

Article

Designer Niches Promote Seedling Survival in Forest Restoration: A 7-Year Study of Whitebark Pine (*Pinus albicaulis*) Seedlings in Waterton Lakes National Park

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Abstract: Designer niches in which environmental variables are controlled are useful in forest restoration to enhance survival of planted tree seedlings. Here, we evaluate particular manipulated habitats, on site variables, and pre-seedling conditions hypothesized to improve the survival rate of whitebark pine (*Pinus albicaulis*) seedlings out-planted in Waterton Lakes National Park. The tree species is in peril due to blister rust and mountain pine beetle infestations in its range; and is a restoration priority in Waterton Lakes because populations in the park are highly infected with blister rust (up to 90%). At Summit Lake, 21 plots were set up and half of each was terra-torched; 1000 seedlings were planted in clusters of three, under four conditions: on burned areas in burned beargrass mats, in burned areas where beargrass mats were not present, in unburned areas where beargrass was present, and in unburned areas without beargrass. This study reports data for the seventh year after planting, and overall, survival was 53% for individual seedlings and at least one seedling survived in 60.8% of clusters. Planting in burned areas increased cluster survival (by 34.3%, $p < 0.0001$) and planting near microsites increased cluster survival (by 19.3%, $p < 0.0001$); the type of microsite did not make a difference. Planting in beargrass mats decreased survival, but not significantly (8.9%, $p = 0.11$) and this was true for burns, not unburned areas. Inoculation with native ectomycorrhizal fungi did not enhance survival most likely because controls on lightly terra-torched and unburned areas had access to local native fungi. This is the first study to report statistics on the planting of seedlings in clusters; the results need to be compared with studies where seedlings are planted individually.

Keywords: Alberta; five-needle pines; forest management; burns; beargrass understory; microsite; mycorrhizal inoculation

1. Introduction

The survival of tree seedlings in forest situations depends on a myriad of environmental details that comprise niches when regeneration is natural [1], and designer niches when they are controlled. Ecological variables include site conditions such as location, climate, topographic aspect, canopy openness, soil type, burn status, understory vegetation type, potential tree competition, soil microbial community composition, and the presence of local protective shelter objects (microsites) such as logs, snags, and rocks. Previous conditions such as those where thinning, logging, post-fire salvage, soil compaction, insect kill, or invasion of pathogens have occurred, can also play a

role in fostering or slowing seedling regeneration [2,3]. When designing restoration procedures, the autecology of each needs to be taken into account [4]. Evaluating success in forest restoration currently relies on assessing the proportion of planted seedlings that survive over time, for various treatments; gauging ultimate functional success is more elusive [5].

Whitebark pine (*Pinus albicaulis* Engelm.) is a five-needle pine with a range limited to the high elevations of particular mountain ranges in Western North America, including those in Alberta, British Columbia, Washington, Oregon, California, Nevada, Utah, Idaho and Montana [6]. The pine exists as rugged lone individuals on open slopes, as krummholz with other tree species, and in extensive, pure or mixed stands at treeline. Whitebark pine is a pioneer species and an important structural component in these high elevation habitats; its seeds are a significant food source for animals, including squirrels, birds, and grizzly bears [6,7]. Unlike most other conifers, cones do not open and seeds are accessed and planted by Clark's nutcrackers (*Nucifraga columbiana* Wilson, 2011), the primary dispersers [8]. Typically, birds cache seeds in clusters for future use as food, often in open areas including on burns where they regenerate when left back [9]; individual seedlings merge into a genetic chimera represented as one mature tree [10,11]. This autecology along with the fact that seeds mature at different rates and germinate over years [12] makes whitebark pine unique among conifers and adds challenges to its regeneration under any conditions. Added to this are the current threats to its existence, which include white pine blister rust, mountain pine beetle, climate change and changing fire regimes [13,14].

Around 1900, the exotic pathogen white pine blister rust (*Cronartium ribicola* J.C. Fisch) was inadvertently brought to North America in seedling stock, where it proceeded to ravage first western white pine (*Pinus monicola* Douglas ex D. Don) and then whitebark pines [15,16]. The rust, in conjunction with native bark beetles (*Dendroctonus ponderosae* Hopkins), has severely reduced whitebark populations across its limited range [14]. In addition, the upper cone-bearing branches are killed before the tree succumbs to rust, rendering the tree unable to reproduce and exacerbating the impact of the disease [17]. In response to warnings from researchers [18], full-blown restoration efforts are trying to save the species, which is currently listed as endangered in Canada under the Species at Risk Act and Committee on the Status of Endangered Wildlife in Canada (COSEWIC) [19]; it awaits comparable designation in the U.S. Development of local and range-wide restoration strategies are directed towards preservation of already-established mature trees and particularly those showing signs of resistance to the rust, fostering natural regeneration, and planting putatively rust-resistant genetic strains in seed zones where they originated [17,20,21].

Niches conducive to natural seedling regeneration have provided clues for designing niches appropriate for restoration of whitebark pine [4,11,22–24]. There is evidence that certain planting techniques [25], planting in clusters (Parks Canada, unpublished), use of shelter objects or microsite [26,27], inoculation with ectomycorrhizal fungi [27,28], planting on burns [26,27,29], planting in areas with particular understories [24,29,30] and on particular soil types [24], has the potential to improve survival of out-planted nursery-produced whitebark seedlings. It is anticipated that these strategies, along with the use of putatively rust-resistant seedlings placed in appropriate seeds zones, will foster enhanced regeneration [31].

The edge of a species range is particularly vulnerable to ecological upset, and northernmost populations of whitebark pine lie within Alberta and British Columbia. One of the first locations to initiate restoration of whitebark pine in Canada was Waterton Lakes National Park where assessments have estimated rust infection rates to be 70% from 2003–2004 surveys [32]. In addition, 78% of trees in 2009 based on permanent transect monitoring [33] and up to 90% in 2016 based on stand surveys were infected, which further emphasizes the decimation [34]. Restoration in the park began in 2010 with a large study that monitored 983 whitebark pine seedlings planted in plots with varying site conditions. Seedling survival was 95% after one year, 69% after two, and 47% in year three [27,28]; the study found that planting in burned areas, particularly where beargrass was absent and with shelter objects (microsite), was the most beneficial treatment combination by year three. The benefit of inoculation

with ectomycorrhizal fungi was site dependent. Here, data from seven years of monitoring on this site are analyzed and presented. Only a few studies have examined whitebark pine survival after planting for restoration over the long term [26], although this data is starting to filter in. This current study is one of the few in which seedlings were planted in clusters; we analyze the survival of both individual seedlings and seedling clusters as a means for eventually evaluating any benefit from cluster planting to simulate bird caches.

2. Study Area and Methods

2.1. Study Area

The study area is located near Summit Lake in Waterton Lakes National Park (WLNP), Alberta Canada ($49^{\circ}0' N$, $114^{\circ}1' W$) (Figure 1) at elevations between 1950 m to 2000 m; the site is relatively flat. The climate is characterized by deep, persistent snow packs and short cool summers with average annual precipitation around 152 cm. The soil composition is an orthic humo-ferric podzol derived from glacial till and the texture is loam to gravelly loam with 10–70% coarse fragments. The overstory vegetation is mixed coniferous forest comprising of subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.) and Engelmann spruce (*Picea engelmannii* Parry ex Engelm) with scattered whitebark pine. The understory vegetation consists mostly of thick mats of beargrass (*Xerophyllum tenax* (Pursh) Nutt.) with scattered huckleberry shrubs (*Vaccinium membranaceum* Douglas ex Torr.) [35]. White pine blister rust infection has caused a high level of mortality in mature whitebark pines at the site. Many of the naturally regenerating seedlings are dying from rust infection and competitive exclusion from the faster-growing, shade-tolerant conifers such as spruce and fir.

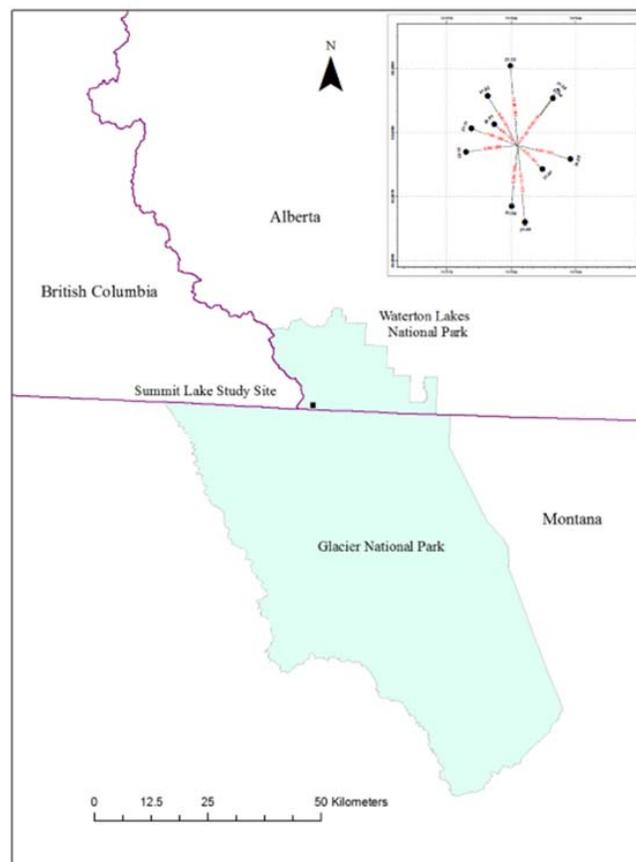


Figure 1. Location of the study site at Summit Lake, Waterton Lakes National Park ($49^{\circ}00'26' N$ $114^{\circ}01'31'' W$). Inlay is an example of the layout of seedling plantings for one of 21 plots.

2.2. Nursery Seedlings and Mycorrhizal Inoculation

Seedlings for this study were grown at the Forest Service Coeur d'Alene nursery under standard nursery conditions [36] from potentially rust-resistant seeds (entered in the USFS genetic testing program) collected at Preston Park (48°34'45" N, 113°39'03" W, 2719 m) in Glacier National Park (GNP), approximately 40 km away from the test plots. Five months prior to out-planting, 1000 14-month-old seedlings were transferred to an outdoor site at the GNP Native Plant Nursery, where fertilization was stopped so that conditions would be beneficial for subsequent mycorrhizal colonization.

A spore slurry was made from cleaned fresh sporocarps (mushrooms) of the native ectomycorrhizal fungus *Suillus sibiricus* Singer (CLC 2640) collected in 2010. The slurry was diluted to a spore count of approximately 1×10^6 spores/mL [37]. Approximately 500 seedlings were inoculated with the slurry at a rate of three million spores/seedling (1×10^6 spores/mL slurry \times 3 mL) at the GNP Native Plant Nursery on 19 August 2010 and were tagged as 'inoculated'. Approximately 500 uninoculated seedlings were designated as controls. It was not possible to pre-check seedlings for mycorrhizal colonization; both nursery-originated and native mycorrhizal fungi are known to colonize whitebark pine seedlings at the nursery.

2.3. Site Preparation

Twenty-one plots each with a diameter of 50 m were established at the Summit Lake study area. A custom-built terrestrial torch was used to burn approximately half of each plot (25 m diam) to reduce living Engelmann spruce, subalpine fir, and understory vegetation [38]. Areas burned by the terrestrial torch were small, patchy, and of a mixed severity ranging from "no lethal fire" to "greater than 50% consumption of mature trees and surface fuels such as grasses, juvenile trees and dead and downed material". Plots 1–12 were burned in 2009 and plots 13–21 in 2010. When present, beargrass was lush in unburned areas and remained as a thick root mat in burned areas. Each plot typically contained four site condition combinations: (1) burned without beargrass, (2) burned with beargrass root mats, (3) unburned with beargrass, and (4) unburned without beargrass [37].

2.4. Planting Strategy and Treatments

On 28 September 2010, 983 whitebark pine seedlings were planted in clusters of three within the 21 plots. Approximately 16 seedling clusters were planted in each plot, with four replicate clusters in each of the four site condition combinations described above. Seedlings in each cluster were designated as either A, B, or C to ensure individual seedling recognition during monitoring. Clusters were planted near shelter objects (called microsites) when possible, although this was influenced by availability and site condition type. Clusters planted within 30 cm of a snag, stump, large rock, or large log were considered positive for the presence of a shelter object [27].

Zero, one, two or three inoculated seedlings were included in each cluster to determine the minimum number of inoculated seedlings needed to improve survival. Individual seedlings were considered 'inoculated' if they had initially received spore slurry. Uninoculated seedlings placed in clusters adjacent to inoculated seedlings were considered 'exposed' to mycorrhizal treatment. Seedlings placed in clusters without any inoculated seedlings were in the 'uninoculated' (and unexposed) group.

The final design consisted of two burn treatments (burn/no burn) \times two beargrass treatments (XETE/no XETE) \times two microsite treatments (yes/no) \times three inoculation treatments (inoculated, exposed, not inoculated).

2.5. Monitoring

Seedlings were monitored on a yearly basis after planting, over seven years. Monitoring consisted of scoring the health of each seedling on a scale from 1 to 5, where 1 = dead, 2 = missing, 3 = barely alive, 4 = good health, and 5 = excellent health. In some years, seedling height was also recorded by measuring from the lowest limb to the apical bud, referred to as height from the lowest limb.

Data correlated with each seedling was: (1) plot, (2) site condition within the plot, (3) presence or absence of microsite and (4) the original mycorrhizal condition of individual seedlings and of the cluster in which it was planted.

2.6. Statistical Analysis

For analysis purposes, all living seedlings (scores of 3–5) were combined into one category. Seedling clusters with 1, 2, or 3 inoculated seedlings were considered exposed to mycorrhizae. All analyses were performed using the statistical software R [39]. Survival data were right censored because seedlings sometimes could not be re-located, and because many seedlings survived past the end of the study period. To account for this, survival curves were estimated using the Kaplan-Meier method [40]. The proportional hazards assumption was tested using scaled Schoenfeld residuals and since the data failed to meet this assumption, the use of Cox's proportional hazards model was ruled out. Parametric distributions were also explored using a maximum likelihood estimation specific to censored data. Akaike's Information Criterion (AIC) and log-likelihood scores were used to compare the fit of log-normal, normal, Weibull and gamma distributions. The exponential distribution was not used because it has a constant hazard function. Based on the AIC and log-likelihood scores, the log-normal distribution was selected for further investigation. However, visual comparison of the Kaplan-Meier survival curve and the log-normal model found that the model did not provide a good approximation of the data (Figure 2). Therefore, parametric methods were ruled out, and analyses were carried out on the Kaplan-Meier curves using the log-rank test with a significance value of $\alpha = 0.05$.

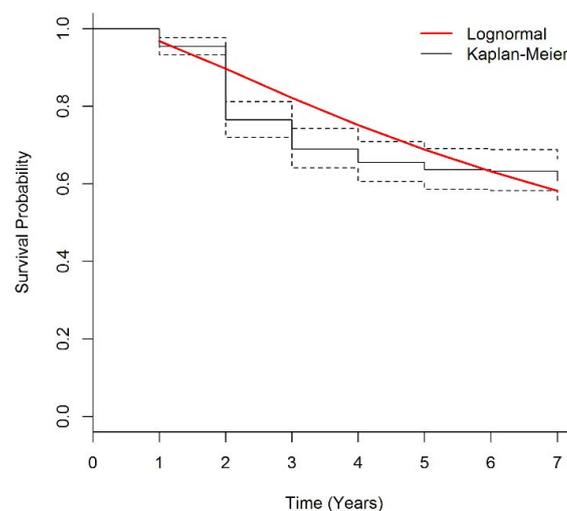


Figure 2. Survival of seedling clusters over seven years. Comparison of the log-normal model fit with the Kaplan-Meier curve. Dashed lines represent 95% confidence intervals.

3. Results

The probability of survival of whitebark pine seedlings after seven years, for all treatments, was analyzed for individual seedlings and seedling clusters (Table 1). Seedlings inoculated or exposed to inoculation are combined in the table. The table also gives the actual number of events (number of seedlings or clusters that have died) for each set of conditions. Overall survival in year seven was $53\% \pm 3\%$ for individual seedlings and $60.8\% \pm 5\%$ for clusters. The greatest mortality was between the 2nd and 3rd year after planting, which then leveled off with a greater proportion of seedlings surviving in the following years (Table 1, Figure 2). In all cases, the cluster survival probability is higher because even though individuals within clusters die, the cluster itself is counted as living if one seedling survives. Only one seedling was noted to be infected with rust. Of those counted as still

alive, 59.2% were in the 5 = healthy category, 18.5% in the 4 = fair category, 2.2% in the 3 = almost dead category, and the 20% in the 2 category were counted as missing.

Table 1. Sample size, and probability of survival (%) after 7 years for each treatment. ‘Seedling Individuals’ refers to the analysis of each seedling separately. ‘Seedling Clusters’ refers to the analysis of each group of three seedlings as a single entity; even if only one seedling survives in a cluster, the whole cluster is considered alive.

		Seedling Individuals		Seedling Clusters	
	Treatment	N	Survival	n	Survival
Overall		990	53.0	331	60.8
Habitat	Burn, No Beargrass	366	67.3	122	79.0
	Burn, Beargrass	374	53.3	125	59.7
	No Burn, Beargrass	169	39.9	57	50.3
	No Burn, No Beargrass	81	12.9	27	3.9
Microsite	Present	658	62.1	219	70.7
	Absent	332	35.1	112	41.5
Beargrass	Present	543	49.2	182	56.9
	Absent	447	57.6	149	65.5
Burn	Present	740	60.3	247	69.3
	Absent	250	31.2	84	35.1
Microsite Type	Stump	74	67.1	25	79.6
	Snag	231	71.9	77	76.9
	Rock	78	54.4	26	69.2
	Log	275	54.8	91	63.2
	None	332	35.1	112	41.5
Inoculation and Burn	Exposed, Burn	552	59.2	182	69.0
	Un-inoculated, Burn	188	63.5	65	70.3
	Exposed, No Burn	169	33.5	57	35.8
	Un-inoculated, No Burn	81	25.8	27	33.3

After seven years, survival probability was highest for seedlings planted in burned areas without beargrass (67.3% for individuals, 79% for clusters), followed by burned areas with beargrass (53.3% and 59.7%); survival probability was lower in unburned areas with beargrass (39.9% for individuals and 50.3% for clusters) and without beargrass in marginal areas (12.9% for individuals and 3.9% for clusters) (Table 1, Figure 3).

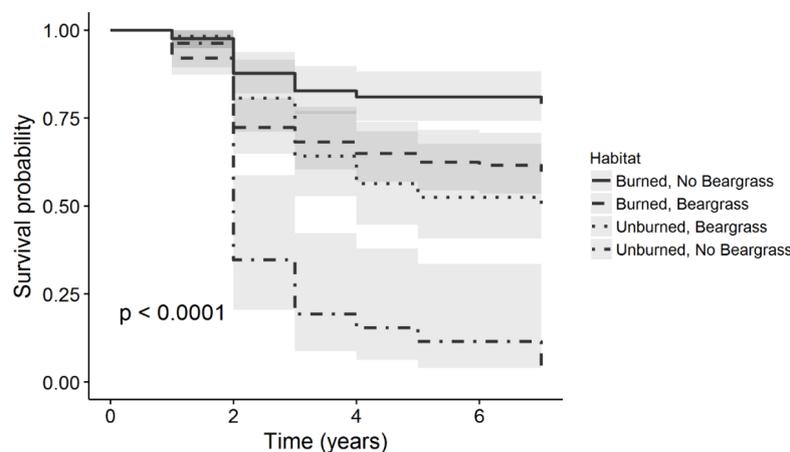


Figure 3. Cluster survival for four habitat treatment types: Burned and beargrass, burned and no beargrass, unburned and beargrass, and unburned and no beargrass ($X^2 (3, n = 331) = 64.8, p < 0.0001$) over seven years.

On the burned areas after seven years, survival probability was 60.3% for individuals and 69.3% for clusters, versus 31.2% and 35.1%, respectively, for seedlings in unburned areas; probability of survival was significantly higher on burns ($X^2(1, n = 331) = 64.6, p < 0.0001$) (Table 1, Figure 4A). Seedlings planted in areas where beargrass was present (whether as living plants or burned roots) had a lower probability of survival, 49.2% (individuals) and 56.9% (clusters) compared to 57.6% and 65.5%, respectively, for those planted in areas without beargrass (Table 1, Figure 4B). Seedling clusters planted in areas without beargrass did not have a significantly higher survival rate ($X^2(1, n = 331) = 2.6, p = 0.11$), and the effect was not as great as that of the burning or microsite treatments. Survival probability was higher—62.1% (individuals) and 70.7% (clusters) when a shelter object was nearby and lower—35.1% and 41.5%, respectively—when it was not (Table 1, Figure 4C). While shelter objects did make a significant difference ($X^2(4, n = 331) = 91.1, p < 0.0001$), differences among types of shelter objects were not significant ($p = 0.143$). However, there was a trend for both individual seedlings and clusters, from highest to lowest survival rates being: stumps > snags > rocks > logs > none (Table 1, Figure 4D).

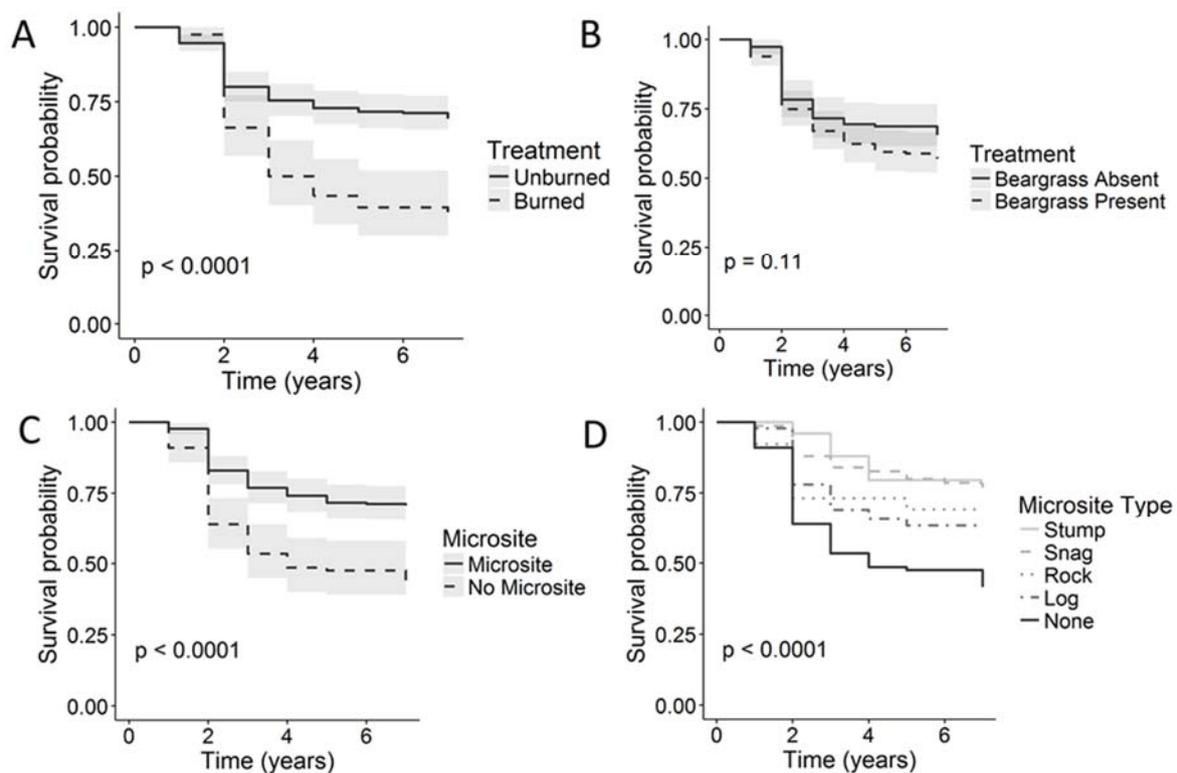


Figure 4. Seedling cluster survival over time. (A). on burned and unburned areas $X^2(1, n = 331) = 27, p < 0.0001$. (B). with and without beargrass $X^2(1, n = 331) = 2.6, p = 0.11$. (C). with and without microsite $X^2(1, n = 331) = 27.4, p < 0.0001$. (D). among microsite (shelter object) types $X^2(3, n = 219) = 5.4, p = 0.143$, but is the same as C between “None” and “Present”.

Individual treatment effects for the four main variables are summarized in Figure 5A. There was no overall effect on seedling survival whether comparing individual seedlings in the three mycorrhizal categories $X^2(1) = 27.4, p = 0.92$, just in burned areas ($p = 0.39$); or in exposed or uninoculated clusters in burned areas ($p = 0.73$). While there was higher overall seedling survival when seedlings were not planted in beargrass, this mainly reflected the higher survival rates in burned areas; survival was higher with beargrass in unburned areas and when there was no microsite available (Figure 5C). Unburned areas without beargrass also lacked other understory plants and were considered poor planting sites in general.

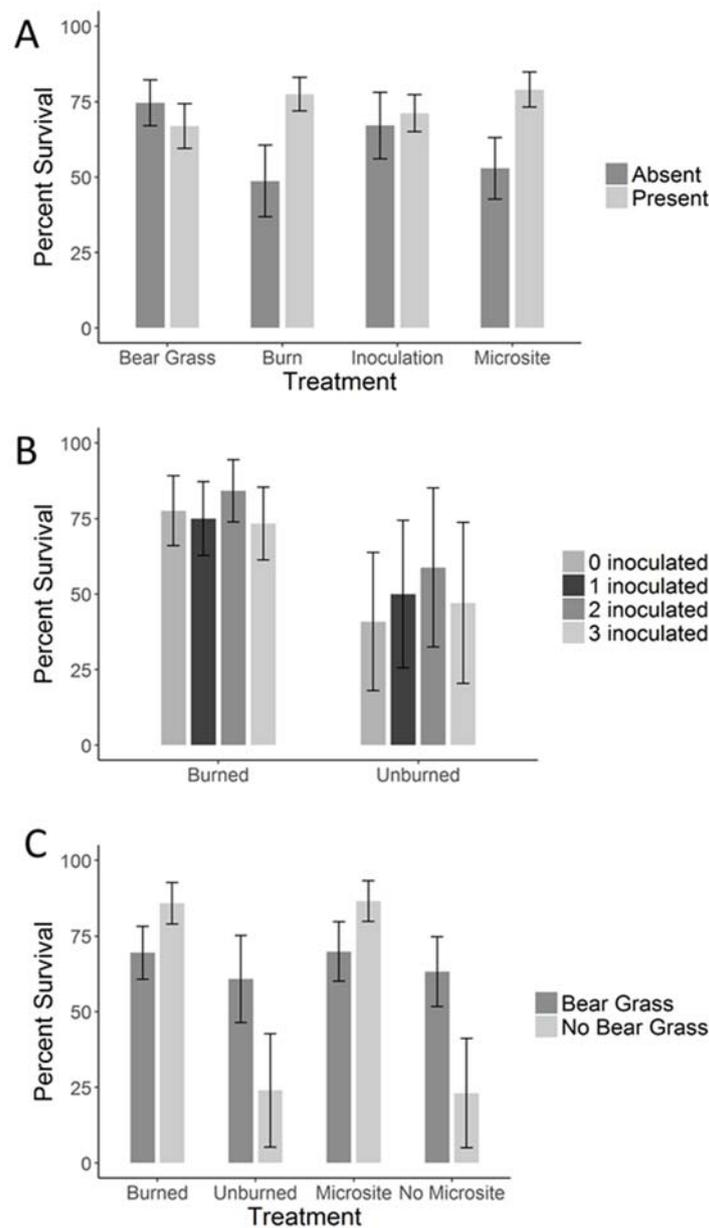


Figure 5. Survival of whitebark pine seedling clusters after seven years summarized for (A). individual variables: with and without beargrass $X^2(1, n = 307) = 2.20, p = 0.14$, with and without burning $X^2(1, n = 307) = 22.75, p < 0.001$, with and without inoculation $X^2(1, n = 307) = 2.2, p = 0.14$, with and without inoculation $X^2(1, n = 307) = 0.49, p = 0.49$, with and without a microsite $X^2(1, n = 307) = 22.22, p < 0.0015$. (B). with and without ectomycorrhizal inoculation of 1, 2, or 3 seedlings in each cluster on burned and unburned sites. For burned sites $X^2(3, n = 231) = 2.27, p = 0.52$, for unburned sites $X^2(3, n = 76) = 1.26, p = 0.74$. (C). burned with and without beargrass $X^2(1, n = 231) = 8.85, p = 0.003$, unburned with and without beargrass $X^2(1, n = 76) = 9.09, p = 0.003$, microsite present with and without beargrass $X^2(1, n = 205) = 8.56, p = 0.003$, microsite absent and without beargrass $X^2(1, n = 102) = 12.49, p < 0.001$.

4. Discussion

Whitebark pine has a unique autecology, occurring at high elevation habitats in low-nutrient soils [6]. The pine is difficult to grow from seed in the nursery because stratification procedures are lengthy and embryos mature at different rates [36], all of which complicates the restoration process.

In addition, a large portion of seedlings do not survive after planting [26]. Whitebark pine survival rates are known to decline for two or three years post planting, after which survival numbers level off [26,41]. This was also true in the current study where the steepest decline occurred between years two and three. Overall, 53% of the planted seedlings were still alive after seven years, which is comparable to results for restoration projects in nearby Glacier Park (41% after 5 years, 34% after 11 years), and at three planting sites in Montana where survival ranged from 22% to 62% [26]. The 53% survival rate at seven years is higher than the 47% measured by Cripps et al. [28] three years post planting for the same set of seedlings on the 21 plots at Summit Lake. In year three, many of seedlings were not located and missing trees were counted as dead. However, a number of missing seedlings were re-located during subsequent monitoring. In addition, the Kaplan-Meier method that attempts to estimate how many of the missing seedlings are alive was used in the present analysis, which also contributed to the higher survival estimate.

After seven years, particular designer niches resulted in the highest survival of whitebark pine seedlings and clusters; these prized niches were on burns where beargrass roots were absent and microsite (preferably stumps and snags) were situated in proximity to seedlings. Conversely, seedlings planted in designer niches on unburned areas where beargrass mats were present and microsites were absent had the lowest survival rates for individuals and clusters. Results for inoculation with ectomycorrhizal fungi was site dependent.

In this study, 60% of the seedlings and 69% of clusters planted on burns were alive after seven years, while only 31% of individuals and 35% of clusters situated in unburned areas survived. Other studies have also found that planting whitebark pine seedlings on burns can result in higher seedling survival rates [26,29,41]. It has been suggested that a pulse of nutrients after a fire benefits seedlings [27], and while this may be true, the positive effect of terra-torching continued over the seven-year period in our study. The removal of tree and understory competition, which also results in additional light reaching the forest floor may help explain the sustained positive effect. Gelderman et al. [24] found that selective removal of competing trees was preferred to fire for natural establishment of whitebark pine seedlings. In a non-fire system, opening of the canopy from beetle infestation promoted higher numbers of natural whitebark pine seedlings, which was explained by gap dynamics [22]. Moody [42] found that fire was not required for recruitment. These studies suggest that optimal regeneration may not be on burns per se but in areas where the canopy is opened or competition removed. However, slash left from thinning can prevent bird caching and increase fuel load [43]. Factors important in warming climates will also need to be considered. For other conifers, drought after fire was found to be a strong negative factor in natural regeneration [44].

Whitebark pine seedlings regenerate naturally on burns after wildfire often because these open areas attract bird caching, but seedlings can be absent where birds or a seed source are lacking, or there is full retrieval, or severe site conditions [43,45,46]. A study using paired burned and unburned sites in Wyoming found that the density of whitebark pine seedlings regenerating naturally was actually higher in most unburned areas [47]. A recent large range-wide study found that 75% of naturally regenerating whitebark pine seedlings are now found in forest types other than whitebark pine, and 35% of these are in lodgepole pine forests [48]. Results may reflect comparatively less regeneration in ghost forests since 50% of the whitebark pine assessed was dead. Lodgepole pine is ultimately the faster-growing tree in these situations. Thus, while natural niches where regeneration is high can be informative, individual factors must be teased apart when designing niches in restoration. Planting can help overcome natural obstacles such as lack of seed source, absence of birds, high seed retrieval, delayed germination, and episodic moisture.

In natural settings, where whitebark pine exists in forests, natural regeneration has been reported to be prevalent in *Vaccinium scoparium* and rare in *Xenax tenuifolium* (beargrass) [11,26,48]. In our study, after seven years of planting, whitebark pine seedlings in beargrass root mats in the burned areas reduced seedling survival by 15.1%, $X^2(1, n = 685) = 16.11, p < 0.001$ and cluster survival by 16.3%, $X^2(1, n = 231) = 8.85, p = 0.003$. Other studies have also shown that a beargrass understory

is not conducive to establishment of planted seedlings or seed germination [25,29]. A difference here is that for burned areas, only roots were present, and we can attest that whether burned or not, these consist of thick, dense, rhizomatous mats. Seedlings that initially survived in these mats are likely to succumb to beargrass competition or lack of soil access, unless roots dive deep. Studies have hypothesized that when a *Vaccinium* understory promotes survival it is because of mycorrhizal facilitation; however, currently there is no evidence to support this hypothesis for whitebark pine. Greenhouse studies with *Vaccinium* and *Pinus strobus* L. have shown definitively that *Vaccinium* is not a source of mycorrhizal fungi for the pine since *Vaccinium* species associate with ericaceous fungi and pines with ectomycorrhizal fungi [49]. Interestingly, in the Kohout study, adjacent ericaceous plants did promote colonization of *P. strobus* root tips by the pine-specific ectomycorrhizal fungus *Rhizopogon salebrosus* A.H. Sm.; the mechanism is not known.

Protective microsites are interesting components of designer niches because available shelter objects are inherently different on burns and unburned areas. In burned areas, microsites consist primarily of burned snags, burned stumps, burned movable charred wood, and rocks. In unburned areas, shelter objects can be living or dead standing trees, recent or old logs, rocks, or even clumps of vegetation. In nature, Tomback et al. [23] found a shelter object within 15 cm of 85% of the seedlings regenerating naturally on a burn. The presence of microsite has been found to be important on unburned sites as well [25–27]. In this study, planting near microsites increased overall survival by 27% for individuals and 29.2% for clusters at the end of seven years. Stumps and snags were the most effective, regardless of site, and rocks and logs were found to be less effective in this study. The category ‘logs’ also included movable wood, especially in areas where large logs were not available, which might have affected results. Stumps and snags are initially more permanent and may offer organic or microbial resources. Here, the observation that microsites are important in natural regeneration has been useful in informing restoration. However, while initial data suggest snags as excellent microsites, McCaughey et al. [25] wisely recommended avoiding these as shelter objects since these can ultimately topple and reduce survival.

Inoculation with the native ectomycorrhizal fungus *Suillus sibiricus* did not improve overall survival after seven years. There is evidence that ectomycorrhizal fungi can improve the survival of conifers on burns and inoculation is recommended on severe burns [50]. Results for mycorrhizal studies are often site dependent for specific abiotic and biotic conditions, including variables such as soil, host and fungus [51]. In the case of Summit Lake, terra-torching was effective in removing overstory trees but burning was superficial and localized. Therefore, it is likely that ectomycorrhizal fungi survived deeper in the soil and on the roots of nearby living whitebark pines. Controls would also have access to ectomycorrhizal fungi, dampening any inoculation effect. In addition, inoculation does not guarantee colonization, and it was not possible to assess the roots of individual seedlings before planting. Benefits might be better tested on larger, more continuous and severe burns, as these can be devoid of ectomycorrhizal fungi [52]. We do know that whitebark pine seedlings colonized with the ectomycorrhizal fungus *Suillus sibiricus* promote nitrogen uptake and biomass increases in seedlings planted in burn soil devoid of ectomycorrhizal fungi [52,53]. Asebrook et al. [53] found that while survival was not enhanced on some sites in Glacier National Park by inoculation, height and stem diameter (not assessed here) increased with inoculation. Trusty and Cripps [54] found that over 90% of whitebark pine seedlings planted in a severe burn were colonized with ectomycorrhizal fungi after five years, however host specific fungi such as *Suillus* were severely reduced in comparison to the unburned adjacent site. The question is, under what conditions does jump-starting with inoculation improve seedling survival, and when is it necessary? Areas devoid of fungi appropriate for whitebark pine [55,56], such as those not previously in whitebark pine, those lacking mature living trees as a fungal source (ghost forests), and those where fire, beetle kill, or other disturbance have decimated the soil mycota [17,57] are candidates for use of inoculation. *Pinus cembra* L. (another stone pine) inoculated with *Suillus* species 50 years ago in Europe still hosts these fungi, highlighting the fact that inoculation can be sustainable [58].

5. Conclusions

As restoration of whitebark pine seedlings continues in an effort to save the species, it is important to understand which planting strategies produce the best survival outcomes to conserve resources. From our results, planting on lightly burned areas in microsites (shelter objects) is recommended for future plantings. Ideally, restoration sites should be monitored on a yearly basis to assess planting success. Where this is not feasible, monitoring 2 and 5 years after planting could capture the most significant decline in survival, as well as the period after the curve has stabilized, and give a reasonable prediction of long-term survival, according to the survival curve for our data.

This is one of the first studies to evaluate whitebark pine seedling planted in clusters. The number of individual seedlings that survived is comparable to that of other studies in which seedlings were planted individually. However, if seedlings in each cluster grow together as happens in nature, the maximum number of trees that might potentially survive to maturity is currently 331 and the actual number of clusters surviving is 205, of which 84% are on burns (in part reflective of the higher initial N in burns). Evidence from limber pine plantings in the Waterton Lakes National Park showed cluster planting to be advantageous to survival (data not shown). However, analyzing long-term advantages and disadvantages of planting in clusters should be considered in future plantings. The development of local designer niches must also be done with consideration for more general factors such as climate [59,60], genetics [61], and avoidance of areas where rust infection is too high [34]. Long-term monitoring can then begin to identify how some designer niches might promote early germination (such as on south slopes for some studies) while others (such as on north slopes) are more effective in promoting growth and sustainability on a local or regional level.

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