches intact. In this analysis trees rated 1 were compared with all other trees.

2: Least square means are reported for categorical values instead of estimates from model

3: least square means are the proportion of trees classified as a health status 1, compared to all other categories. P-value<0.0001 for comparing mean of seedlings around an object (north, east, south, west) to those without an object.

4: Indicates seedlings with no nurse object.

Table 8: Linear multiple regression model parameters for limber pine seedlings planted in 2009, predicting terminal length (cm) at six sites, two growing seasons after planting (2010). These parameters are from a backwards stepwise regression modeling 2010 terminal growth length, based on site characteristics. Positive estimates indicate a positive relationship with longer terminals. R^2=0.61. Generalized Chi-Square / df = 0.35.

Parameter	Estimate	s.e.	d.f	p-value
Intercept	2.442	0.458	16.97	<0.0001
Mean Canopy Cover (%)	0.022	0.007	31.64	0.0018

Table 9: Linear multiple regression model parameters for limber pine seedling planted in 2009, predicting needle length (cm) at six sites, two growing seasons after planting. These parameters are from a backwards stepwise regression modeling 2010 needle length based on site characteristics. Positive estimates indicate a positive relationship with needle length. R^2=0.70. . Generalized Chi-Square / df = 0.16.

Parameter	Estimate	s.e.	d.f.	p-value
Intercept	2.78	0.5439	85.71	<0.001
Mean Canopy Cover (%)	0.01529	0.00459	31.66	0.0022
Planting Height (cm)	-0.0446	0.01891	163.7	0.0196

Table 10: Site characteristics of 18[1] natural regeneration transects near the six limber pine planting sites, sampled in 2009 and 2010.

	Elevation (m)		Aspect (º)		Slope (%)		Canopy Cover (%)	
Site	mean	std	mean	std	mean	std	mean	std
Buffalo Peak	3385	199	179	28	12.4	7.9	38.9	29.5
Columbine	3116	59	222	141	11.9	6.5	26.4	29.4
Killpecker	2849	292	213	86	10.6	7.0	51	31.5
Mosca Pass	2972	40	220	65	12.4	5.9	62.8	27.3
Pilot Hill	2652	33	244	44	4.5	3.8	39.7	35.5
Trout Creek	2919	33	170	46	39.3	77.1	52	28.6

1: Three transects at each site.

Table 11: Stand characteristics of 18[1] natural regeneration transects near the six limber pine planting, sampled in 2009 and 2010.

			Basal area (m^2/hectare)		Diameter breast height (cm)		Stems per hectare	
Site	Species	n (trees)	mean	std	mean	std	count	percent live
Pilot Hill	fir	2	0.0	0.1	1.8	0.4	20	100
	Rocky Mountain bristlecone pine	0	0.0	0.0	0.0	0.0	0	
	lodgepole pine	0	0.0	0.0	0.0	0.0	0	
	limber pine	98	63.1	87.6	11.7	10.5	980	97
	spruce	0	0.0	0.0	0.0	0.0	0	
	ponderosa pine	0	0.0	0.0	0.0	0.0	0	
	aspen	29	2.6	10.1	3.6	4.7	290	100
	Douglas-fir	0	0.0	0.0	0.0	0.0	0	
	unknown pine species	0	0.0	0.0	0.0	0.0	0	
	total	129	65.7	94.5	9.7	10.1	1290	98
Killpecker	fir	16	2.8	10.7	5.5	6.3	160	100
	Rocky Mountain bristlecone pine	0	0.0	0.0	0.0	0.0	0	
	lodgepole pine	138	32.1	46.2	6.3	7.0	1380	96
	limber pine	94	122.1	89.2	18.1	13.1	940	97
	spruce	11	53.7	183.5	35.7	25.5	110	36
	ponderosa pine	14	22.0	45.5	19.3	15.0	140	100
	aspen	30	4.5	12.7	4.6	6.9	300	100
	Douglas-fir	4	0.3	1.3	3.8	4.9	40	100
	unknown pine species	1	0.1	0.3	5.0		10	0
	total	308	237.6	174.9	11.3	12.9	3080	95

Table 11 continued:

			Basal area (m^2/hectare)		Diameter breast height (cm)		Stems per hectare	
Site	Species	n (trees)	mean	std	mean	std	count	percent live
Columbine	fir	1	0.0	0.1	3.0		10	100
	Rocky Mountain bristlecone pine	3	1.1	3.6	10.0	7.8	30	100
	lodgepole pine	90	24.7	62.3	10.1	7.6	900	100
	limber pine	259	88.1	67.2	7.8	8.3	2590	99
	spruce	16	6.5	19.2	7.4	9.2	160	100
	ponderosa pine	0	0.0	0.0	0.0	0.0	0	
	aspen	158	32.9	93.5	4.6	7.7	1580	82
	Douglas-fir	0	0.0	0.0	0.0		0	
	unknown pine species	1	0.2	0.6	8.0		10	0
	total	528	165.6	190.3	7.2	8.2	5280	94
Buffalo Peak	fir	0	0.0	0.0	0.0	0.0	0	
	Rocky Mountain bristlecone pine	113	129.4	123.0	15.1	14.6	1130	99
	lodgepole pine	1	0.9	3.7	19.0		10	100
	limber pine	95	20.5	30.3	6.3	6.5	950	100
	spruce	62	34.6	53.8	11.7	8.8	620	100
	ponderosa pine	0	0.0	0.0	0.0	0.0	0	
	aspen	254	11.9	32.3	3.3	2.7	2540	72
	Douglas-fir	0	0.0	0.0	0.0	0.0	0	
	unknown pine species	2	0.5	1.8	9.5	0.7	20	0
	unknown	4	7.0	17.9	22.2	15.4	40	0
	total	531	204.8	115.0	7.5	9.5	5310	85

Table 11 continued:

			Basal area (m^2/hectare)		Diameter breast height (cm)		Stems per hectare	
Site	Species	n (trees)	mean	std	mean	std	Count	percent live
Trout Creek	fir	0	0.0	0.0	0.0		0	
	Rocky Mountain bristlecone pine	24	30.3	51.0	18.5	12.0	240	100
	lodgepole pine	1	0.0	0.2	4.0		10	100
	limber pine	71	65.5	56.8	17.4	7.2	710	100
	spruce	7	15.0	49.4	23.0	16.6	70	100
	ponderosa pine	1	5.9	23.0	47.6		10	0
	aspen	45	20.1	48.0	9.3	9.2	450	62
	Douglas-fir	6	6.2	16.7	15.5	13.7	60	100
	unknown pine species	1	1.8	6.9	26.0		10	0
	unknown	4	3.4	13.2	13.3	14.2	40	0
	total	160	147.3	89.0	15.5	10.6	1600	86

Table 11 continued:

			Basal area (m^2/hectare)		Diameter breast height (cm)		Stems per hectare	
Site	Species	n (trees)	mean	std	mean	std	Count	percent live
Mosca Pass	fir	0	0.0	0.0	0.0	0.0	0	
	juniper	5	0.5	2.0	5.9	2.3	50	40
	Rocky Mountain bristlecone pine	5	0.9	2.5	7.6	3.8	50	100
	lodgepole pine	0	0.0	0.0	0.0	0.0	0	
	pinyon pine	5	0.1	0.3	3.0	1.3	50	100
	limber pine	161	219.6	265.3	14.6	17.6	1610	97
	spruce	0	0.0	0.0	0.0	0.0	0	
	ponderosa pine	11	17.2	37.6	19.7	15.2	110	100
	aspen	101	9.2	18.3	4.2	4.2	1010	46
	Douglas-fir	0	0.0	0.0	0.0	0.0	0	
	unknown	1	1.6	6.3	25.0		10	0
	total	289	249.1	251.9	10.7	14.7	2890	78

1: Three transects per site.

Table 12: Density and height of limber pine regeneration (<135 cm tall) in three natural regeneration transects located near six limber pine planting sites, collected in 2009 and 2010.

Site	Species	n (seedlings)	Seedlings per hectare		Height (cm)	
			mean	std	mean	std
Pilot Hill	aspen	1	223	865	71	
	fir	1	30	116	54	
	limber pine	9	140	217	67	20
	ponderosa pine	1	3	13	37	
	total	12	99	447	64	19
Killpecker	aspen	4	83	233	81	13
	Douglas-fir	2	20	65	74	16
	fir	2	113	387	80	3
	limber pine	7	117	186	62	20
	lodgepole pine	7	223	349	86	15
	ponderosa pine	3	13	30	98	41
	spruce	3	30	80	95	31
	total	28	86	234	80	23
Columbine	aspen	4	483	1023	87	6
	fir	3	17	41	50	12
	limber pine	14	300	243	77	14
	lodgepole pine	10	110	114	81	25
	ponderosa pine	1	3	13	50	
	Rocky Mountain bristlecone pine	2	7	18	92	60
	spruce	6	150	351	62	17
	total	40	153	442	75	22

Table 12 continued:

Site	Species	n (seedlings)	Seedlings per hectare		Height (cm)	
			mean	std	mean	std
Buffalo Peak	aspen	5	617	1194	69	6
	limber pine	5	53	81	71	21
	Rocky Mountain bristlecone pine	11	100	118	90	31
	spruce	8	53	61	95	20
	unknown	1	3	13	130	
	total	30	165	572	86	26
Trout Creek	aspen	5	220	412	74	15
	Douglas-fir	1	3	13	26	
	limber pine	2	13	35	67	24
	Rocky Mountain bristlecone pine	2	7	18	57	37
	spruce	2	7	18	57	18
	unknown	1	3	13	97	
	total	13	42	183	66	23
Mosca Pass	aspen	5	520	973	66	8
	limber pine	9	130	229	86	28
	pinyon pine	3	47	154	58	19
	ponderosa pine	2	13	40	101	14
	total	19	178	534	78	25

Table 13: Percent canopy cover and ground cover for seedling microsites in the natural regeneration transects near six limber pine planting sites, collected in 2009 and 2010.

Type	n	Percent Canopy Cover		Percent Shrub		Percent Rock		Percent Gravel		Percent Forb		Percent Grass	
		mean	std	mean	std	mean	std	mean	std	mean	std	mean	std
Limber pine seedling	218	43.8	33.7	11.0	19.4	8.8	14.2	10.0	15.0	9.7	9.4	7.8	9.0
Random[1]	270	42.1	32.4	8.1	16.5	6.3	12.8	11.2	16.1	13.1	14.1	13.7	14.0

Type	n	Percent Bare		Percent Litter		Percent Log		Percent Tree		Percent Other A[2]		Percent Other B[2]	
		mean	std	mean	std	mean	std	mean	std	mean	std	mean	std
Limber pine seedling	218	3.5	9.5	38.9	25.9	4.8	19.5	5.5	11.4	1.2	4.8	0.0	0.5
Random	270	9.6	15.0	33.1	25.7	1.5	7.1	1.1	4.4	1.8	8.1	0.0	0.4

1: Random sites were located at 10, 25, and 40 m along 25 m subplots of the natural regeneration transects, and provided a control comparison for the microsites placed at limber pine seedlings.

2: Percent other A and B indicate percents for cover types other than the standard categories. When two additional cover types were present, the one with the higher ground cover was classified as A.

Table 14: Distance to three nearest objects for 1 m^2 microsites in natural regeneration transects near planting plots. occurring in stand structure survey transects located near the six limber pine planting plots, collected in 2009 and 2010.

Type	n	Object A[1] mean	Object A[1] std	Object B[1] mean	Object B[1] std	Object C[1] mean	Object C[1] std
Limber pine seedling	218	40.9	49.6	52.0	50.6	67.1	62.5
Random[2]	270	95.0	152.4	120.4	148.2	142.3	182.1

1:Object A is the closest object; Object B is the second closest object; and Object C is third closest object to either the limber pine seedling or the center point of the microsite.

2: Random sites were located at 10, 25, and 40 m along 25 m subplots of the natural regeneration transects, and provided a control comparison for the microsites placed at limber pine seedlings.

Table 15: Linear multiple regression model of the density of natural limber pine seedlings by microsite and site characteristics in 18[1] natural regeneration transects near the six limber pine planting plots, collected in 2009 and 2010. R^2=0.39. Generalized Chi-Square /df=1.00.

Parameter	Estimate	s.e.	d.f.	p-value
Intercept	23.84	32.14	9.51	0.476
Percent groundcover of other tree/seedling stems	92.2	26.06	65.95	0.0007
Basal area limber pine	0.44	0.13	81.68	0.0021

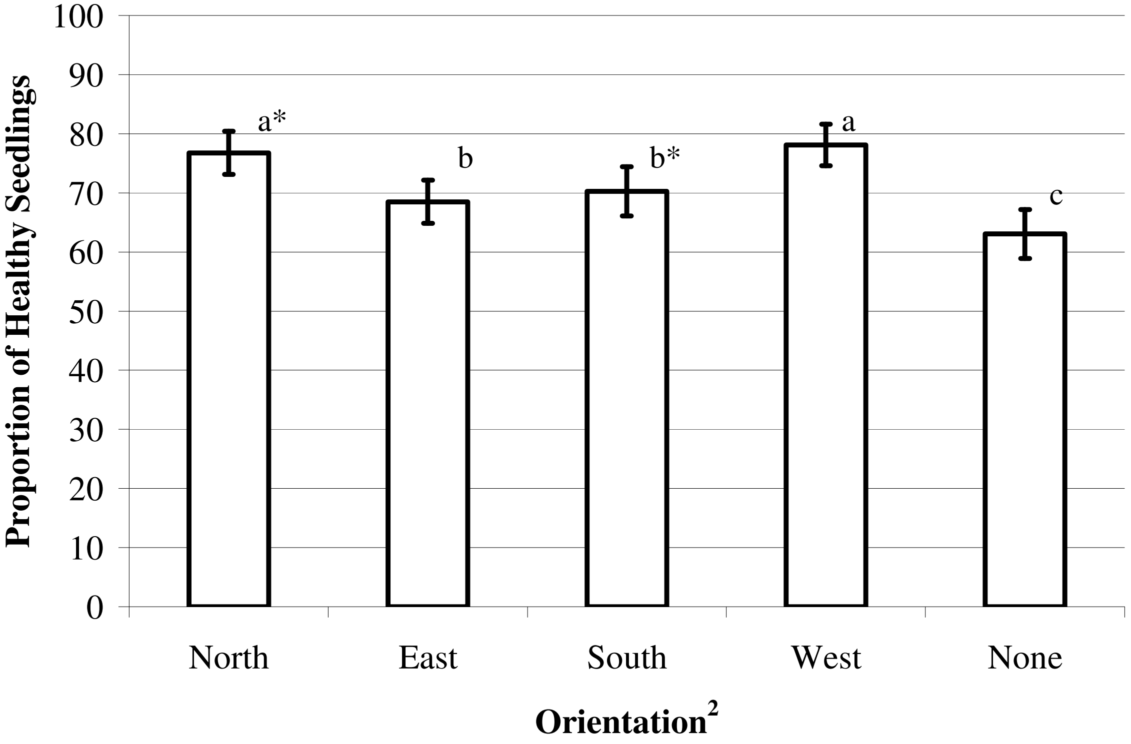

Figure 1: Proportion of healthy[1] planted limber pine seedlings by orientation to nurse object in August 2011, three growing seasons after planting. Different letters indicate a significant difference in pairwise comparison between values (p-value < 0.05). The pair of asterisk indicates significance at p=0.0595. Error bars indicate standard error. Proportions are least-squares means from 36 plots, six in each of the six planting sites along eastern side of Front Range Mountains in WY and CO, 2009

1: Overall seedling health was rated on a 1-5 scale: 1) tree was healthy with less than 5% dead needles or branches, 2) tree had 5-50% dead needles and/or branches, 3) tree had 50-99% dead needles and/or branches, 4) tree was recently dead- <5 years In this analysis trees rated 1 were compared with all other trees.

2: Orientation refers to the position of the tree in relation to the nurse object, where none indicates control trees.

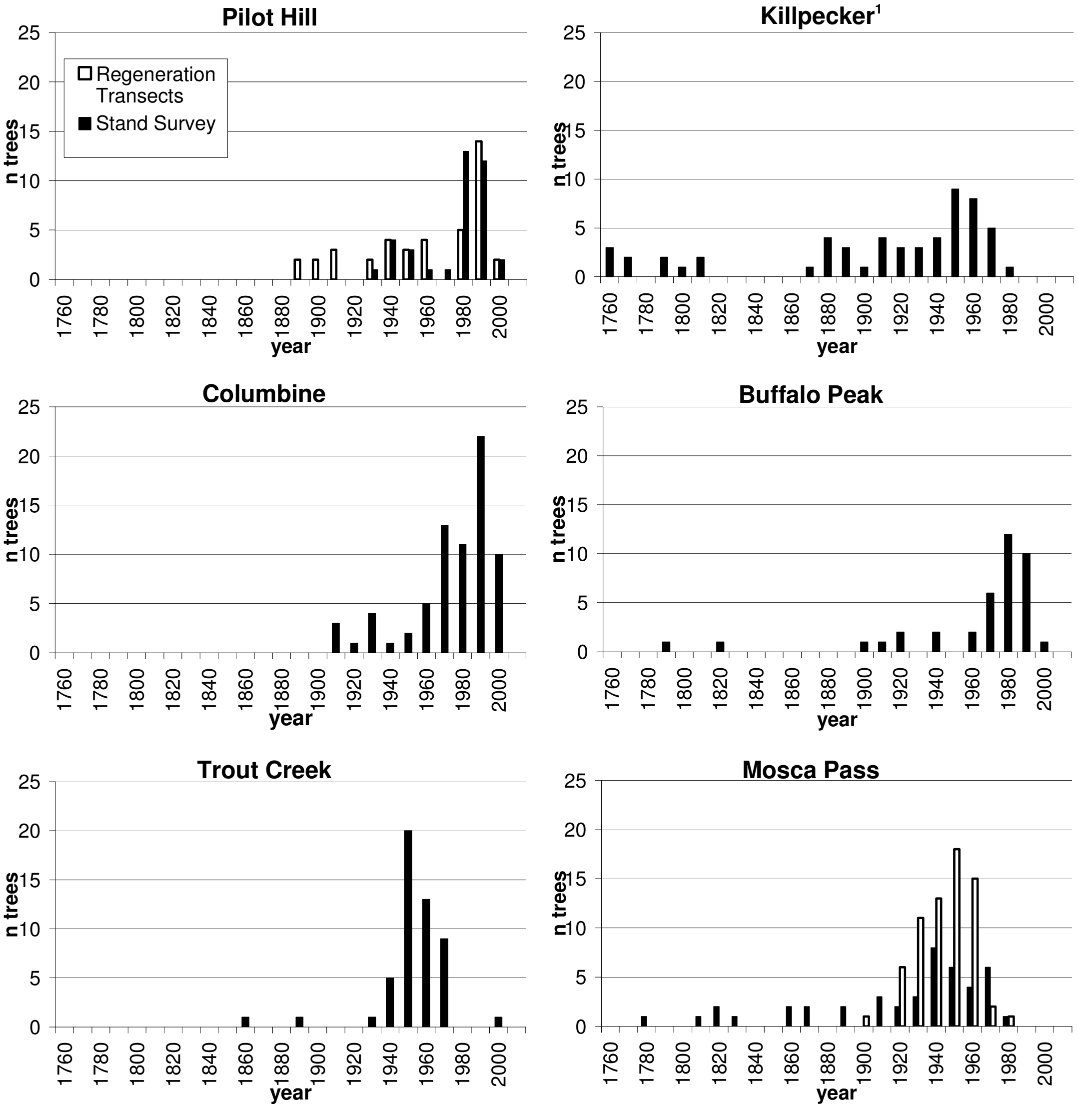

Figure 2: Limber pine tree pith dates binned by decade for trees >135cm tall occurring in stand structure survey transects and natural regeneration survey transects located near and in the six limber pine planting plots, collected in 2009 and 2010. Trees were cored at approximately 20cm height, therefore pith dates do not include the first 20cm of growth.

1: One tree dated prior to 1760 in this site, its pith date was 1279.

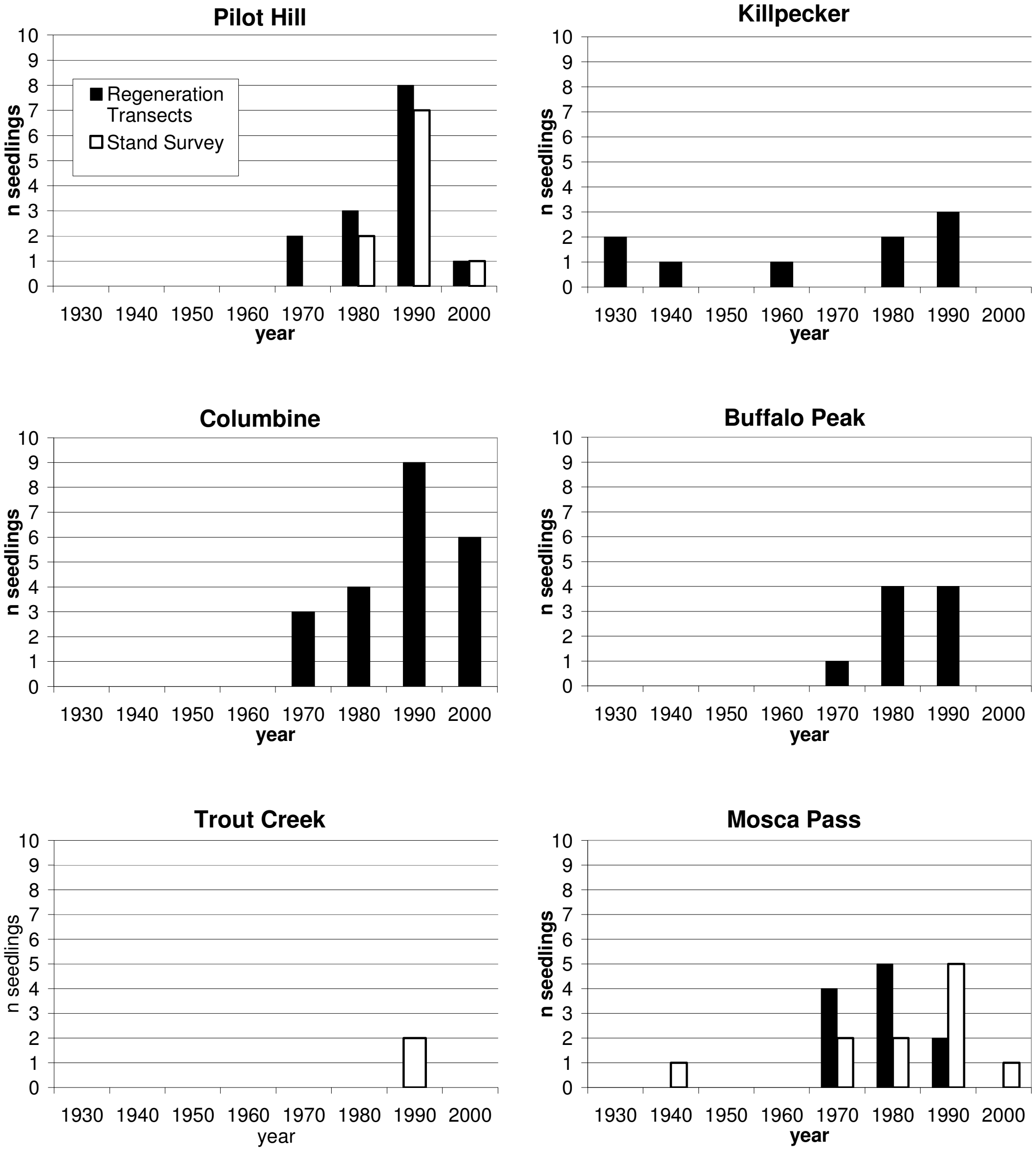

Figure 3: Limber pine seedling root/shoot boundary dates binned by decade for all natural regeneration <135cm tall occurring in stand structure survey transects and natural regeneration survey transects located near and in the six limber pine planting plots, collected in 2009 and 2010. Seedlings were aged at the root-shoot boundary; therefore dates represent time of germination.

Literature Cited

Agaba, H., L. J. B. Orikiriza, J. F. O. Esegu, J. Obua, J. D. Kabasa and A. Huttermann. 2010. Effects of hydrogel amendment to different soils on plant available water and survival of trees under drought conditions. Clean-Soil Air Water. 38:328-335

Barbeito, I., M. Fortin, F. Montes, and I. Canellas. 2009. Response of pine natural regeneration to small-scale spatial variation in a managed Mediterranean mountain forest. Applied Vegetation Science. 12: 488-503.

Benedict, J.B. 1984. Rates of tree-island migration, Colorado Rocky Mountains, U.S.A. Ecology. 65: 820-823.

Benkman, C.W. 1995. The impact of tree squirrels (*Tamiasciurus*) on limber pine seed dispersal adaptations. Evolution. 49:585-592.

Bentz, B. and G. Schen-Langenheim. 2007. The mountain pine beetle and whitebark pine waltz: Has the music changed? In Proceedings of the Conference Whitebark Pine: A Pacific Coast Perspective. U.S. Department of Agriculture, Forest Service R6 NR-FHP-2007-01. Portland, OR: U.S. Department of Agriculture, Pacific Northwest Region, Forest Service. pp. 43-50

Bentz, B., E. Campbell, K. Gibson, S. Kegley, J. Logan and D. Siz. 2010. Mountain pine beetle in high-elevation five-needle white pine ecosystems. In: Keane, R.E., D.F. Tomback, M.P. Murray, and C.M. Smith. (Eds.). The future of high-elevation, five-needle white pines in Western North America: Proceedings of the High Five Symposium. 28-30 June 2010; Missoula, MT. U.S.D. Forest Service Proceedings RMRS-P-63. Rocky Mountain Forest and Range Experimental Station, Fort Collins, CO. p. 78-84.

Bergquist, J., M. Lof, and G. Orlander. 2009. Effects of roe deer browsing and site preparation on performance of planted broadleaved and conifer seedlings when using temporary fences. Scandinavian Journal of Forest Research. 24: 308-318.

Bernier, P. Y. 1993. Comparing natural and planted black spruce seedlings .I. Water relations and growth. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere. 23:2427-2434

Bonnet, V.H., A.W. Schoettle, and W.D. Shepperd. 2005. Postfire environmental conditions influence the spatial pattern of regeneration for *Pinus ponderosa*. Canadian Journal of Forest Research. 35: 37-47.

Burns, K.S. 2006. White pine blister rust survey in the Sangre de Cristo and Wet Mountains of southern Colorado. Biological Evaluation R2-06-05. Denver, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Region. 22 p.

Burns, K.S., A.W. Schoettle, W.R. Jacobi, M.F. Mahalovitch. 2008. Options for the management of white pine blister rust in the Rocky Mountain Region. Gen. Tech. Rep. RMRS-GTR-206. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. 26 p.

Callaway, R.M. and L.R. Walker. 1997. Competition and facilitation: a synthetic approach to interactions in plant communities. Ecology. 78: 1958-1965.

Carsey, K.S. and D.F. Tomback. 1994. Growth form distribution and genetic relationships in tree clusters of *Pinus flexilis*, a bird-dispersed pine. Oecologica. 98: 402-411

Castro, J., R. Zamora, J.A. Hodar, J.M. Gomez, and L. Gomez-Aparicio. 2004. Benefits of using shrubs as nurse plants for reforestation in Mediterranean mountains: a 4 year study. Restoration Ecology. 12: 352-358.

Cerezke, H. F. 1995. Egg gallery, brood production, and adult characteristics of mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Scolytidae), in three pine hosts. Canadian Entomologist. 127:955-965

Colack, A.H. 2003. Effects of microsite conditions on Scots pine (*Pinus sylvestris* L.) seedlings in high-elevation plantings. Forstw. Cbl. 122:36-46

Coop, J.C. and A.W. Schoettle. 2009. Regeneration of Rocky Mountain bristlecone pine (*Pinus aristata*) and limber pine (*Pinus flexilis*) three decades after stand replacing fire. Forest Ecology and Management. 257: 893-903.

Donnegan, J.A., and A.J. Rebertus. 1999. Rates and mechanisms of subalpine forest succession along an environmental gradient. Ecology. 80: 1370-1384.

Dullinger, S., T. Dirnbock and G. Grabherr. 2004. Modelling climate change-driven treeline shifts: Relative effects of temperature increase, dispersal and invasibility. Journal of Ecology. 92:241-252

Elman, E. and D.L. Peterson. 2003. Post-harvest regeneration of montane *Abies amabilis* forests in northern Washington, USA. The Forestry Chronicle. 79:268-273.

Feldman, R., D.F. Tomabck, and J. Koehler. 1999. Cost of mutualism: competition, tree morphology, and pollen production in limber pine clusters. Ecology. 80:324-329.

Furniss, R.L. and V.M. Carolin. 1977. Western forest insects. Washington: Dept. of Agriculture, Forest Service.

Geils, B.W., K.E. Hummer, R.S. Hunt. 2010. White pines, *Ribies*, and blister rust: a review and synthesis. Forest Pathology. 40:147-185.

Germino, M.J., and W.K. Smith. 1999. Sky exposure, crown architecture, and low temperature photoinhibition in conifer seedlings at alpine treeline. Plant, Cell and Environment. 22: 407-415.

Germino, M.J., W.K. Smith, and A.C. Resor. 2002. Conifer seedling distribution and survival in an alpine-treeline ecotone. Plant Ecology. 162: 157-168.

Gibson, K.E., K. Scov, S. Kegley, C. Jorgensten, S. Smith, and J. Witcosky. 2008. Mountain pine beetle impacts in high-elevation five-needle pines: current trends and challenges. Rep. R1-08-020. Missoula, MT: US Department of Agriculture, Forest Service, Northern Region, Forest Health Protection. 32 p

Gomez-Aparicio, L., Z. Regino, C. Jorge, and J.A. Hodar. 2008. Facilitation of tree saplings by nurse plants: microhabitat amelioration or protection against herbivores? Journal of Vegetation Science. 19: 161-172.

Grossnickle, S.C. 2005. Importance of root growth in overcoming planting stress. New Forests. 30:273-294.

Hicke, J.A. J.A. Logan, and J. Powell, and D.S. Ojima. 2006. Changing temperatures influence suitability for modeled mountain pine beetle (*Dendroctonus ponderosae*) outbreaks in the western United States. Journal of Geophysical Research. 111: G02019

Hoff, R.J., R.T. Bingham, and G.I. McDonald. 1980. Relative blister rust resistance of white pines. European Journal of Forest Pathology. 10. 307-316

Hogg, E.H., and A.G. Schwarz. 1997. Regeneration of planted conifers across climactic moisture gradients on the Canadian prairies: implications for distribution and climatechange. Journal of Biogeography. 24:527-534.

Huttermann, A., M.Zommorodi, and K. Reise. 1999. Addition of hydrogels to soil for prolonging the survival of *Pinus halepensis* seedlings subjected to drought. Soil and Tillage Research. 50: 295-304.

Jacobs, D.F., and K. Steinbeck. 2001. Tree shelters improve the survival and growth of planted Engelmann spruce seedlings in southwestern Colorado. Western Journal of Applied Forestry. 16: 114-120.

Johnson, E.A., K. Miyanishi, and H. Kleb. 1994. The hazards of interpretation of static age structures as shown by stand reconstructions in a *Pinus contorta – Picea engelmannii* forest. Journal of Ecology. 82: 923-931.

Kamil, A.C., and J.E. Jones. 1997. The seed-storing corvid Clark's nutcracker learns geometric relationships among landmarks. Nature. 390:276-279.

Kearns, H.S.J. 2005. White pine blister rust in the central Rocky Mountains: modeling current status and potential impacts. Fort Collins, CO: Colorado State University. Dissertation. 243 p.

Kearns, H.S.J., W.R. Jacobi, K.S. Burns, and W.B. Geils. 2008. Distribution of *Ribes*, an alternate host of white pine blister rust, in Colorado and Wyoming. Journal of the Torrey Botanical Society. 135: 423-438.

Kendell, K.C. 1983. Use of pine nuts by black and grizzly bears in the Yellowstone area. International Conference on Bear Research and Management. 5: 166-173.

Kendrick, B. 2000. The Fifth Kingdom. Newburyport, MA: R. Pullins Co. 373 p.

Knowles, P., and M.C. Grant. 1983. Age and size structure analyses of Engelmann spruce, ponderosa pine, lodgepole pine, and limber pine in Colorado. Ecology. 64:1-9

Korner, C., M. Neumayer, S.P. Menendez-Riedl, and A. Smeets-Scheel. 1989. Functional morphology of mountain plants. Flora. 182: 353-383.

Krugman, S.L. and J. Jenkinson. 1974. Pinus L. Pine. in Seeds of Woody Plants in the United States. Tech coordinator C.S. Schopmeyer. Ag handbook number 450. Forest Service, US Dept of Ag Washington, D.C. 598-638.

Lanner, R.M., and S.B. Vander Wall. 1980. Dispersal of limber pine seed By Clark's nutcracker. Journal of Forestry. October 1980: 637-639.

Logan, J.A., and J.A. Powell. 2001. Ghost forests, global warming, and the mountain pine beetle (Coleoptera: Scolytidae). American Entomologist. 47: 160-172.

Mahalovich, M.F., K.E. Burr, and D.L. Foushee. 2006. Whitebark pine germination, rust resistance and cold hardiness among seed sources in the Inland Northwest: Planting Strategies for Restoration. In: National Proceedings: Forest and Conservation Nursery Association; 2005 July 18-20; Park City, UT, USA. Proceedings RMRS-P-43. Fort Collins, CO: US Department of Agriculture, Forest Service, Rocky Mountain Research Station: 91-101.

Maher, E.L., M.J. Germino, and N.J. Hasselquist. 2005. Interactive effects of tree and herb cover on survivorship, physiology, and microclimate of conifer seedlings at the alpine tree-line ecotone. Canadian Journal of Forest Research. 35: 567-574.

McCaughey, W., G.L. Scott, and K.L. Izlar. 2009. Whitebark pine planting guidelines. Western Journal of Applied Forestry 24: 163-166.

McCutchen, H.E. 1996. Limber pine and bears. Great Basin Naturalist. 56:90-92.

McDonald, G.I., B.A. Richardson, P.J Zambino, N.B. Klopfenstein, and M.S. Kim. 2006. *Pedicularis* and *Castilleja* are natural hosts of *Cronartium ribicola* in North America: a first report. Forest Pathology. 36:73-82.

Millar, C.I., R.D. Westfall, and D.L. Delany. 2007. Response of high-elevation limber pine (*Pinus flexilis)* to multiyear droughts and 20th-century warming, Sierra Nevada, California, USA. Canadian Journal of Forest Research. 37: 2508-2520.

Munier, A., L. Hermanutz, J.D. Jacobs, and K. Lewis. 2010. The interacting effects of temperature, ground disturbance, and herbivory on seedling establishment: implications for treeline advance with climate warming. Plant Ecology. 210: 19 30.

Peet, R.K. 1981. Forest vegetation of the Colorado Front Range. Plant Ecology. 45: 3-75.

Perkins, D.L., and T.W. Swetnam. 1996. A dendrochronology assessment of whitebark pine in the Sawtooth-Salmon River region, Idaho. Canadian Journal of Forestry Research. 26: 2123-2133.

Perla, R.I., and M. Martinelli. 1976. Avalanche Handbook. U.S. Dept. of Agriculture. Forest Service. Agriculture Handbook 489.

Rebertus, A.J, B.R. Burns, and T.T. Veblen. 1991. Stand dynamics of *Pinus flexilis* dominated subalpine forests in the Colorado Front Range. Journal of Vegetation Science 2:445-458.

Reinhardt, K., C. Castanha, M. J. Germino and L. M. Kueppers. 2011. Ecophysiological variation in two provenances of *Pinus flexilis* seedlings across an elevation gradient from forest to alpine. Tree Physiology. 31:615-625

Resler, L.M., and D.F. Tomback. 2008. Blister rust Prevalence in krummholtz whitebark pine: implications for treeline dynamics, northern Rocky Mountains, Montana, U.S.A. Arctic, Antarctic, and Alpine Research. 40: 161-170.

Rodriguez-Garcia, E., F. Bravo, and T.A. Spies. 2011. Effects of overstorey canopy, plant-plant interactions and soil properties on Mediterranean maritime pine seedling dynamics. Forest Ecology and Management. 262: 244-251.

Ronco, F. 1961. Planting in beetle-killed spruce stands. Fort Collins CO: USDA Forest Service, Rocky Mountain Forest and Range Experiment Station, Research Note RMRS-RN-60. 6 p.

Ronco, F. 1967. Lessons from artificial regeneration studies in a cutover beetle-killed spruce stand in western Colorado. Research Note RMRS-RN-90. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station 8 p.

Ronco, F. 1970a. Shading and other factors affect survival of planted Engelmann spruce seedlings in central Rocky Mountains. Research Note RMRS-RN-163. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station, 7 p.

Ronco, F. 1970b. Influence of high light intensity on survival of planted Engelmann spruce. Forest Science. 16: 331-339.

Ronco, F. 1972. Planting Engelmann spruce. Research Paper RMRS-RP-89. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station. 25 p.

Rowe, E.C., J.C. Williamson, D.L. Jones, P. Holliman, and J.R. Healy 2005. Initial tree establishment on blocky quarry waste ameliorated with hydrogel or slate processing fines. Journal of Environmental Quality 34:994-1003.

Ruano, I., V. Pando, and F. Bravo. 2009. How do light and water influence *Pinus pinaster* Ait. germination and early seedling development? Forest Ecology and Management. 258: 2647-2653.

Samman, S. and J. Logan. 2000. Assessment and response to bark beetle outbreaks in the Rocky Mountain area. Report to Congress from Forest Health Protection, Washington Office, Forest Service, USDA. USDA Forest Service, Rocky Mountain Research Station, General Technical Report RMRS-GTR-62. 46 p.

Schallenberger, A. and C. Jonkel. 1980. Rocky Mountain east front studies, 1979. Border Grizzly Project, Spec. Rep. 39. Univ. Montana, Missoula. 207 p.

Shoal, R., T. Ohlson, and C. Aubry. 2008. Land managers guide to whitebark pine restoration in the Pacific Northwest region 2009-2013. Olympia WA: U.S. Department of Agriculture, Forest Service, Pacific Northwest Region. 37 p.

Schoettle, A.W., and S.G. Rochelle. 2000. Morphological variation of *Pinus flexilis* (Pinaceae), a bird-dispersed pine, across a range of elevations. American Journal of Botany. 87: 1797-1806.

Schoettle, A.W. and J.F. Negron. 2001. First report of two cone and seed insects on *Pinus flexilis*. Western North American Naturalist. 61: 252-254.

Schoettle, A.W. 2004. Ecological roles of five-needle pines in Colorado: potential consequences of their loss. In: Sniezko, R.A., S. Samman, S.E. Schlarbaum, and B.E. Howard (Eds.). Breeding and Genetic Resources of Five-needle Pines: Growth, Adaptability and Pest Resistance. U.S. Department of Agriculture Forest Service Proceedings RMRS-P-32. U.S. Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experimental Station, Fort Collins, CO. p. 124-135.

Schoettle, A.W. and R.A. Sniezko. 2007. Proactive intervention to sustain high elevation pine ecosystems threatened by white pine blister rust. Journal of Forest Research. 12:327-336.

Schoettle, A.W., B.A. Goodrich, J.G. Klutsch, K.S. Burns, S. Costello, R.A. Sniezko, and J. Connor. 2011. The proactive strategy for sustaining five-needle pine populations: an example of its implementation in the southern Rocky Mountains. In: Keane, R.E., D.F. Tomback, M.P. Murray, and C.M. Smith. (Eds.). The future of high-elevation, five-needle white pines in Western North America: Proceedings of the High Five Symposium. 28-30 June 2010; Missoula, MT. U.S.D. Forest Service Proceedings RMRS-P-63. Rocky Mountain Forest and Range Experimental Station, Fort Collins, CO. p. 323-334.

Schulman, E. 1954. Longevity under adversity in conifers. Science. 119: 396-399.

Schuster, W.S.F., and J.B. Mitton. 1991. Relatedness within clusters of a bird-dispersed pine and the potential for kin interactions. Heredity. 67: 41-48.

Schuster, W.S.F., J.B. Mitton, D.K. Yamaguchi, and C.A. Woodhouse. 1995. A comparison of limber pine (*Pinus flexilis*) ages at lower and upper treeline sites east of the continental divide in Colorado. American Midland Naturalist. 133:101 111.

Schwandt, J.W., I.B. Lockan, J.T. Kliejunas, and J.A. Muir. 2010. Current health issues and management strategies for white pines in the western United States and Canada. Forest Pathology. 40: 226-250

Scott, G.L. and W. McCaughey. 2006. Whitebark pine guidelines for planting prescriptions. In: Riley, L . E.; Dumroese, R. K.; Landis, T. D., Technical coordinators, 2006. National Proceedings: Forest and Conservation Nursery Associations. 2005. RMRS-P-43. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. 160 p.

Shankman, D., and C. Daly. 1988. Forest regeneration above tree limit depressed by fire in the Colorado Front Range. Bulletin of the Torrey Botanical Club. 115: 272-279.

Shea, K. L. and M. C. Grant. 1986. Clonal growth in spire-shaped Engelmann spruce and sub-alpine fir trees. Canadian Journal of Botany-Revue Canadienne De Botanique. 64:255-261

Siepielski, A.M, and C.W. Benkman. 2007. Convergent patterns in the selection mosaic for two North American bird-dispersed pines. Ecological Monographs. 77:203-222.

Steele, R., 1990: *Pinus flexilis* James Limber pine. Silvics of North America, Conifers. Agric. Handb.654. Washington, DC: U.S. Department of Agriculture, Forest Service. 348–354 p.

Strand, M., M.O. Lofvenius, U. Bergsten, T. Lundmark, and O. Rosvall. 2006. Height growth of planted conifer seedlings in relation to solar radiation and position in Scots pine shelterwood. Forest Ecology and Management. 224: 258-265.

Stohlgren, T.J., R.R. Bachand, Y. Onami, and D. Binkley. 1998. Species-environment relationship and vegetation patterns: effects of spatial scale and tree life-stage. Plant Ecology. 135: 215-228.

Taylor, J.E. and J.A. Walla. 1999. First report of *Dothistroma septospora* on native limber and whitebark pine in Montana. Plant Disease. 83:590.

Tomback, D.F. 1982. Dispersal of whitebark pine seeds by Clark's nutcracker: a mutualism hypothesis. Journal of Animal Ecology. 51: 451-467.

Tomback, D.F., and Y.B. Linhart. 1990. The evolution of bird-dispersed pines. Evolutionary Ecology 4: 185-219

Tomback, D.F. 2001. Clark's nutcracker: an agent of regeneration. In: Tomback, D.F., S.E. Arno, R.E. Kearne (Eds.). Whitebark Pine Communities. Island Press, Washington, DC, P 89-104.

Tomback, D.F., A.W. Schoettle, K.E. Chevalier, and C.A. Jones. 2005. Life on the edge for limber pine: seed dispersal within a peripheral population. Ecoscience. 12: 519-529.

Tomback, D.F. and P. Achuff. 2010. Blister rust and western forest biodiversity: ecology, values and outlook for white pines. Forest Pathology. 40: 186-225.

U.S. Department of Agriculture, Forest Service. 2011. Custom Climate Data Requests. Retrieved February 11, 2011 from USDA Forest Service RMRS, Moscow Forest Sciences Laboratory. <http://forest.moscowfsl.wsu.edu/climate/customData/>

Veblen, T.T. 1986. Age and size structure of subalpine rorests in the Colorado Front Range. Bulletin of the Torrey Botanical Club. 113: 225-240.

Webster, K.L. and E.A. Johnson. 2000. The importance of regional dynamics in local populations of limber pine (*Pinus flexilis*). Ecoscience. 7:175-182.

Wood, C.S., and L. Unger. 1996. Mountain pine beetle - a history of outbreaks in pine forests in British Columbia, 1910-1995. Victoria, BC: Natural Resources Canada, Canadian Forest Service, Pacific Forestry Centre.

Woodward, F.I. 1986. Ecophysiological studies on the shrub *Vaccinium myrtillus* L. taken from a wide altitudinal range. Oecologia 70: 580-586.

Appendix I: Data Collection Details

Coring Methods

Cores were secured to the mounts with clear plastic tape while the glue dried. They were sanded with a belt sander using 320 grit sand paper, and sanded by hand with 400 and 600 grit sand paper. When mounting cores it is important to verify that the wood glue used is water soluble, as many new wood glues are water resistant. Water solubility allows mis-mounted cores to be soaked in water overnight and re-mounted.

When it was difficult to determine the location of the seedling root/shoot boundary, we cut the stem just below the estimated boundary and looked for the dark pith indicating shoot growth. If pith color indicated that the section was in the root, approximately 2 mm sections were cut with a band saw until the dark pith of the stem was found.

Water Potential Readings

As an indicator of water stress, pre-dawn water potentials were measured on needles from a subset of trees in 2009. Water potential measures were not repeated due to difficulty in obtaining accurate measurements on small size seedlings and their needles.

Needle Color and Burn

Initially we collected needle color and tip burn data, however this was discontinued due to subjectivity of data collection.

Appendix II: Additional Tables and Figures

Table 16: Soil nutrients for limber pine planting sites, collected in 2009

		pH		% Clay[1,2]		% Organic Matter		Nitrate (ppm)[3]		Phosphorus (ppm)[2]		Potassium (ppm)[2]	
Site	Canopy[1]	mean	std	mean	std	mean	std	mean	std	mean	std	mean	std
Pilot Hill	Dense	7.2	0.2	12.2	1.4	2.0	0.6	4.0	0.0	11.7	2.8	111	17
	Open	7.0	0.1	7.2	2.8	2.5	0.1	6.5	0.7	11.6	4.2	113	5
Killpecker	Dense	6.3	0.2	6.1	3.1	1.6	0.2	1.7	0.6	28.6	18.7	90	30
	Open	6.3	0.3	3.5	3.1	1.8	0.5	2.0	0.0	50.7	10.3	78	10
Columbine	Dense	6.4	0.1	6.8	2.0	5.6	1.9	1.7	1.2	31.7	10.2	189	24
	Open	6.6	0.2	5.5	1.2	1.8	0.4	2.3	0.6	26.7	7.8	134	14
Buffalo Peak	Dense	6.9	0.0	8.5	3.1	2.4	0.3	1.7	0.6	16.5	6.0	111	23
	Open	6.8	0.0	9.9	7.6	3.8	0.9	1.0	0.0	20.1	1.9	164	19
Trout Creek	Dense	7.5	0.0	29.9	6.1	5.5	0.1	1.0	0.0	38.7	3.8	228	38
	Open	7.5	0.0	26.5	2.3	5.5	1.2	1.0	0.0	39.0	12.2	213	8
Mosca Pass	Dense	6.9	0.3	8.8	2.0	1.9	0.4	2.0	1.0	57.0	26.6	134	20
	Open	7.1	0.1	6.5	1.2	2.9	0.5	3.7	0.6	25.8	6.7	144	22

1: Each canopy category has an n=3 plots unless otherwise specified.

2: Soil Textures: Pilot Hill and Mosca Pass – sandy loam; Killpecker and Columbine– sandy loam/loamy sand; Buffalo Peak – sandy loam/loam; Trout Creek – clay loam/loam;

Table 17: Soil moisture data by collection date for limber pine planting sites, collected in 2009 and 2010.

			May 2009		June 2009		July 2009		August 2009			June 2010		August 2010	
Site	Canopy	n	mean	std	mean	std	mean	std	mean	std		mean	std	mean	std
TroutCreek	Dense	9	17.78	1.35	15.88	1.96	10.45	0.50	8.75	0.75		14.16	3.13	11.12	2.61
TroutCreek	Open	9	18.28	3.10	16.48	3.67	11.56	3.20	9.38	1.96		12.04	3.60	11.43	4.27
Killpecker	Dense	9			14.13	2.57	7.76	3.01	5.92	2.23				8.31	2.27
Killpecker	Open	9			17.30	1.76	12.21	1.90	7.61	2.44				9.78	2.45
Columbine	Dense	9	17.08	2.98	12.80	2.83	6.88	0.72	7.37	1.87		14.63	2.47	15.26	2.77
Columbine	Open	9	10.99	1.76	13.99	9.42	8.98	3.06	6.75	1.99		9.72	1.97	11.44	1.71
Buffalo Peak	Dense	9			16.84	2.58	14.79	2.31	11.16	1.80		14.71	2.44	17.03	10.71
Buffalo Peak	Open	9			21.18	2.60	18.27	5.21	10.87	2.35		14.29	1.58	14.89	5.03
PilotHill	Dense	9	11.42	1.99	15.12	2.98	8.30	2.00	8.34	1.98		12.98	1.53	7.31	1.73
PilotHill	Open	9	12.84	0.63	14.59	1.55	8.68	1.04	8.29	1.65		12.51	2.90	7.03	1.41
MoscaPass	Dense	9	8.47	1.75	10.69	2.10	7.00	1.29	4.15	0.59		8.16	1.71	10.63	0.91
MoscaPass	Open	9	7.80	1.16	10.59	1.21	6.35	1.56	3.68	0.69		6.78	1.90	12.44	1.26

Table 18: Frequency of types of three closest objects in 1 m^2 microsites occurring in stand structure survey transects located near the six limber pine planting plots, collected in 2009 and 2010. "Object a" is the closest object; "object b" is the second closest object; "object c" is third closest object.

		Object A[1]		Object B[1]		Object C[1]	
Type	Object	n	percent	n	percent	n	percent
Limber pine seedling	Ground						
	Juniper	0	0.0	0	0.0	2	0.9
	Log	20	9.2	12	5.5	12	5.6
	Rock	15	6.9	22	10.1	28	13.0
	Sagebrush	4	1.8	3	1.4	3	1.4
	Shrub	40	18.3	48	22.0	42	19.4
	Stump	12	5.5	6	2.8	14	6.5
	Tree	127	58.3	127	58.3	115	53.2
Random[2]	Forb	0	0.0	0	0.0	1	0.4
	Ground						
	Juniper	0	0.0	2	0.7	1	0.4
	Log	22	8.1	19	7.1	9	3.4
	None	4	1.5	6	2.2	9	3.4
	Other	0	0.0	0	0.0	1	0.4
	Rock	54	20.0	43	16.0	44	16.4
	Sagebrush	13	4.8	16	5.9	14	5.2
	Shrub	70	25.9	72	26.8	70	26.1
	Stump	3	1.1	2	0.7	7	2.6
	Tree	104	38.5	109	40.5	112	41.8

1: Object A is the closest object; Object B is the second closest object; and Object C is third closest object to either the limber pine seedling or the center point of the microsite.

2: Random sites were located at 10, 25, and 40 m along 25 m subplots of the natural regeneration transects, and provided a control comparison for the microsites placed at limber pine seedlings.

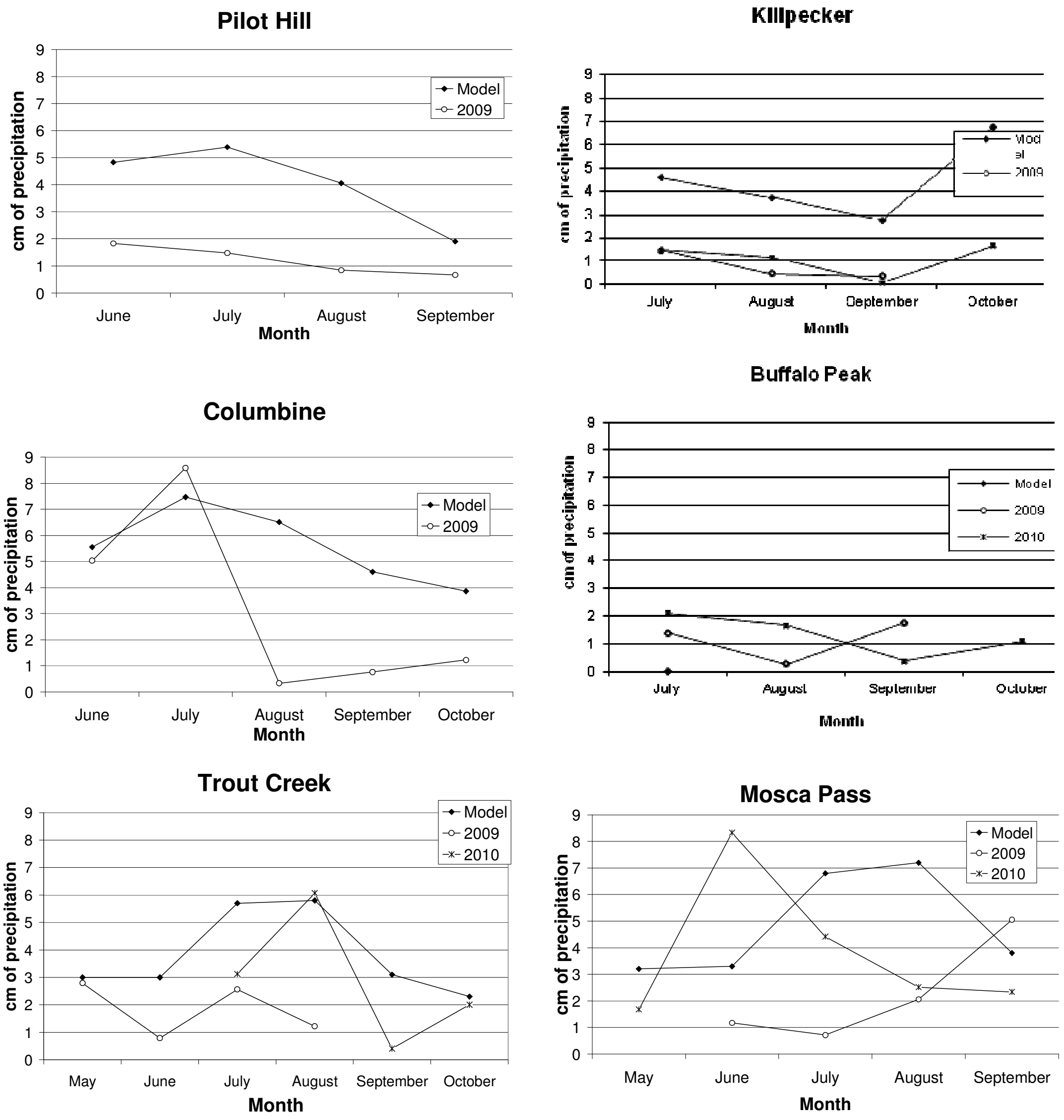

Figure 4: Total monthly precipitation in six limber pine planting for 2009 and 2010, compared to the 30-year average.

Appendix III: Data Sheets

Site:			Date:								New years growth							
Row	**Col**	**Side**	**ht (cm)**	**New Growth lngth cm**	**New Needle lngth cm**	**Basal Diameter**	**Densiometer (N, E, S, W)**				**% burn /band**	**severity of burn/band**	**% color**	**severity of color**	**% insects**	**Severity of insects**	**% herbivory**	**Severity of Herbivory**
1	**1**	**N**																
1	**1**	**E**																
1	**1**	**S**																
1	**1**	**W**																
[redacted]	[redacted]	[redacted]	Comments								2nd Years Growth							
1	**1**	**N**																
1	**1**	**E**																
1	**1**	**S**																
1	**1**	**W**																

Figure 5: Sample of data sheet used for 2009 monitoring of planted limber pine seedlings. Table was expanded to include all rows, columns, and sides in each planting plot

Site:__________________ Date:____________							**New years growth**							
Row	**Col**	**Side**	**ht (cm)**	**New Growth lngth cm**	**New Needle lngth cm**	**Basal Diameter**	**% burn /band**	**severity of burn/band**	**% color**	**severity of color**	**% insects**	**Severity of Insects**	**% herbivory**	**Severity of Herbivory**
1	**1**	**N**												
1	**1**	**E**												
1	**1**	**S**												
1	**1**	**W**												
			Comments				2nd Years Growth							
1	**1**	**N**												
1	**1**	**E**												
1	**1**	**S**												
1	**1**	**W**												
1	2	**N**												

Figure 6: Sample of data sheet used for 2010 monitoring of planted limber pine seedlings. Table was expanded to include all rows, columns, and sides in each planting plot.

Site	Date	Plot	Row	Col	Side	Health	Damage Type	Comments
		1	**1**	**1**	**E**			
		1	**1**	**1**	**W**			
		1	**1**	**2**	**N**			
		1	**1**	**2**	**E**			
		1	**1**	**2**	**S**			
		1	**1**	**2**	**W**			

Figure 7: Sample of data sheet used for monitoring of planted limber pine seedlings when seedlings were only monitored for health class. Table was expanded to include all rows, columns, and sides in each planting plot and site.

Cover Sheet - Plot Data

Site:_______________________________ **Transect:**________ Date:____________

	Start					End					
Plot	GPS		Tree#	Aspect	Slope	GPS		Tree#	Aspect	Slope	Elevation
1											
2											
3											
4											
5											

Figure 8: Sample data sheet for plot data in natural regeneration transects.

LINES BELOW ARE A CONTINUATION OF ABOVE!

	Disturbance						Comments
Plot	Other	Insects	BR	Misletoe	Gopher	Stand Canopy Type	
1							
2							
3							
4							
5							

Transect Location		
FS Rd #	District	Forest

Figure 8 continued

Site:_______________________________ Transect:________ Plot:_____________

Date:____________________________ **Tree Data Sheet**

#	Species	DBH/ht cm	Health	Comments	#	Species	DBH/ht cm	Health	Comments
1					51				
2					52				
3					53				
4					54				
5					55				
6					56				
7					57				
8					58				
9					59				

Figure 9: Sample data sheet for tree data in natural regeneration transects. Table was expanded to fit 100 trees on a sheet.

Site:______________________ Transcect:________ Plot:____________

Date:__________________

T=tree

L=Log

S=stump

G=small rocks(gravely)

Microsite Data Sheet

R=Rock

Do 3 objects per seedling!

Seedling info			%Canopy	Percent Ground Cover								3 Nearest Objects									
#	Stat	Ht (cm)	cover	Shrub	Rock	Forb	Grass	Bare	Litter	Log	Tree	T	S	R	G	L	Distance	Ht	Diam	direction	Comments
1		RANDOM																			
2		RANDOM																			
3		RANDOM																			

Figure 10: Sample data sheet for microsites in natural regeneration transects.

Site:________________________ Transect______________ Date:______

Tree Core/Cookie Data Sheet

Sample # is UNIQUE to each sample !
canopy stat- where in canopy is tree?

Plot	Tree#	Sample#	DBH/HT(cm)	Health	CanopyStatus		Height
		1					
		2					
		3					
		4					
		5					
		6					
		7					
		8					
		9					
		10					

Figure 11: Sample data sheet for age samples in natural regeneration transects.