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Selection and Propagation of Pinyon Pine

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SELECTION AND PROPAGATION OF PINYON PINE

by

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ABSTRACT

Selection and Propagation of Pinyon Pine

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Single-leaf pinyon pines (*Pinus monophylla*) are xeric trees native to the Great Basin. This species is a source of wild-collected, edible pine nuts that are in great demand. With no previous research apparent, this thesis aimed to identify high yielding wild trees as scion sources, to evaluate graft types to establish a stock block, and topworking onto mature trees. Four wild stands were identified by pine nut collectors and six productive trees were selected from each stand based on visual assessment. Cone production was quantified by collecting six branches from the top of each tree and counting cone abscission scars for the past decade along the leader branch. Analysis of the cone scar data showed that trees with high cone production can be identified. Three trees per stand were selected for grafting based on cone productivity and healthy scion wood. Scions from each selected *P. monophyla* accession were grafted onto *P. edulis* seedling rootstocks using side-wedge and side-veneer grafts. Eleven months after grafting, side-wedge and side-veneer grafts had 90.9% and 80.7% survival rates respectively, showing that grafting is a reliable method of clonal propagation. Grafting

techniques for topworking *P. monophylla* scions onto mature *P. edulis* rootstock were evaluated. In 2017, scion treatments, timing of grafting event, and graft type were evaluated. The treatments with the highest survival rates were scions with needles, spring timing of grafting, and side-wedge graft type. In 2018 and 2019, further graft evaluations were done to compare side-wedge and side-veneer graft types with final evaluations on March 11, 2020 (2019 grafts had survival rates of 67%, side-veneer and 80%, side-wedge).

(86 pages)

PUBLIC ABSTRACT

Selection and Propagation of Pinyon Pine

Kylie Lawson

Single-leaf pinyon pines are drought tolerant trees native to the Great Basin. This species is a source of wild-collected, edible pine nuts that are in great demand. With no previous research apparent, this thesis aimed to identify wild trees with high cone production as sources for evaluating grafting onto immature and mature trees. Wild sources of scions were identified from four wild stands and six trees per stand. Counting and analyzing the number of scars left by mature cones along the leader branch provided an estimate for each tree's productivity and identified trees with greater productivity within a stand. The three best trees in each stand were selected as a scion source grafting on the estimated cone production and health of the new growth of each tree. Scions from each selected single-leaf pinyon tree were grafted onto two-leaf pinyon seedling rootstocks using two graft types. After one year, survival rates were above 80% with no difference between graft types. Grafting onto mature trees with treatments including scion treatments, timing of grafting event, and graft type were evaluated in 2017. The treatments with the highest survival rates were scions with needles, spring timing of grafting, and side-wedge graft type. In 2018 and 2019, further graft evaluations were done to compare graft types with final evaluations on March 11, 2020 (2019 grafts had survival rates over 67%).

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CHAPTER 1

SELECTION OF SUPERIOR CONE PRODUCING *PINUS MONOPHYLLA* ACCESSIONS

Introduction

Pine nut production in the United States is very low compared to worldwide production. China currently produces the most, with a five-year average of 42% of world-wide pine nut production and the United States importing 23% of all exported pine nuts worldwide (INF, 2011). Pine nuts are a highly valuable crop regardless of source, and production is increasing. As of the 2016/2017 season, world production reached 23,600 MT and increased by 20% from the previous year. Pine nuts are used for cooking, cosmetics, as high-quality massage oil, as a base for paints and treating leather, and even for finishing wood (Geisler and Romero, 2018).

Pine nuts in the United States are gathered from naturally occurring trees in the Southwest. These pine nuts are from two species of native pinyon pines (*Pinus edulis* Engelm. and *P. monophylla* Torr. & Frém.). Both species have twenty amino acids in the nut protein and their fats are composed of oleic, linolenic, and linoleic fatty acids. Pine nuts from *P. edulis* are composed of 14% protein, 62-71% fats, and 18% carbohydrates, whereas pine nuts from *P. monophylla* are composed of 10% protein, 23% fats, and 54% carbohydrates (Lanner, 1981). Pine nuts from *P. monophylla* are larger than from *P. edulis* and have softer shells. Pinyon pines are incredibly xeric trees that grow in rocky soils typically found on slopes and ridges. These trees are found mixed with Utah juniper (*Juniperus osteosperma*) and Rocky Mountain juniper (*J. scopulorum*) at elevations

ranging from 1000 m and 2800 m across their range and 1520 m to 2130 m in the Great Basin (Meeuwing et al. 1990).

Seed cones of pine nuts take over two years to mature. During the first spring, the microscopic buds form. It is not until the second spring that the buds grow large enough to be seen and are pollinated. The immature cones continue to grow through the summer, averaging about 1.27 cm in diameter by the time they go dormant in the fall. In the third spring, the cones are fertilized and turn green. They expand and grow through the growing season, reaching maturity in late summer. In the fall, the cones turn brown and open. Good pine nuts have dark brown shells, while tan-colored shells typically are empty. Seed cone crops occur in large quantities every 3-5 years, called masting events, making collection of pine nuts unpredictable (Lanner, 1981).

Pine nuts have been a food source for people and animals for centuries. In the Great Basin, pine nuts were a vital food source during the winter for humans and other animals. Predicting a good crop in upcoming years based on immature cone counts and other cone characteristics is unreliable at times due to animal predation and weather, but it is possible to know where a crop would not be if cones were absent. The Shoshone people would keep track of *P. monophylla* stands with immature cones to know where to harvest and prepare for winter months. The harsh winter environments were too dangerous to hunt in, making pine nuts the main food source for the people until spring came. In order to avoid losing the crop to animals, the Shoshone would collect the seed cones while they were still green and closed, then open them by roasting them in a fire. Some other Native American tribes with other food sources would often wait until the

cones opened before collecting them. Whether the pine nuts were historically a main source of food for survival or an excellent dietary supplement, the pine nuts and pinyons feature prominently in the culture of many Native American peoples (Lanner, 1981).

Despite the food value and the monetary value of pinyon pine nuts, less are being gathered and sold in the United States than in the past because of the labor-intensive harvesting process, increasing labor costs, and periodic episodes of widespread pinyon pine tree mortality (Parks, 2017; Redmond et al. 2017). *Pinus edulis* trees have experienced large diebacks in the recent past, with as much as 50% mortality in some areas during and after severe drought events, as well as decreased seed cone production in the surviving trees due to increased average air temperatures during the growing season (Biondi and Bradley, 2013; Redmond et al. 2012). *Pinus monophylla* trees during similar droughts only experience a 10% mortality rate and are otherwise minimally affected by drought events. Pinyon-juniper (PJ) stands have the potential to encroach on bordering plant communities, a pattern that has been widely noted and worked against by land managers who claim that PJ encroachment is human-caused due to fire suppression. They attempt to favor other habitat types that are thought to be more "natural" through the use of chaining and prescribed fire, when actually this pattern may have existed prior the influence of Euro-American settlers in the West (Biondi and Bradley, 2013).

Most pinyon pines used for landscaping are dug and transplanted from native sites into the landscape. Some selection for ornamental features has been done with clonal propagation by grafting scions from trees with desired traits onto seedling rootstocks. At

one nursery, six cultivars of *P. edulis* and seven cultivars of *P. monophylla* are being produced commercially (Fiesler, 2016).

To date, selection of accessions for superior cone and pine nut production has not been documented. This may be due in part to a lack of incentive because of the time investment required for these trees to reach maturity and produce cones. *Pinus edulis* can take as long as 25 years before they begin to produce cones with seeds, and 75 years before they produce a good crop (Ronco et al. 1990). *Pinus monophylla* trees take even longer to produce, with mature cone production beginning between 35 and 100 years (Meeuwing et al. 1990).

With the high value of pine nuts, increasing demand for this crop world-wide, and its potential use in drought tolerant landscaping; knowledge of superior accessions and propagation methods of *P. monophylla* and *P. edulis* may now be worth the effort and time. If superior producing accessions were identified, then a crop production system could be established to allow greater production of these native pine nuts. For this study, *P. monophylla* was chosen for selection of superior accessions because of the larger size and softer shells of their nuts and because there is a stronger tradition of collecting *P. monophylla* nuts in the northern Great Basin than *P. edulis* nuts.

In order to determine which trees produce the most cones with measurements taken at a single point in time, we used a cone abscission scar method that was developed in 1930 in Asia to measure *P. sibirica* seed cone production. It was later developed independently in 1974 by Forcella and Weaver while working with *P. albicaulis* (Forcella, 1981). With this method, counting mature seed cones, conelets, and historical

seed cone scars on a branch at a single point in time can provide a 11-year estimate for the cone production of a tree.

The cone abscission scar method was further evaluated by Redmond et al. (2016) who validated that it was a reliable method of determining seed cone production among slow growing conifers such as pinyons. From 2003-08 they evaluated 19 *P. edulis* trees from late July to early September by counting the conelets and mature cones on six to ten branches per tree. In October 2015, the cone abscission scars on the 19 trees were counted for the years 2004-08. The number of observed conelets and mature cones was compared to the number of cone abscissions scars for each corresponding year. The most efficient method of analyzing the cone scar data required counting the cone scars on five branches per tree and six trees per stand. Their analysis had nearly a one-to-one ratio of cone scars to observed mature cones. The aborted conelets did not typically leave scars large enough to be seen several years later, which shows that the cone scar count is at worst an underestimation of the total number of cones produced by a tree, which just means that it is a conservative method. Counting cone scars proved to be an accurate way to estimate how many mature cones were produced on each tree. It allowed for comparisons between trees with high, medium, and low cone production, though it was not as reliable for comparison among trees with low cone production.

The goal of the study described here was to accurately identify superior cone producing accessions of *P. monophylla* from around the Great Basin. Stands with moderate to high amounts of cone production were identified, from within which accessions could be selected. The selection of accessions was done first by visual

assessment of the trees' health and productivity. Once the initial cohort of accessions were selected, branches were cut from the upper canopy of each tree, and cone scars were counted along each leading branch. The resulting data provided an estimate of the productivity of each accession and stand.

Materials and Methods

Stand Selection

Stands were selected from around the Great Basin based on historically high cone production and pine nut harvesting for cultural reasons and a history of hobby and/or commercial harvesting. The resulting four stands that were identified span much of the Great Basin, increasing the genetic diversity of sampled trees and the potential of finding superior producing accessions from each unique local environment (Figure 1).

The height of the trees was measured using a Trupulse® 200 rangefinder from Laser Technology, Inc. In order to be more accurate, the height was measured twice, then averaged. The age of trees was determined by collecting cores from each tree near ground level, since *P. monophylla* can have low branches or multiple trunks. Cores were collected using a Haglof® 5.15 mm diameter increment borer. The cores were mounted with glue on separate 1 cm wide, grooved boards and then sanded until smooth in order to see the tree rings. The average crown spread of each tree was determined to be the spread from canopy edge to edge measured at the widest point of the canopy and the spread measured perpendicular to the widest point. The two measurements were averaged to determine overall crown spread. Climate data including precipitation and temperatures

(Figures 2 and 3). for each stand was collected using Oregon University's PRISM data (PRISM, 2019). Accessions were collected at the following sites:

Lander County, Nevada

The westernmost stand of *P. monophylla* included in this study was in Lander County, Nevada, and was introduced by Johnny Bobb, Chief of the Western Shoshone National Council. Pine nuts have been historically gathered from this stand by the Shoshone tribe. Trees were sampled and scions collected with permission of the Toiyabe National Forest. Soils in the stand are gravelly to very gravelly loam, composed of colluvium from andesite, tuff and, volcanic rock (Web Soil Survey, 2019). The elevation of the stand is 2195 m, at 39.4575 N latitude and 116.99306 W longitude (Figure 4). Typical plant communities in this stand include black sagebrush (*Artemisia arbuscula*), mourning milkvetch (*Astragalus atratus*), thickspike wheatgrass (*Elymus lanceolatus*) and *P. monophylla* (Intermountain Region Herbarium Network, 2020). The average age of trees sampled in this stand on 2 February 2018 was 91 yrs (Table 1). For this site the mean precipitation is 31.4 $cm \cdot yr^{-1}$ and the average maximum, mean, and minimum temperatures for the period 2007 to 2018 are 30.9° C, 9.3°C and -5.8°C respectively (PRISM, 2019; Figures 2 and 3).

Box Elder County, Utah

The northernmost stand evaluated is in the Raft River Mountains in Box Elder County, Utah. This stand has a history of being a source of pine nuts and is close to northern Utah population centers. It is one of the most northern stands of *P. monophylla* in the Great

Basin, with the sampled trees being at 1920 m elevation and 41.95278 N and 113.32167 W (Figure 5). Soils in these stands are gravelly loam derived from sandstone, limestone, quartzite, and mica schist parent materials (Web Soil Survey, 2019). Plant communities in the area include bluebunch wheatgrass (*Pseudoroegneria spicata*), Indian ricegrass (*Achnatherum hymenoides*), arrowleaf balsamroot (*Balsamorhiza sagittata*), mountain big sagebrush (*Artemisia tridentata* subsp. *vaseyana*), antelope bitterbrush (*Purshia tridentata*), alderleaf mountain-mahogany (*Cercocarpus montanus*), Utah juniper (*Juniperus osteosperma*), and *P. monophylla* (Stanley et al. 2019). Trees were sampled by permission of the Sawtooth National Forest on 12 February 2018. The mean age of the trees was 74 years (Table 1). This stand is remarkably cooler and wetter than the other sample locations, with a mean precipitation of 67.2 $cm \cdot yr^{-1}$ and maximum, mean and, minimum yearly temperatures of 28.1°C, 6.2°C and -7.9°C respectively (PRISM, 2019; Figures 2 and 3).

Iron County, Utah

The southernmost stand that we sampled is in Hamlin Valley in Iron County, Utah, which is managed by the Bureau of Land Management. The stand has an elevation of 2047 m and is located at 37.99833 N and 113.96944 W (Figure 6). This stand was introduced to the project by Chad Reid, Extension Professor for Iron County. Both hobby and commercial collection of pine nuts occurs in western Iron County. The soil in this stand of trees is a gravelly loam, formed from fan remnants and alluvium from igneous rock (Web Soil Survey, 2019). Plant communities in this stand include Indian ricegrass,

bluebunch wheatgrass, arrowleaf balsamroot, mountain big sagebrush, antelope bitterbrush, *P. monophyla,* and Utah juniper (Stanley et al. 2019). The trees were sampled on 23 February 2018. The average age of the trees sampled in 2018 was 157 years (Table 1). The mean precipitation is 31.9 cm/year and the maximum, mean, and minimum yearly temperatures are 30.5°C, 10.1°C, and -6.2°C respectively (PRISM, 2019; Figures 2 and 3).

Juab County, Utah

The easternmost stand that we sampled is in Juab County near Eureka, Utah, at 39.97889 N and 112.17500 W and 1967 m elevation, on land managed by the Bureau of Land Management (Figure 7). The soils in this stand are formed from alluvial fans, with parent material from sandstone, igneous rock, and quartzite (Web Soil Survey, 2019). Plant communities include bluebunch wheatgrass, arrowleaf balsamroot, antelope bitterbrush, mountain big sagebrush, Utah juniper, and *P. monophylla* (Stanley et al. 2019). This stand was selected based on a history of heavy use by hobby pine nut collectors. The trees were sampled on 23 August 2017 and 5 March 2018, with the average age of the 2018 sampling being 86 yrs (Table 1). The mean precipitation is 46.2 $cm \cdot yr^{-1}$ and maximum, mean, and minimum yearly temperatures are 30.4°C, 8.1°C, and -7.6°C respectively (PRISM, 2019; Figures 2 and 3).

These four stands provide a sampling of productive trees from around the Great Basin, as demonstrated by their utilization by pine nut collectors. Six to twelve trees per stand in each stand except for Lander County, NV were initially sampled in 2017 to determine if the cone abscission scars could be identified on *P. monophylla* and to

develop the sampling method. Example trees from each stand are shown in Figure 8. A second sampling of six trees per stand was done in mid to late winter of 2018 to collect scion wood while also gathering a final sample for cone scar counts and to compare with the previous cone scar counts from 2017. Some of the trees sampled in 2017 were evaluated again in the 2018 sampling, though some were excluded from the second sampling due to excessive age, poor health, or time constraints.

Growth measurements were collected concurrently with cone scar counts. Cores were taken from each tree to estimate tree age, along with height, crown diameter, trunk diameter, and circumference (Table 1). Experimental trees are identified throughout this manuscript by the first letter of the county name, the United States Postal Service state abbreviation, and the number of the tree from 1-6. For example, tree number five from Box Elder County, Utah would be identified as BUT5, while tree number two from Lander County, Nevada would be identified LNV2.

Accession Sampling

Sampling of individual trees consisted of cutting six branches from the top half of the canopy from six aspects around the canopy (N, NE, SE, S, SW, and NW). Each branch was a minimum of 0.6 m long to ensure that enough wood was collected to count at least 10 years of cone scars, especially for trees with vigorous vegetative growth. Branches with intact mature cones were selected when possible to ensure that samples were consistent. Once the apical branch selected for a cone abscission scar count was removed from the tree, lateral branches suitable for use as scions were also removed and stored for later grafting. The remaining apical shoots of the six sampled branches from

each tree were bundled together and stored dry, while the scion wood was wrapped in damp paper towels and stored in a cooler on ice.

Cone Abscission Scars

Counting cone scars began with the apical bud being designated as year one and correlating with the next year's potential mature cone crop. Since cones take two years to mature, at year one conelets may be found adjacent and basipetal to the apical bud. Mature cones with ripe pine nuts can be found just below the bud scar of the previous year, year two, concurrent with the current year's mature cone crop. Moving back along the branch and noting the presence or absence of cone scars affiliated with each bud enables an accurate estimate of cone production for each year. Lateral branches emerge just above the bud scar, which helps to differentiate between a cone scar and an aborted lateral branch. *Pinus monophylla* are slow growing trees, with 1-5 cm of annual growth on mature wood, and potentially 7-10 cm of growth on immature wood. After 10-11 years, it becomes very difficult to find cone scars since the bark begins to stretch and crack as well as accumulate wounds.

Cone scar data was compiled by listing the stand location, individual tree, branch number, estimated year for each bud along the branch, and the number of or absence of cone scars. Comparisons of the cone abscission scars provide an estimate of the productivity of individual trees and of each stand. Using ANOVA and Tukey's Honestly Significant Difference (HSD) in JMP (Version 13.2, SAS Institute, Cary, NC) significant differences of cone production were independently identified between trees within the same stand for both the 2017 and 2018 data collections.

Results

Average cone scars per year for a given tree or stand were typically less than one scar per year because cones are not produced every year. Differences between individual trees within each stand were compared using ANOVA and TukeyHSD tests in RStudio. The trees sampled in Lander County, NV averaged 0.48 cones per branch per year over the 11 years. The cone scar counts ranged from an average of 0.242 cone scars to 0.712 scars for a sampled year, with accession LNV6 having higher cone scar counts than LNV2, LNV4, and LNV5 ($p < 0.02$, Figure 9). Trees selected for grafting were LNV1, LNV2 and LNV6. Tree LNV6 was chosen because it was the tree with the highest cone production within the stand. Trees LNV1 and LNV2 were selected due to slightly higher cone counts and healthy scion wood. Healthy scion wood was determined by the length and diameter of the previous year's growth. Some of the trees had 1 cm of previous year's growth or less, making grafting using the new wood nearly impossible. Trees with greater growth were more likely to have grafting success.

The sampled trees in Box Elder County, UT averaged 0.84 cones per branch per year over 11 years. Analysis comparing the cone scar counts from the Raft River Mountains, UT trees showed no significant difference between trees ($p = 0.569$, Figure 10). Trees selected for grafting from this stand were BUT1, BUT3 and BUT4.

The sampled trees in Iron County, UT averaged 0.39 cones per branch per year for 11 years. Tree IUT1 is significantly more productive than IUT2, IUT3 and IUT4, with 0.71 cone scars per year compared to 0.35, 0.18 and 0.32 cone scars per year, respectively ($p < 0.02$, Figure 11). Trees selected for grafting were IUT1, IUT4 and

IUT5. Tree IUT1 had the highest cone production estimate, and trees IUT4 and IUT5 had healthy scion wood.

The sampled trees in Juab County, UT averaged 0.58 cone scars per branch per year for 11 years. Tree JUT3 is significantly more productive than trees JUT2 and JUT5, with 0.85 cone scars per year compared to 0.45 and 0.32 cone scars per year respectively (Figure 12). Trees selected for grafting were JUT1, JUT3 and JUT4. Tree JUT3 had the highest cone scar estimate, and trees JUT1 and JUT4 had slightly higher cone scar estimates and healthy scion wood.

Differences between the cone productivity of each stand were compared using ANOVA and TukeyHSD. The Box Elder County stand had significantly more seed cone production than the other stands, with 0.838 mean cone abscission scars per year (Lander = 0.48, Iron = 0.39, Juab = 0.58, $p < 0.00012$). The Juab County stand had significantly greater cone production than the Iron County stand ($p = 0.03$, Figure 13). The age of each tree was compared to the average cone productivity for a given year of each tree. Based on the literature, it was expected that younger trees would have less cone production than older trees. However, no correlation was found between tree age and cone scar counts (Figure 14).

It is important to recognize that differences between stands are likely to be influenced by both environment and genetics, making comparisons between trees from different stands biased due to the different environments of each stand. By comparing cone production of trees within a stand to one another, the need to compensate for environmental differences is removed and we can be more certain that the differences in

cone production are likely influenced by the natural genetic variability of the individual trees.

The preliminary collection in 2017 was to see if the cone scar method could reliably find better trees. It also helped in the development of the methods for the 2018 sampling. Methods used in 2017 were less reliable and less uniform than for 2018 – branches were cut from easy to reach points around the trees, and some trees were sampled despite being either marginally productive or too young to produce large numbers of cones. The 2018 methods are described above and were much more uniform and reliable. When we compared the data from trees sampled in both 2017 and 2018, there was no significant differences between the samples ($p \geq 0.05$, Figure 15). This is more evidence that the cone scar count method is a reliable way of estimating cone production.

Discussion and Conclusion

Six superior producing trees were identified from each of the four sampled stands around the Great Basin based on visual assessment. Counting cone scars, the method developed by Forcella (1981) and used with success by Redmond et al. (2016), appeared to accurately estimate the cone production of individual trees and provided a method of finding the tree or trees with higher current cone production. Producing an orchard for pine nuts with the gathered accessions from this study would provide a common garden

of superior genetics from around the Great Basin for future studies and potentially high pine nut production.

At least one tree with superior cone production was identified in each stand except the Box Elder County stand, where all trees were statistically equivalent. The goal was to select three superior trees from each stand for use in grafting and to establish an accession pool. Considering that the six trees selected were healthy and had visual signs of greater cone production than surrounding trees, then it is likely that all of them have high potential for cone production. That we could find at least one tree that was significantly more productive than the other five visually superior trees, proves that the hypothesis was correct, and superior trees could be identified. In the case of the Box Elder County stand, no individual tree had significantly superior seed cone production because all of the accessions had superior cone production, with mean cone scars per branch for a sampled year ranging from 0.74 to 0.99 as compared to the upper range of the other stands' cone scar production (Lander = 0.71, Iron = 0.71, Juab = 0.85). Accessions BUT1, BUT3 and BUT4 had higher cone production than several accessions from each of the other stands (LNV4, JUT5, IUT3 and IUT4 specifically, $p \leq 0.05$, Figure 13) The high cone production of the trees from the Box Elder County stand, which ranged from 54-107 years old, challenges the idea that *P. monophylla* trees need to be 100 years old before they have reliable and relatively abundant cone production. The lack of correlation between the age of a tree and cone productivity also shows that increased age does not imply a decrease in cone production (Figure 14), at least within the age ranges of these populations.

All the stands produced large amounts of cones in synchrony with each other except for the Box Elder County stand. This stand often had high and low producing years just before or after the other stands had high and low production. Though the reasons for masting years are still being researched, it is believed that climate is the main trigger for the masting events (Redmond et al. 2012). Perhaps the reason that the Box Elder County stand masting events are not synchronous with the other three sampled stands is due to the difference in climate at that location.

The cone scar counts used in this study are estimates of the cone production of individual trees and whole stands. Though it has been proven to be a reliable, robust method, further research of the cone production of these sampled trees, both the parent plant and the grafted scions would provide further validation of the cone scar count method. Further evaluation of grafted scions would provide information on what effects if any that grafting scions of *P. monophylla* onto *P. edulis* rootstock has on cone production.

This sampling and gathering of scion wood from multiple stands around the Great Basin will provide a germplasm bank in case a natural disaster or land management actions remove these trees. In the time it took to identify the four stands for this study and selecting potential trees for sampling, one tree from the Lander County stand and two from the Box Elder County stand were cut down for various reasons. Though the removal of these trees was inconvenient for this project, pinyon-juniper stands are not static and are believed to have moved and encroached on neighboring landscapes since long before Europeans settled in the western states (Biondi and Bradley, 2013). Collecting samples of

highly superior trees, then grafting them onto seedling trees and growing them in an orchard setting, could compensate for the potential loss of genetics that is part of the constantly changing *P. monophylla* ecosystem.

An interesting aspect of the estimated climate data for the sampled locations is the difference in precipitation. The temperatures of each stand are not that different (Figure 3) but the precipitation each stand experiences is different (Figure 2). The Box Elder County stand has significantly more precipitation than each of the other stands (p = 0), as well as higher cone production ($p \leq 0.001$). The climate data and cone production are both estimates, making correlations between the two tenuous at best and requiring further research. However, it looks as though precipitation has more of an effect on cone production than temperature. In a study with *P. pinea*, the effects of irrigation were evaluated, and it was determined that drought has a greater effect on plant growth than temperature (Loewe and Delard, 2016). Further research could help determine the same is true for *P. monophylla*. Supplemental irrigation in an orchard of *P. monophylla* could potentially increase the cone production. This added to the possibility that the trees may begin mature cone production earlier than has been previously documented, may be enough reason to encourage the cultivation of *P. monophylla* for pine nut production rather than relying solely on harvest from wild grown stands.

Figure 1. A map of the sampled stands from around the Great Basin (Google, 2019).

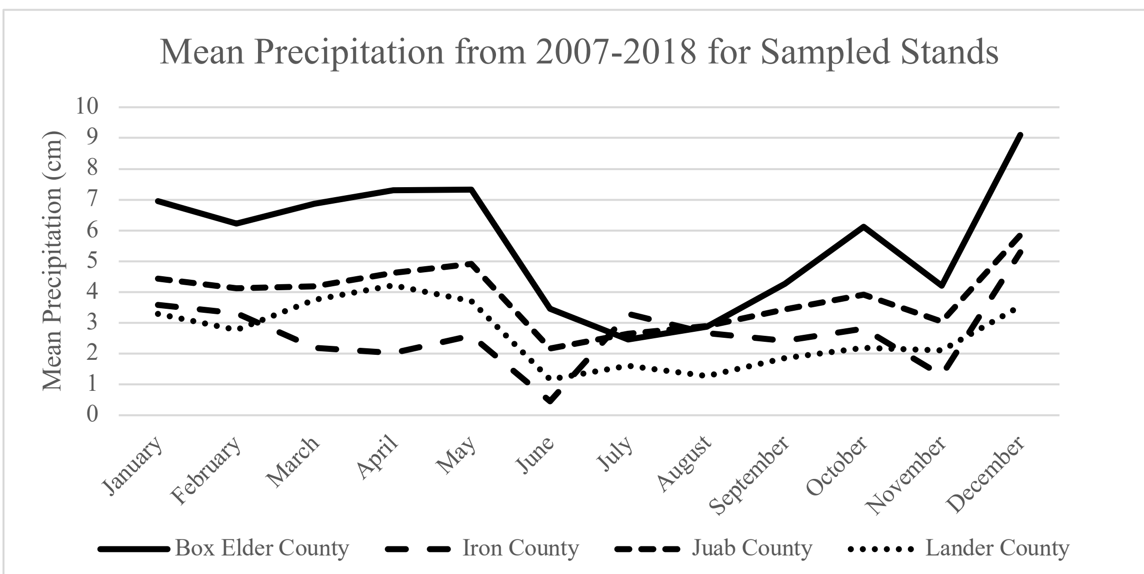

Figure 2. Estimated precipitation averages over 2007-18 (PRISM, 2019) by month for each of the sampled stands. The Box Elder County stand has the greatest precipitation, at almost double that of the Lander and Iron County stands.

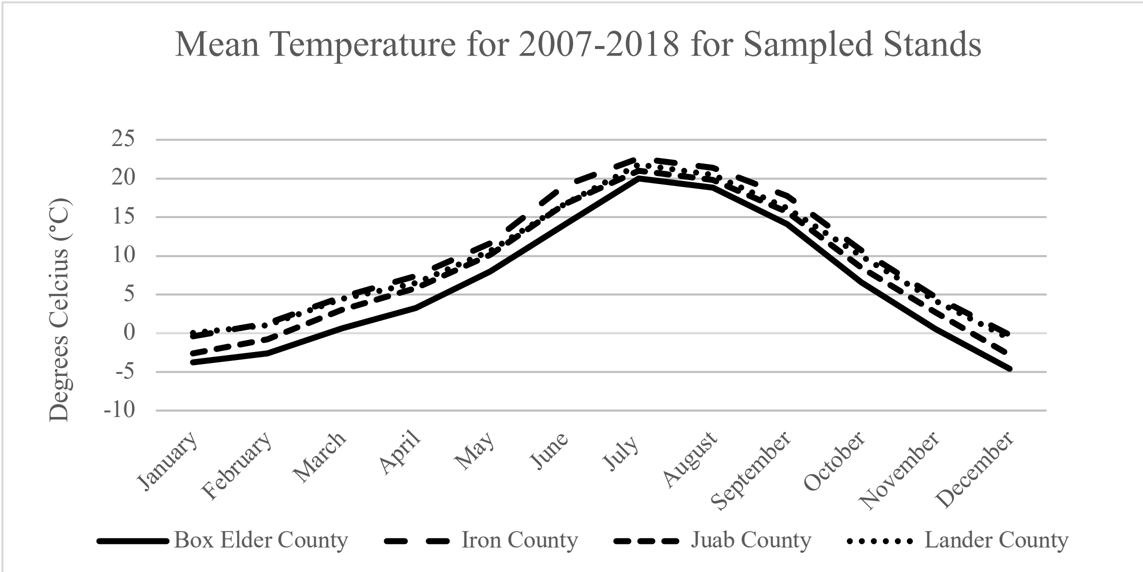

Figure 3. Estimated mean temperature by month for sampled stands. The Box Elder County stand has lower temperatures than each of the other stands for all months.

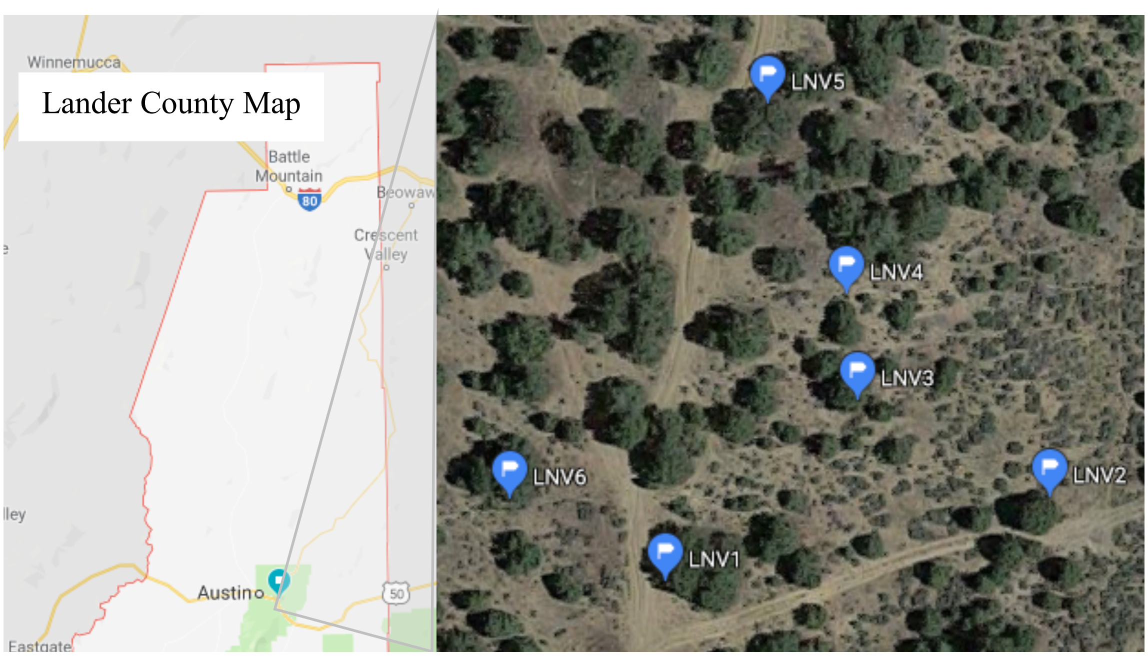

Figure 4. The location of the sampled stand in Lander County and the trees selected for cone scar count evaluations.

Table 1

Measurements of Sampled Trees

Location	Tree Name	Trunk Diameter (cm)	Trunk Circumference (cm)	Height (m)	Average Crown Spread (m)	Age (yrs)	Height to Core (cm)
Box Elder	BUT1*	40.7	128	5.1	6.4	59	104.1
Box Elder	BUT2	38.3	120	5.5	6.5	65	40.6
Box Elder	BUT3*	31	98	6.6	6.25	59	40.6
Box Elder	BUT4*	34	107	5.8	5.95	54	20.3
Box Elder	BUT5	51.8	163	9.1	7.3	100	71.1
Box Elder	BUT6	54	170	10.7	8.35	107	99.1
Juab	JUT1*	61.5	193	7.8	9.45	259	48.3
Juab	JUT2	66.5	209	8.8	11	138	30.5
Juab	JUT3*	53.5	168	9.2	7.3	146	27.9
Juab	JUT4*	45.3	142	7.6	7.3	137	30
Juab	JUT5	60	188	8.8	8.55	111	38.1
Juab	JUT6	65.2	205	7.4	7.9	151	25.4
Iron	IUT1*	57.5	180	11.1	9.2	92	68.6
Iron	IUT2	63	198	8.6	7.1	96	25.4
Iron	IUT3	36.8	119	8.1	4.95	62	43.2
Iron	IUT4*	28.8	91	7.2	4.9	82	38.1
Iron	IUT5*	42.5	134	8.6	7.2	98	30.5
Iron	IUT6	53	166	7.5	5.75	86	48.3
Lander	LNV1*	49.2	155	8.8	9.15	131	124.5
Lander	LNV2*	39	123	7.2	7.45	64	50.8
Lander	LNV3	49.5	156	7.4	7.55	77	63.5
Lander	LNV4	20.5	65	4	3.85	44	22.9
Lander	LNV5	52.8	166	7.9	9.2	141	78.7
Lander	LNV6*	32	100	6.6	6.65	91	45.7

Note: "*" denote trees selected for use as scion source in grafting

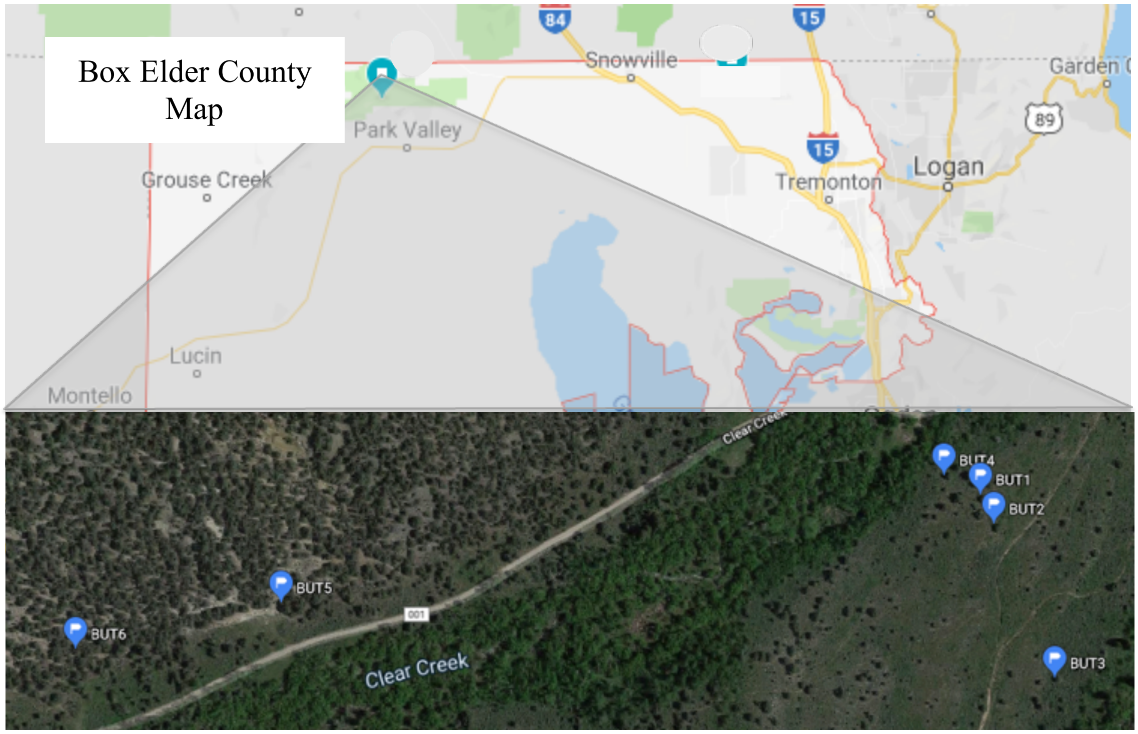

Figure 5. The location of the sampled stand in Box Elder County and the trees selected for cone scar count evaluations.

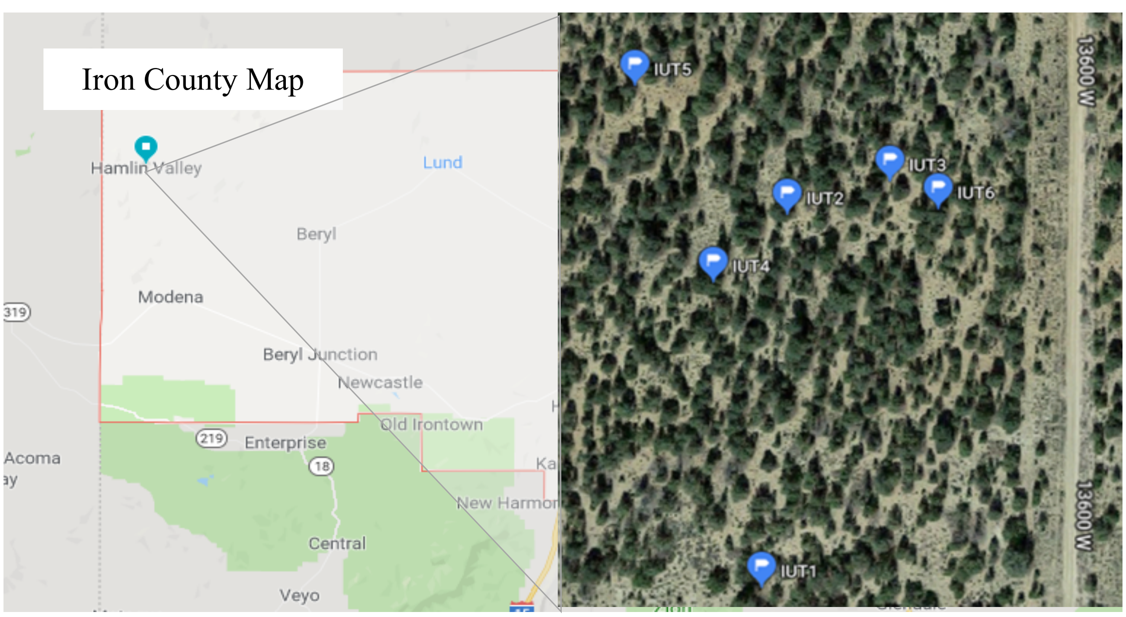

Figure 6. The location of the sampled stand in Iron County and the trees selected for cone scar count evaluations.

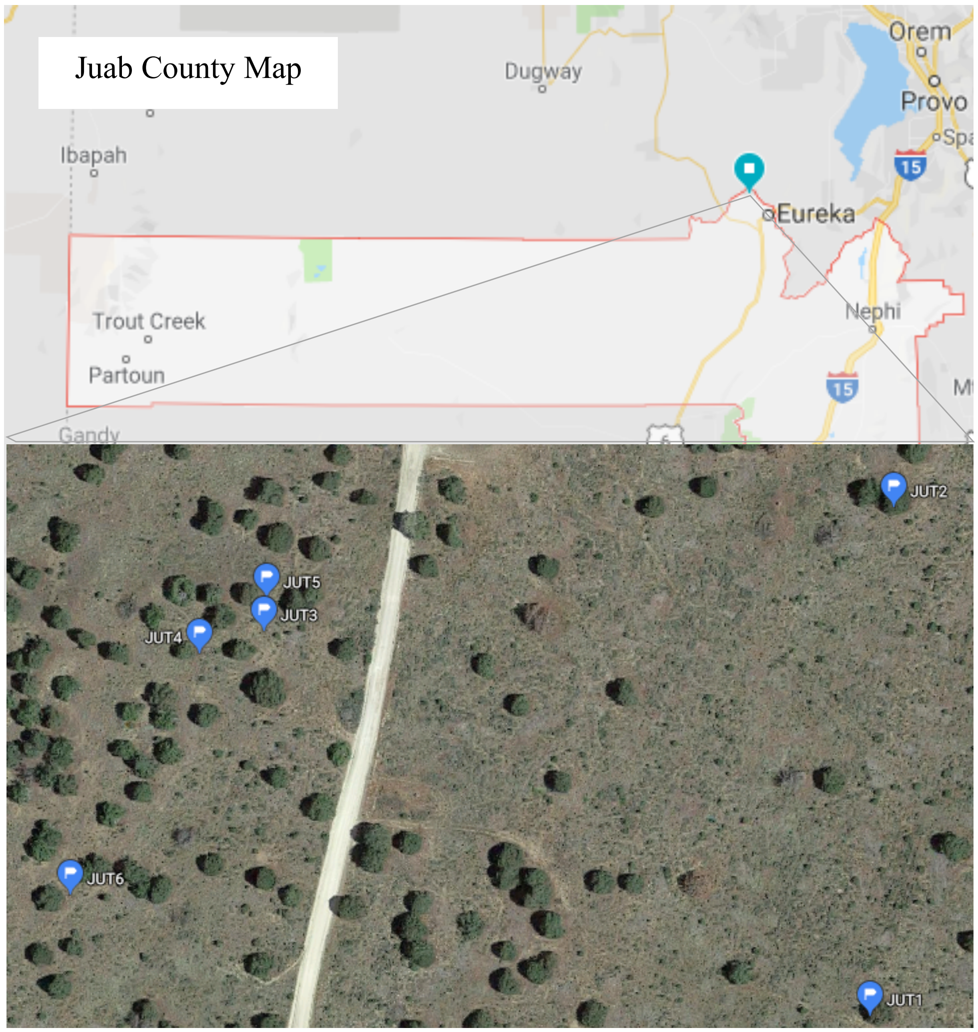

Figure 7. The location of the sampled stand in Juab County and the trees selected for cone scar count evaluations.

Example Trees from Sampled Stands

Figure 8. Photos of trees from each of the four sampled stands. From left to right and top to bottom are example trees from the Lander County, Box Elder County, Iron County and Juab County stands.

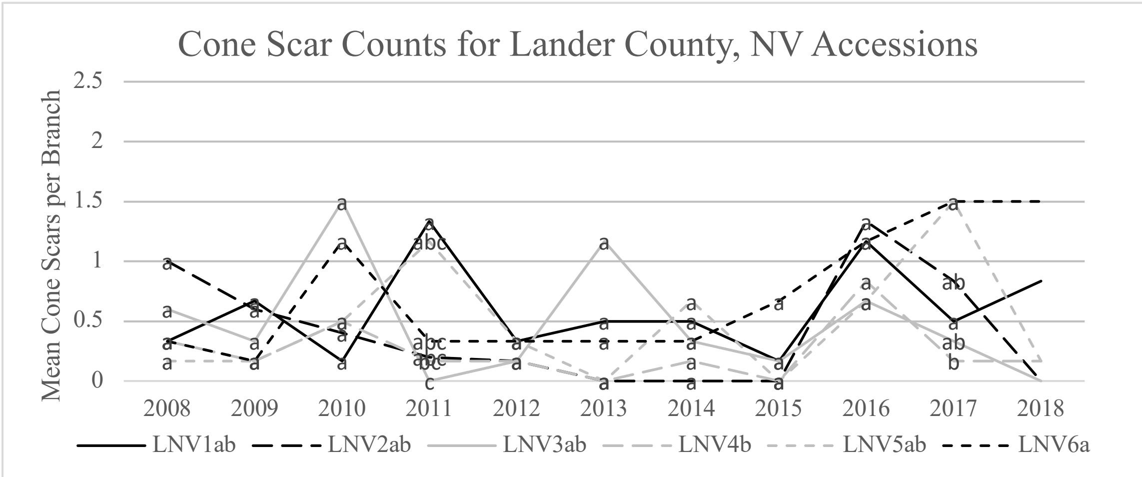

Figure 9. Average cone scars per branch for each given year for the six accessions (labeled in the right legend) sampled in Lander County, NV. Trees which were selected for further use in grafting have black lines. Mean cone scars of accessions with similar letters are not statistically significant ($p < 0.05$).

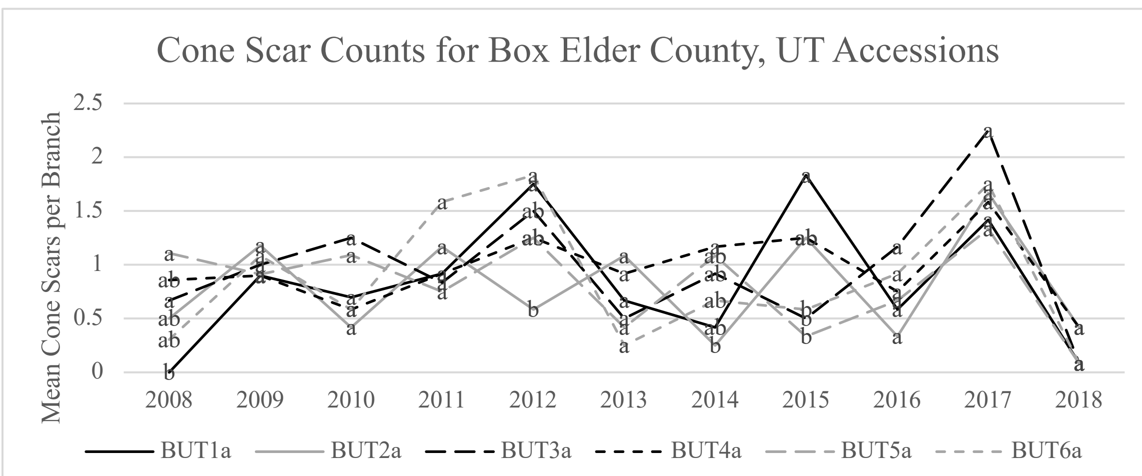

Figure 10. Average cone scars per branch for each given year for the six accessions sampled in Box Elder County, UT. Trees which were selected for further use in grafting due to high cone production and healthy bud wood have black lines. Mean cone scars of accessions with similar letters are not statistically significant ($p < 0.05$).

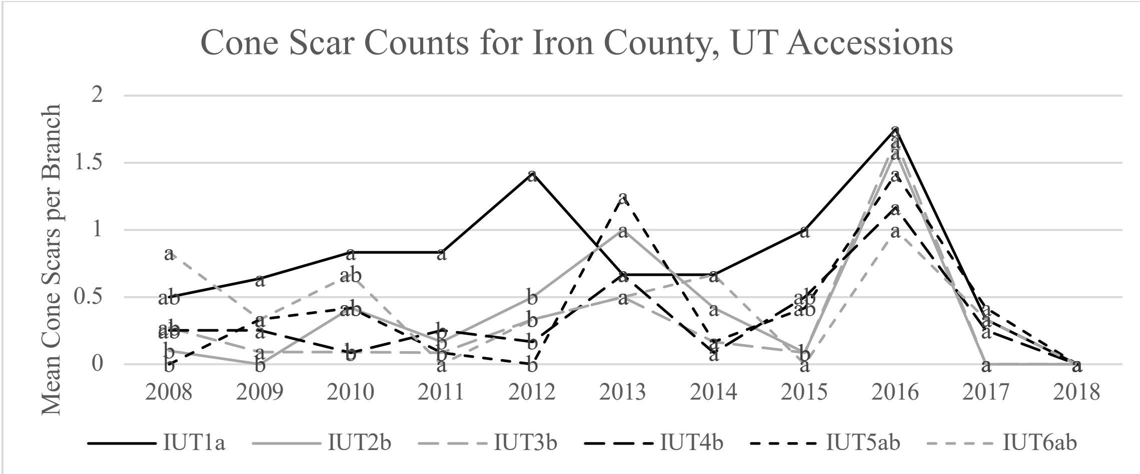

Figure 11. Average cone scars per branch for each given year for the six accessions sampled in Iron County, UT. Trees which were selected for further use in grafting due to high cone production and healthy bud wood have black lines. Mean cone scars of accessions with similar letters are not statistically significant (p < 0.05).

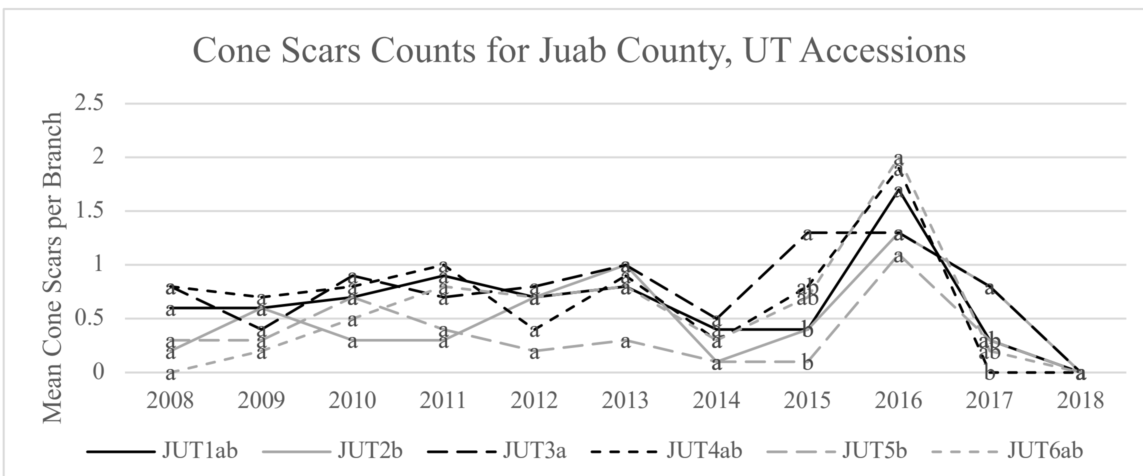

Figure 12. Average cone scars per branch for each year for the six accessions sampled in Juab County, UT. Trees which were selected for further use in grafting due to high cone production and healthy bud wood have black lines. Mean cone scars of accessions with similar letters are not statistically significant (*p* < 0.05).

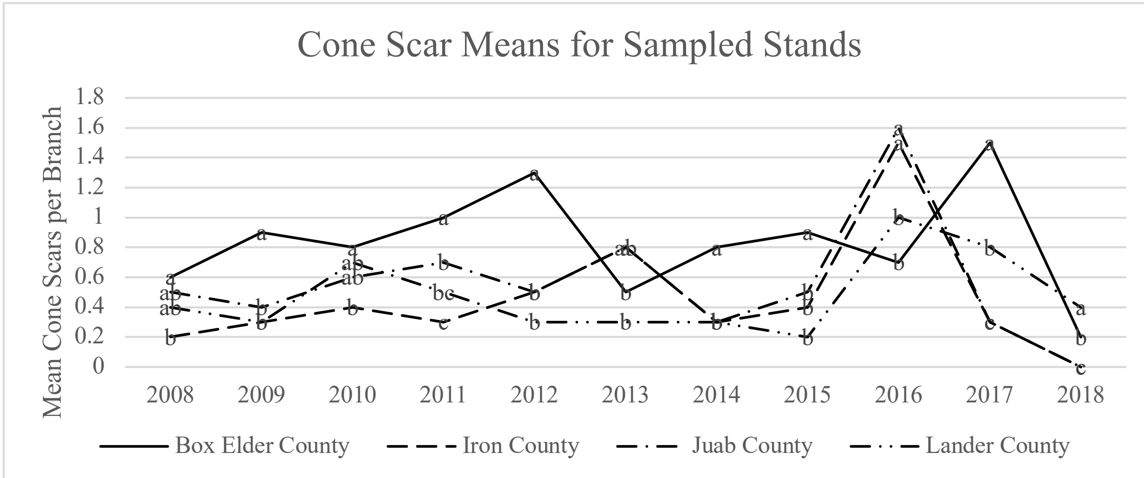

Figure 13. Average cone scars per branch for each given year of the four sampled stands. Mean cone scar counts of stands with similar letters are not statistically different ($p < 0.05$).

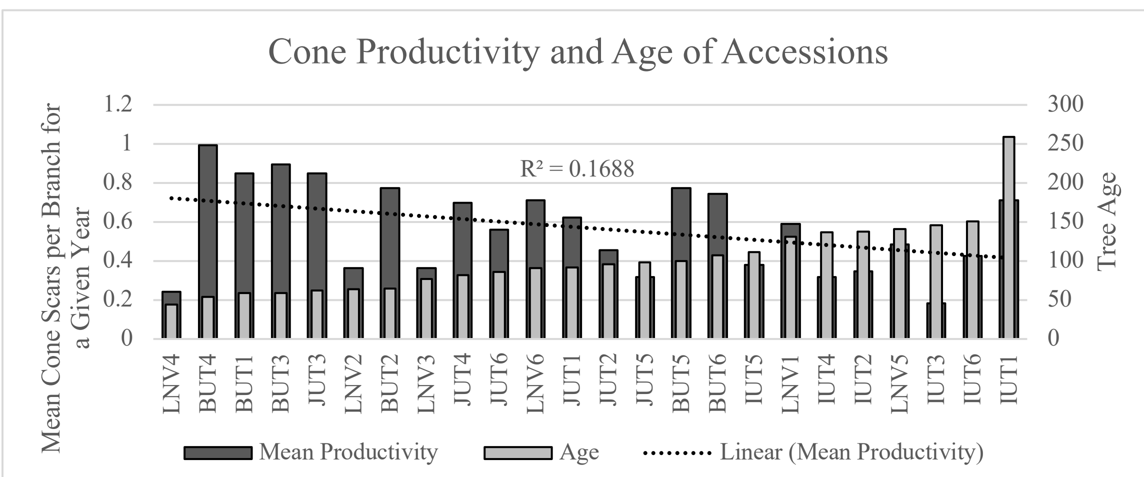

Figure 14. Correlation between tree ages as determined by tree ring count compared to the estimated cone production for each tree. No correlation was found between tree age and cone productivity.

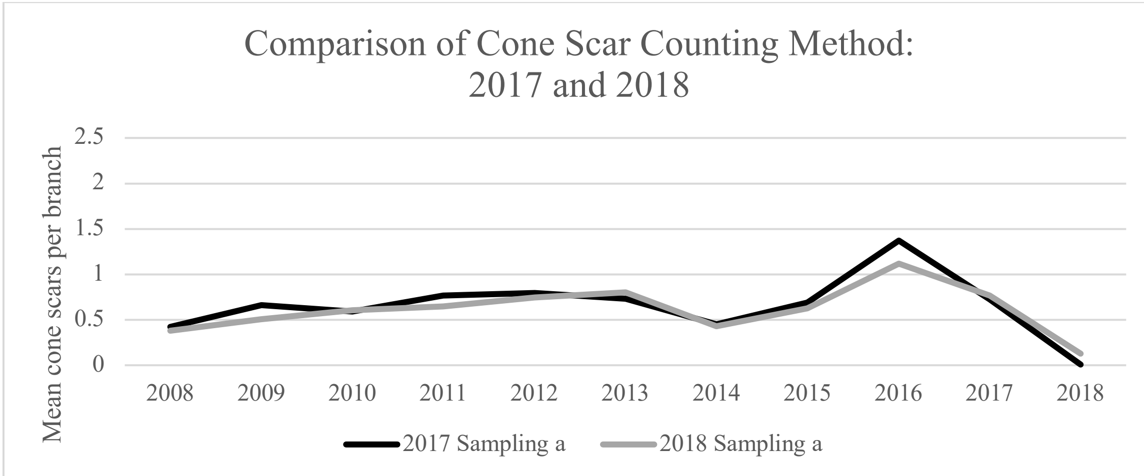

Fig 15. Cone scar data for the 2018 and 2017 cone scar samplings show that the cone scar counting method is viable. Only trees that were sampled in both 2017 and 2018 were used in this comparison. Means of cone scar counts over the entire sampling period with different letters are statistically different ($p < 0.05$).

CHAPTER 2

TOPWORKING PINUS EDULIS TO PINUS MONOPHYLLA

Introduction

Pine nuts are a highly valuable crop, under increasing demand as a gourmet food product, and a luxury oil source world-wide. Two species of pinyon pine that produce pine nuts are native to the western United States – *Pinus edulis* and *Pinus monophylla*. Propagating these species by grafting scions to seedling rootstocks is done commercially with great success (Fiesler, 2016), but with little published documentation. Further, little to no research has been published on grafting pinyon pine onto mature rootstock (topworking). Topworking is commonly done in fruit and breeding orchards to change trees from one selection to another. Topworking involves grafting new genetic material onto a mature tree. The resulting commercial fruit tree typically contains two or three distinct genetic portions: the rootstock, the interstock which was the original grafted scion, and the scion which is the new genetic material (Davies, et al. 2018).

The trunk side-wedge graft, cleft graft, and the bark graft are established methods of topworking fruit trees (Davies et al. 2018). The trunk side-wedge graft is performed on the main trunk of a tree. The scion is cut into a wedge shape with the terminal bud removed, and a sloping cut is made into the rootstock. The scion is placed along one side of the cut on the rootstock to match the cambium of both pieces. The cut portions of the graft are covered to prevent desiccation. The cleft graft is performed by cutting off the end of a 1.3 to 2.5 cm diameter branch of the rootstock tree. A vertical cut is made 2.5 cm down into the center of the branch. The scion wood is cut into a 2.5 cm long wedge and is

then placed inside the cut space of the rootstock, then the graft union is wrapped with grafting rubbers. A bark graft is performed by cutting back a branch on the rootstock tree to a stub when the bark is in the slip stage. The bark on the stub is then sliced perpendicular to the main cut. The scion is prepared by cutting one side with a long, sloping cut. The scion is inserted under the bark along the cut with the exposed side of the scion pressed against the wood of the rootstock. The scion is then tied in place.

Research involving other species of pine can provide insight. *Pinus elliotii* was used in a study to measure if grafting could be used to speed up the change from a juvenile to a mature pine tree. It was determined that top grafting can shorten the time by up to 5 years. This study also determined that graft location on the rootstock has a significant effect on what type of cones – male or female – are produced. Female cones are much more likely to grow in the upper two thirds of a pine tree, whereas male cones are more likely to grow in the lower third (Perez et al. 2007).

Almqvist (2013) reported on the survival rate and cone production of *P. sylvestris* scions of different ages. The scion material was from one, two or three-year-old seedlings grown in either pots or raised beds. The scions were grafted onto 16 to 25-year-old *P. sylvestris* trees and the grafted trees were grown for five years and evaluated throughout the process. In the first fall, 75% of the grafts were alive, but after five years, only 54% of the grafts had survived. The older the seedlings were that scion pieces were taken from, the more successful the grafts were. This study also confirmed that location of the graft on the rootstock affects the gender of cones produced, with female cones growing higher

on the tree and male cones growing lower on the tree. It also showed that the lower a graft was placed on the tree, the higher the success rate of the graft.

In a long-term experiment using loblolly pine (*Pinus taeda*), the effects of rootstock on cone production were examined in detail. Scion trees for this study were measured each year for cone productivity, with a range of highly productive to almost sterile. The cones were counted each spring throughout the study, including the cones on the grafted branches as well as from the original ungrafted crown of the trees. This study found that interstock did effect cone production – as high as 15%– but only on female cone production. Interstock is created when a grafted tree, with a distinct rootstock and scion portion, has a new, distinct scion grafted on top of the previous scion. The resulting tree is a compound of three distinct genotypes. The interstock had no effect on male cone production in *P. taeda*, though. The effect of the scion on cone production was much higher at a range of 24 to 52%. This showed that even if the interstock had a negative effect, topworking trees is still an efficient method to increase cone production on mature trees and is worth the risk of the potential variance that interstock can cause on cone production (McKeand and Raley, 2000).

Another study using loblolly pine examined the effects of seedling rootstock on scion cone production. In this long-term study, it was found that the genetic variation in the seedling rootstock was minor, and that scions had more impact on the trees' ability to produce cones (Jayawickrama et al. 1997).

Specifics of graft types and care were harder to find in the literature, especially for topworking mature conifer trees. A video by McKeand and the North Carolina State

Cooperative Tree Improvement Program shows both scion care and graft types used in topworking loblolly pines planted in a plantation for a breeding program. The grafting was done to change the cultivars in the breeding program and was found to stimulate flowering in these pines. Grafts were completed in February to ensure that both the scion and rootstock were fully dormant. The scions, from cultivated trees, were prepared by removing all the needles and dipping the bud into paraffin wax to prevent desiccation. The graft type was a cleft graft. The scion was cut into a long sloping wedge with the cut about 5 cm long on both sides. Rootstock shoots from the previous season's growth had the needles removed to facilitate grafting. The top of the branch was cut off, then a 5 cm cut was made straight down the center of the branch. The scion was inserted into the cut cleft in the rootstock, then the graft was wrapped with grafting rubbers, long thin rubber bands that hold the graft together while the scion and rootstock connect, then sealed with Parafilm®, a thin waxy plastic that helps seal the graft to prevent desiccation (McKeand, 2011).

A YouTube video demonstrated another method of caring for conifer grafts. The scion was prepared by removing the needles on the scion except for those closest to the bud. The graft type used in the video was a side-veneer graft, and the completed graft was wrapped with grafting rubbers. In order to prevent desiccation, the grafted scion was covered with a lightweight plastic bag and sealed with twist-ties. Occasionally, the top twist-tie was removed to ventilate the graft union (Coniferblacklab, 2014).

A common practice for grafting conifer scions to seedling conifers is a side-wedge or side-veneer graft (Nelson, 2017). The side-wedge graft is performed by cutting

the scion into a wedge shape with two long, sloping sides. The immature rootstock, typically the stem of a seedling plant between 2-3 years old, is cut into at an angle but no deeper than 1/3 the diameter of the stem. The scion is placed inside the cut rootstock and then tied in place. The side-veneer grafts consisted of scion wood cut shallowly along one side and cut back at the bottom. A corresponding shallow cut was made in the rootstock and the scion set into the rootstock with cut sides facing. Grafts are tied with grafting rubbers or other material depending on the propagator's preference.

In order to determine which graft types would be most successful for grafting *P. monophylla* onto mature or maturing *P. edulis* rootstocks, side-wedge, side-veneer, and bark graft types were tried in multiple trials. A variant of the trunk side-wedge graft was also tried by preparing a scion similarly to side-wedge or side-veneer grafts, but with it inserted in the trunk similarly to a trunk side-wedge graft. This graft is referred to as a trunk side-wedge graft. Scion preparation and treatment methods, buds with needles (B+N) and buds without needles (B), were used to determine if these methods would improve the survival rates of topworking these species.

Materials and Methods

2017 Grafts

The purpose of this experiment was to compare graft type and graft treatments. The preliminary experiments were designed to evaluate a variety of methods common to topworking other species to determine what would work best in topworking *P. monophylla* onto *P. edulis* rootstock trees. The experiments were done at the USU Botanical Center in Kaysville, Utah on mature *P. edulis* rootstock trees. The rootstock

trees were transplanted from wildland settings to the Botanical Center in 2012. The trees were hand watered once per month during the growing season of the first year, then once per month through the growing season with overhead sprinklers for the second and third year, after which no supplemental water was applied. The landscape around the trees is mowed one to two times per year. The trees ranged from 1.8 to 2.4 m tall, though exact measurements were not taken at the time of grafting. Based on the height and presence of cones on some of the trees, it is likely that the rootstock trees were at least 30-40 years old, though the exact age could not be determined as the trees were small enough we felt taking cores for dating could damage the trees.

Pinus monophylla scion material was collected on March 7, 2017 from the Toiyabe National Forest and on August 23, 2017 near Eureka, Utah in Juab County. Scion care consisted of wrapping the cut scions in damp paper towels, placing them in plastic bags on ice during travel then keeping the scions in a cooler at approximately 4° C until use in grafting. Seven scion accessions were selected from each location. Since mid to late summer is the typical time for budding deciduous trees, this experiment looked at the possibility of grafting with dormant conifer buds during summer months. Grafts were completed on April 19, 2017 using the scions collected from the Toiyabe National Forest and August 25-28, 2017 using the scions collected from Juab County to evaluate the effect of seasonality on graft survival. Three accessions were randomly assigned to each rootstock tree, and four different types of grafts were completed for each accession.

Scions were prepared as buds only (B) or buds with needles (B+N). The B treatment was modeled after McKeand (2011), by removing all the needles from the

scion wood and then dipping the buds in paraffin wax (April) or diluted by one part water to two parts Doc Farwell® Grafting Sealant (August) to prevent bud desiccation. Once grafted, the graft was wrapped with 1 x 12.7 cm grafting rubber bands, and the graft and bands covered with Doc Farwell® Grafting Sealant to prevent graft union desiccation (Figure 1). The B+N treatment was used with bark, side-wedge and trunk side-wedge grafts. Grafts were tied with grafting rubber bands and then enclosed in a 1 mil clear plastic sleeve to prevent needle desiccation. A layer of 3 mil opaque white plastic tied with twist ties (April) or 6 mil tented and stapled white plastic (August) was used to reduce heat from solar radiation (Fig 2).

Three graft types were evaluated, bark grafts, side-wedge grafts and trunk side-wedge grafts. Bark grafts were performed by cutting off the end of a rootstock tree branch then making a vertical cut through the bark about 2.5 cm in length down from the cut end. A sloping cut of similar length was made on one side of the B scions which were then slid inside the bark with the cut surface against the wood. Side-wedge grafts were completed on 1-2-year-old rootstock tree shoots. The scions were cut into a V-shape about 1.3 to 2.5 cm long. A corresponding cut of similar length was made at downward angle into the rootstock tree penetrating about one third the width of the stem. Trunk side-wedge grafts consisted of B+N scions and were almost identical to side-wedge grafts, with the exception that the graft was performed on the main trunk of the tree rather than newer wood (Fig 3). Grafts were evaluated on July 13, 2017 (April grafts) and November 8, 2017 (August grafts). All grafts also were evaluated on May 18, 2018 to determine overwintering survival. Grafts were considered to have survived if the needles

(for B+N treatments) were still green and the bud had either grown or stayed dormant. Dead buds were differentiated from dormant buds by nicking the bark or bud scales with a scalpel. If the bark or bud scales had green beneath them, then the graft was considered dormant rather than dead. Dead scions typically had shrunken and/or wrinkled bark, whereas living and dormant scions had unwrinkled bark. Data were analyzed using PROC GLIMMIX or PROC MIXED procedures of SAS/STAT 14.3 in SAS (Version 9.4, SAS Institute, Cary, NC).

2018 Grafts

The preliminary results were used to develop an experiment to determine the difference in survival between side-wedge and side-veneer graft types that took place on April 18, 2018, at USU's Blue Creek Experiment Farm. This farm sits at an elevation of 1575 m, latitude 41.9622 N, longitude 112.4392 W. The soils at this site are well-drained silt loam, composed of parent material from sandstone, limestone and quartzite (Web Soil Survey, 2019). Three scion accessions were collected from the Juab County stand on March 5, 2018 and stored at 4 °C until use in grafting. The scions were all prepared and maintained using the B+N treatment. The rootstock trees were 10 unirrigated *P. edulis* trees, purchased in early spring of 2002 from the New Mexico State Forestry seedling program as seedlings and then held in one-gallon containers until October 2002 when they were planted at the Blue Creek Experiment Farm. Once planted, they were hand watered twice per month from June through September for two years. Since then, no supplemental water has been supplied to the trees. The soil around the trees was tilled annually to control weeds until the trees were too large to allow equipment between

them, after which spot treatment with glyphosate herbicide was used to control weeds. Trees were selected based on suitability for grafting.

Side-wedge and side-veneer grafts were performed as described for the 2017 grafts, with one of each graft type completed for each scion accession per rootstock tree. Scion accessions were collected from the Juab County stand from trees JUT2, JUT3 and JUT6. This resulted in 6 grafts per rootstock tree and 60 grafts in total. Each graft was wrapped with grafting rubber bands and Parafilm®. The graft was then covered by a 1 mil transparent plastic bag with the bottom cut off, then slipped over the branch and sealed with twist ties above and below the graft. White, 6 mil opaque plastic was then tented over the graft and stapled to reduce solar radiation and heat buildup inside the clear plastic. Half of the rootstock trees were randomly selected to be cut back at the time of grafting, with the remaining five left to be cut back until 16 weeks after grafting. Cutting back refers to cutting off the top of the rootstock tree above the whorl of branches on which the grafts were completed. The rootstock trees on which grafts were completed were identified as trees 1-10.

Four weeks after grafting on May 16, 2018, the inner, transparent plastic was cut to reduce the humidity around the graft, and all plastic layers were removed one week later on May 23, 2018. Pruning of all the grafts and cutting back of the remaining five rootstock trees occurred on August 8, 2018, 16 weeks after the grafting was completed. Pruning involved cutting the branches with grafts 1 cm above the graft union to ensure apical dominance of the scion in the following year. Final survival evaluations were completed on March 11, 2020.

2019 Grafts

In 2019, this experiment was replicated at the same location, but using different trees for rootstocks (identified as trees 11-20), and a different source of scion wood. In 2019, the scions were collected from the Box Elder County stand, from trees BUT1, BUT3 and BUT4 on March 27, 2019. The period of holding the grafts in a cooler before grafting during the 2019 grafts was three weeks, compared to six and a half weeks for the 2018 grafts.

Grafts were completed on April 17, 2019, as before. Plastic was slit on May 15, 2019 and the plastic layers were removed on May 22, 2019. Temperatures inside the plastic layers around the grafts were monitored using two HOBO Temperature Data Loggers®, recording the temperature every five minutes. The sensors were placed at the time of grafting by tying them to the grafted branch adjacent to the graft and inside the plastic cover. Temperature data was downloaded when the plastic was removed from around the grafts. Ambient climate data was collected from the Blue Creek, UT weather station (Utah Climate Center, 2019).

Unlike the 2018 grafts, the apical bud of each branch (but not the branch itself) with a completed graft was removed on June 13, 2019 to reduce the apical dominance of the rootstock tree on the grafted scion. Survival counts and measurements of candle and needle length were recorded on July 16, 2019. Final survival evaluations were completed on March 11, 2020.

Results

Scion treatments (B vs B+N treatments) influenced grafting success in 2017 for grafts with B+N scion treatments resulting in higher survival rates than B scion treatment (Figure 4). Graft mortality increased after each winter as shown by measurements made on May 18, 2018 and July 4-5, 2019. By July 2019, all the grafts completed in August had died, with April grafts having an overall survival rate of 21.5% and B+N scion treatment being more successful than B scion treatment (31% and 12% respectively, $p = 0.01$, Figure 4).

Graft type also affected the graft survival rate (Figure 5). Initially, the side-veneer grafts and trunk side-wedge grafts had significantly higher survival rates than the bark grafts. By May 2018, the overall survival rate dropped to 27%. Side-wedge grafts with B+N scion treatment had a survival rate of 64% compared to the other graft types (side-wedge grafts with B scion treatment 18%, trunk side-wedge grafts with B+N scion treatment 22% and bark grafts with B scion treatment 3%, $p < 0.0003$). By July 2019, survival rates had dropped to 21%, with side-wedge grafts with B+N scion treatment continuing to be more successful than the other graft types (50%, side-graft with B treatment 19%, trunk side-wedge graft with B+N scion treatment 13%, and bark graft with B scion treatment 3%, $p < 0.002$).

Timing of grafting initially had no effect on graft survival rate (April 47.27%, August 40.47%). However, when the grafts were evaluated a third time on June 4, 2019 no grafts completed in August survived. This may be due to the higher temperatures in August compared to April (Figure 6), and it may be due to the lack of dormancy in either

the scion or the rootstock tree. Either way, spring grafts were the most successful method of grafting onto mature rootstock trees.

The Blue Creek grafts completed in 2018 were evaluated on June 29, 2018 and had much higher success rates than the grafts completed at the USU Botanical Center, with an average of 82.5% survival rate. Neither graft type (side-wedge 82% vs side-veneer 83% survival) was significantly better than the other. The only significant effect was the scion selection, with JUT3 having less bud break and scion growth than JUT2 and JUT6 ($p = 0.002$, Table 1). These grafts were evaluated for survival after one year on June 13, 2019 and accession source was again the only factor that had a significant result, with JUT3 had less candle growth, needle length and survival than JUT2 and JUT6 scion accessions ($p \leq 0.05$ for each, 37, 80 and 86% survival rates respectively, Table 1).

Preliminary survival of the Blue Creek grafts completed on April 17, 2019 was evaluated on June 29, 2019 and showed 100% survival based on presence of green needles. On July 16, 2019, survival rates were again evaluated along with the length of one candle and needle of each graft measured. No difference was found between the survival rate of the two graft types (side-veneer = 90%, side-wedge = 83%, Table 2). Differences in candle measurements were seen when comparing scion accessions, with BUT4 grafts having longer candle lengths than the BUT1 grafts (3.3 cm average compared to 1.7 cm average respectively, $p = 0.009$). Despite the mild temperatures during April and May 2019 (Figure 7), inside the plastic wraps maximum temperatures were at least 27°C, reaching a high of 47°C on May 13, 2019 (Figure 8). Even with the

high temperatures inside the plastic wraps, the overall survival rate of the 2019 grafts was 73.5% as of March 11, 2020.

Discussion and Conclusion

Grafting of *P. elliotii* shortened the time to mature cone production by 5 years (Perez et al. 2007) and can stimulate cone production in *P. taeda* (McKeand, 2000). In our 2019 study, conelets were found on grafted scions on the USU Botanical Center 2017 spring grafts and the Blue Creek 2018 grafts. This is a good sign, showing that grafting may induce cone production on the grafted scions of *P. monophylla*. Further study is needed to determine if the cone production is due to the mature scion wood used or stimulation from being grafted. Such a study would also indicate if mature cone production can be expected more rapidly from the grafted scions than from the rootstock itself.

After evaluating a variety of scion treatments (B+N vs B) and grafting methods, valid methods of propagating *P. monophylla* onto *P. edulis* rootstock trees have been determined. Timing of grafts plays a critical role in graft survival. Grafts done in the spring, even with less than optimal methods, have a much higher chance of surviving than those done in the summer. Some fruit trees are budded in the late summer, and this option was evaluated in grafting *P. monophylla*. To have no summer grafts survive after two years while having very good success with the initial evaluation of the spring grafts (47.3% USU Botanical Center (2017), 82.5% Blue Creek (2018), 86.7% Blue Creek (2019)) shows that we have found valid methods for grafting the mature trees of this species. Scions prepared by removing all the needles and then dipping in wax or grafting

sealant (scion preparation B) had much less success than scions prepared by removing all but a few apical needles and wrapping in polyethylene enclosures (scion preparation B+N). These results were unexpected, considering that the B scion treatment method was modeled after a successful method of topworking *P. taeda* (McKeand, 2011). These results may have been confounded by using a bark graft rather than a cleft graft as was used by McKeand. The cleft graft required using much larger rootstock branches than the bark graft which is why it was used instead. In order to verify this, future trials could be done by comparing the bark graft and cleft graft with B scion treatment.

Another factor that may have led to low survival rates is the difference in climate, with the *P. taeda* grafts being done in North Carolina while the *P. monophylla* grafts were done in northern Utah, which is much drier. Both the dry climate and the slow growth of *P. monophylla* rootstock trees resulted in growth of current season's scions being very much reduced, typically less than 2.5 cm, as compared to *P. taeda* scions that had at least 5 cm of current season's stem growth to be used in the grafting process. Scion stock plant management may also be a factor to consider. Scions for the *P. monophylla* grafts were collected from wild-grown trees and were likely under greater stress than plantation-grown stock. Graft success with the B scion treatment may be higher if scions were collected from cultivated, irrigated trees.

An increase in survival rates was seen in spring grafted scions with B+N scions in all experiments. These rates are encouraging, but there is still room for improvement, especially considering the high temperatures recorded inside the layers of plastic for the 2019 Blue Creek grafts. One method of decreasing the temperature inside the sealed

plastic may be to wrap the area in aluminum foil to reflect solar radiation as is done with air layering (Davies et al. 2018). However, the lack of sunlight around the graft union may have adverse effects on scion health and would need to be evaluated to determine whether the cost of lower temperatures is worth the risk.

Interestingly, the graft types listed as being good for topworking fruit trees, trunk side-wedge and bark grafts (Davies et al. 2018) were the least successful in topworking *P. monophylla*. Instead, graft types most often used in propagating seedling conifer trees, side-wedge and side-veneer, (Coniferblacklab, 2014; Davies et al. 2018; Nelson, 2017; Fieseler) were more successful in grafting *P. monophylla* onto mature rootstock. It may be that the graft types used in topworking fruit trees will not end up being suitable for grafting conifers. Further research is be required to determine why the seedling graft types were more successful than topworking graft types in topworking *P. monophylla* scions onto *P. edulis* rootstocks.

This study has shown that topworking of these species is reliable using both side-wedge and side-veneer graft types. Topworking can be used to increase pine nut production by grafting trees with superior cone production onto mature trees with inferior cone production. Adequate cone production could be seen within a few years rather than the decades necessary for seedling pinyons to reach mature cone production. Some of the scions from the USU Botanical Center (2017) and Blue Creek (2018) graft events have produced immature cones (Figure 9). Further study would be needed to evaluate the cone production of the completed grafts. Marginal lands with stands of mature pinyons with varying levels of cone production would benefit most by topworking ttrees with the

lowest cone production. The overall cone production of the stand would be improved and provide a valuable food crop where cultivation of other crops is not reasonable. Further research would be needed on social and economic levels to engage and educate landowners and the public about the value of pine nuts and the potential to increase this native food crop.

Figure 1. The bud without needles treatment (B). The graft union was wrapped in rubber bands and painted with Doc Farwell® Grafting Sealant.

Figure 1. The bud with needles treatment (B+N). The graft union in wrapped with rubber bands and parafilm (left image), then covered by a thin, clear plastic bag (middle image) and then a thick, white plastic is stapled over the whole area (right image).

Figure 3. These images show the three graft types (side-wedge, a; trunk side-wedge, b; bark, c).

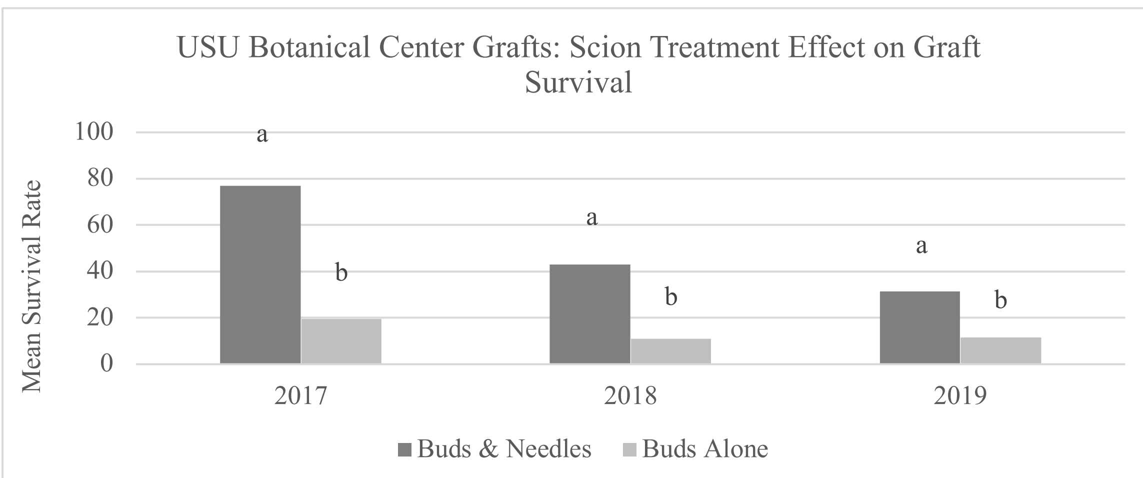

Figure 4. The results for grafts completed at the USU Botanical Center during the current and following seasons. The scion treatment with buds with needles (B+N) consistently had a higher success rate than the scion treatment with buds without needles (B, $p \leq 0.01$). Means with same lowercase letters within not significantly different among years and between scion treatments by Tukey's method for multiplicity at $\alpha = 0.05$.

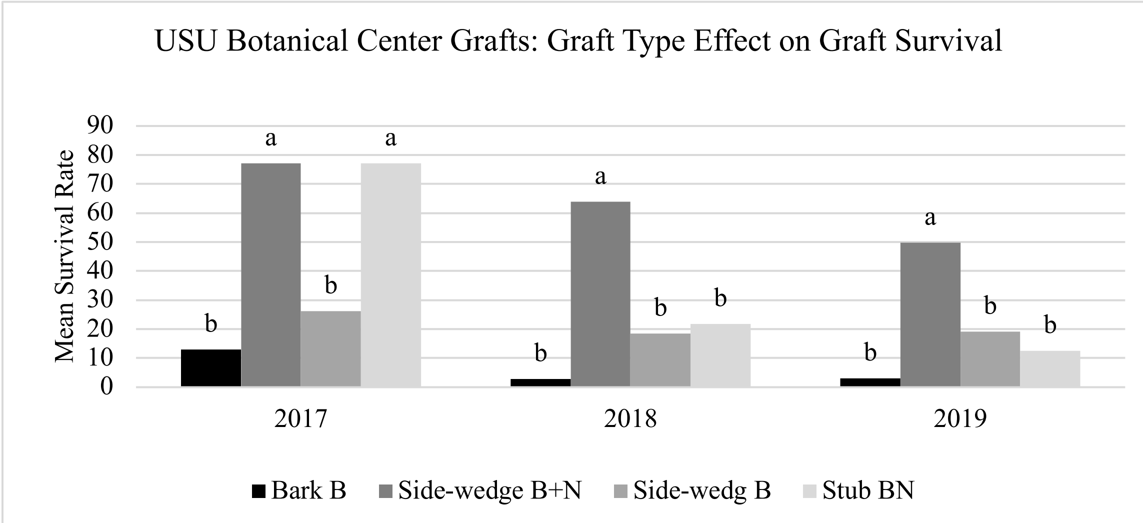

Figure 5. This shows the results of the USU Botanical Center grafts completed in 2017, based on graft type. As of 2017 evaluations, spring grafts showed that side-wedge (B+N) and trunk side-wedge (B+N) grafts were better than bark grafts (B, $p < 0.0001$). After one winter, graft survival decreased, with side-wedge grafts (B+N) having higher survival rates than the other graft types ($p \leq 0.02$). Means with same lowercase letters are not significantly different among a year or between graft types by Tukey's method for multiplicity at $\alpha = 0.05$.

Table 1

USU Blue Creek Experiment Station Farm 2018 Grafts: Measured March 2020

	Accession			Graft Type	
Measurement	JUT2	JUT3	JUT6	Side-Veneer	Side-Wedge
Survival (%)	70 a	25 b	75 a	63 a	50 a
Candle Length (mm)	346 a	152 a	434 a	345 a	356 a
Needle Length (mm)	40 a	45 a	41 a	42 a	40 a

Table 2

USU Blue Creek Experiment Station Farm 2019 Grafts: Measured March 2020

	Accession			Graft Type	
Measurements	BUT1	BUT3	BUT4	Side-Veneer	Side-Wedge
Survival (%)	65 a	70 a	85 a	67 a	80 a
Candle Length (mm)	42 b	90 a	107 a	96 a	71 b
Needle Length (mm)	21 b	36 a	42 a	38 a	30 b

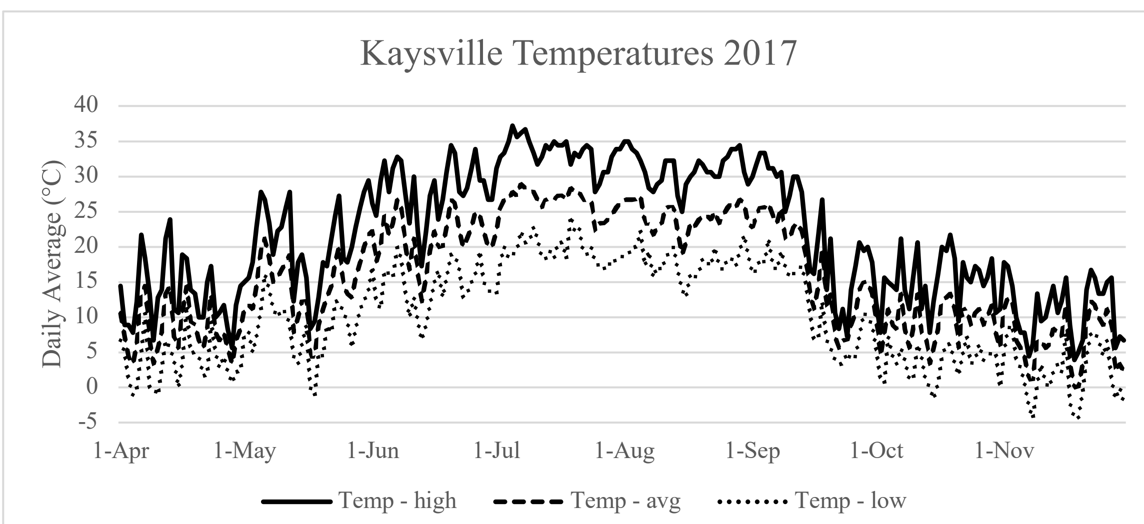

Figure 6. Temperature data collected from a weather station in Kaysville, UT during the growing season for 2017.

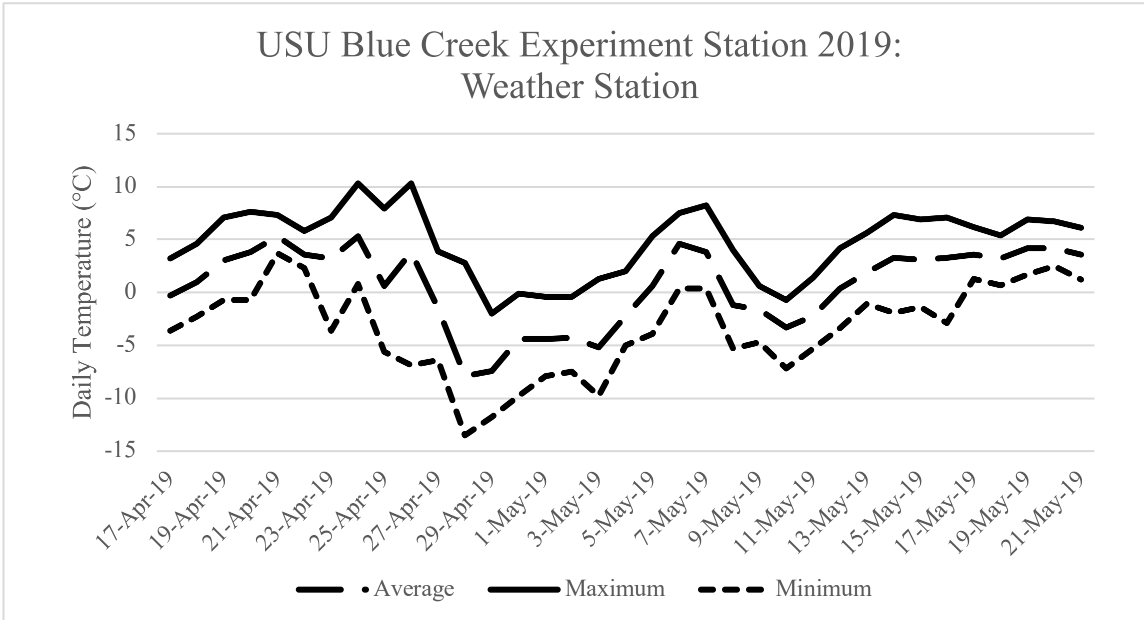

Figure 7. Temperature data collected from the weather station at the USU Blue Creek Experimental Farm. This time period was often overcast, with frequent precipitation totaling 3.8 cm from April 17, 2019 to May 21, 2019. (Blue Creek, UT, 2019).

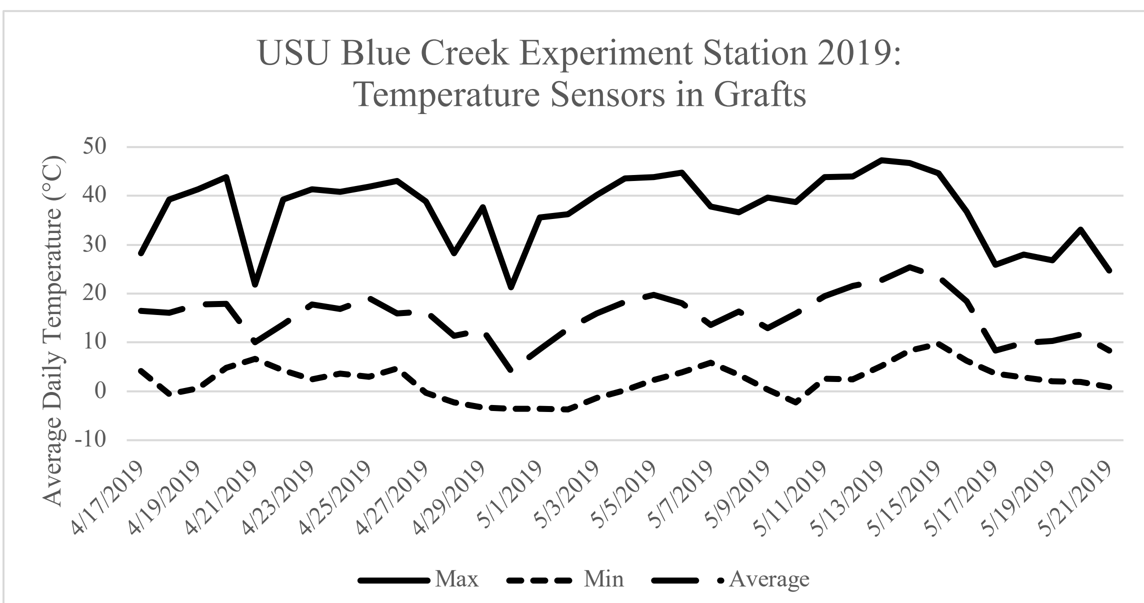

Figure 8. Average temperature data collected from two temperature sensors placed at the grafts.

Figure 9. Conelets on grafted scions from 2017 at the USU Botanical Center (left photograph) and 2018 at the USU Blue Creek Experiment Station (right photograph).

CHAPTER 3

GRAFTING *PINUS EDULIS* SEEDLING ROOTSTOCKS WITH *PINUS MONOPHYLLA* SCIONS

Introduction

Two-needle and single-leaf pinyon pine (*Pinus edulis* and *Pinus monophylla* respectively) are xeric trees native to the western United States and are often found mixed with Utah juniper or Rocky Mountain juniper (*Juniperus osteosperma* and *J. scopulorum* respectively). Both species of pinyon produce highly valuable pine nuts despite growing in marginal lands which are not suitable for production of most foods or plants. No documentation is found of efforts to cultivate or select for individual trees with high pine nut production. This is likely because these trees do not reach mature cone-production until they are 50-75 years old. It appears it has been more economical and convenient to harvest pine nuts from wild grown trees rather than cultivated in an orchard.

Pinus monophylla is native to the Great Basin, covering approximately 7 million hectares (Biondi and Bradley, 2013). No other species of pine in the United States grows in such xeric conditions as those experienced by *P. monophylla*. The average annual precipitation ranges from 20-46 cm, but is highly variable, with most of the precipitation occurring during winter months. The temperatures range from -6 to 30°C, with an average of 10°C. This species is one of the slowest growing conifers. They can grow 6-12 m tall, with lateral roots that spread to be three times the size of the crown. Cone production doesn't begin until trees are 35 years old and mature cone production doesn't begin until near 100 years (Meeuwing et al. 1990). The pine nuts of *P. monophylla* have

thin shells that can be cracked with bare hands and are about 30% the overall pine nut weight. The nuts have a composition of 10% proteins, 23% fats and 54% carbohydrates with all the amino acids present and fats consisting of unsaturated oleate, linoleate and linolenate fatty acids (Lanner, 1981).

Pinus edulis is native to the southwestern United States, covering approximately 24.7 million hectares. In covering such a broad area, from California to Texas, this species experiences a wide range of environments, which is more likely influenced by the species low tolerance of water stress than to temperature or other factors. Precipitation ranges from 25 cm in lower elevations to 56 cm in higher elevations. Temperatures average from 4 to 16°C, with extremes ranging from -35 to 27°C, with 90-205 frost free days. This species grows slowly with mature sizes of 3-15.5 m in height. Cone production begins when trees are about 25 years old and 1.5-3 m tall, but mature cone production can take up to 75-100 years to occur. Cone production occurs sporadically every 4-7 years, though some trees and locations can produce every 2-5 years (Ronco, 1990). The shells of pine nuts from *P. edulis* trees are hard enough to need tools to crack and are about 42% of the overall weight of the unshelled pine nuts. The nuts are composed of 14% protein, 62-71% fats and 18% carbohydrates. With nearly three times the amount of fats than *P. monophylla*, the pine nuts of *P. edulis* are often pressed for oil. The proteins in the nuts are composed of all the amino acids, and the fats are unsaturated oleate, lineate and linolenate fatty acids (Lanner, 1981).

No plantations or orchards of either *P. monophylla* or *P. edulis* have been documented. Success of cultivation of other nut bearing *Pinus* species could provide

insight on the potential of planting an orchard of *P. monophylla* for pine nut production. A study of *P. pinea* L. in Chile looked at the effects of irrigation on cone production. Stands of unirrigated trees were compared to stands of irrigated trees. Irrigation resulted in increases in both growth and seed cone production, and a reduction in alternating bearing cone production cycles. It was found that drought conditions had greater effect on the growth of the trees than temperature (Loewe and Delard, 2016). This study may indicate that an orchard of *P. monophylla* with supplemental irrigation could also have increased cone production and reduced time between cone bearing years.

Both species of native pinyon pines mature slowly, with nuts being produced only after several decades. This makes breeding and selecting for high producing genetics a very time intensive process. Grafting selected scions onto seedling rootstocks is used in commercial horticulture and fruit production (Davies et al. 2018), ensuring that grafted trees will have the features that were selected for, such as color of foliage, growth habit, fruit flavor, or productivity. Grafting superior producing accessions of pinyon pine onto seedling rootstocks could result in high producing trees, without the genetic variability of seedling plants. Very little if any research has been published on grafting of *P. edulis* or *P. monophylla*, though grafting of these species and other conifers for ornamental use is done commercially with great success (Fiesler, 2016).

For grafting seedling conifers, the graft types most often used are side-wedge and side-veneer (Davies et al. 2018; Nelson, 2017). The side-wedge graft is a modified wedge graft where the scion is inserted into the side of the rootstock, and the rootstock plant above the graft remains until after the graft union has knit together, rather than being cut

off as in typical wedge grafts. It is performed by cutting the scion on both sides to form a wedge about 2 cm long starting 1-2 centimeters below the apical bud, with 5-10 needles retained just below the bud. A straight, downward sloping cut is made in the rootstock shoot that is approximately the same length as the cut surface of the scion and that penetrates no more than 1/3 the diameter of the rootstock (Figure 1). The side-veneer graft is performed by cutting the scion on one side with a small notch back towards the cut side. The rootstock is done with a long, straight cut made just into the wood. The scion is placed with the cut side facing the cut side of the rootstock (Figure 1). After inserting the scion, both types of grafts are tied with a grafting rubber band and parafilm until healed. To determine what graft will work best for grafting pinyon pine, both the side-wedge and side-veneer grafts were used in this project.

Selection of high producing trees was completed by counting cone abscission scars, a method that was developed independently in 1930 in Asia for estimating *P. siberica* cone production and 1947 by Forcella and Weaver for estimating *P. albicaulis* cone production (Forcella, 1981). In 2016, the method was used in evaluating *P. edulis* cone production, and was found to be a reliable method for estimating cone production for slow growing conifers, with only 5-6 branches per tree and 6 trees per stand to evaluate the stand production and estimate the differences in production between trees (Redmond et al. 2016).

Rootstock and scion interaction can have effects on both graft survival and cone production. Knowing that the scion has a greater effect on cone production than the rootstock, this study aims to use *Pinus monophylla* selected for high cone production as

the scion source, and *Pinus edulis* as the rootstock in a grafting experiment to determine the best methods for grafting these species. *P. monophylla* were chosen as the source of scions for grafting because its nuts are large and have a shell soft enough to be cracked with bare hands, whereas *P. edulis* has smaller nuts with a shell hard enough that tools are required to crack it. The native range of *P. monophylla* was also closer than the *P. edulis* range to Utah State University, making them much more accessible for collecting wild-grown scions. In commercial ornamental propagation, clonal propagation of *P. monophylla*, which is a more attractive tree as an ornamental than *P. edulis*, is commonly done by grafting these scions onto *P. edulis* rootstock (Fiesler, 2016, personal communication). These three factors were the basis for the decision to use wild-grown *P. monophylla* as the scion and seedlings of *P. edulis* as the rootstock for this project. Future study will be needed to determine if the scion/rootstock interaction affects cone production (amount and timing) for this species interaction.

Materials and Methods

Second-year container-grown seedling *P. edulis* rootstocks were obtained from the University of Idaho Franklin H. Pitkin Forest Nursery. A total of 300 trees were shipped on November 14, 2017 and potted on November 17 and 20, 2018. The trees were 0.567 cu. meter Super Stock plants shipped as plugs with a small rootball, five trees per plastic bag and about 100 trees per box. Trees were stored in a cooler at 4° C until potted. The rootstocks were potted in 7.3 x 7.3 x 22.7 cm containers with a bark-based media mix (Metro-Mix 900 with 45-55% composted bark, Canadian sphagnum peat moss, vermiculite, perlite, dolomitic limestone, and 0.0001% silicon dioxide soil conditioner)

and placed in a polyethylene covered Quonset-style greenhouse where they were kept at temperatures slightly above freezing to prevent complete dormancy.

Scion wood from wild *P. monophylla* trees was collected from four stands with long histories of pine nut collection from within the Great Basin. One stand of *P. monophylla* is in Lander County, Nevada, with an elevation of 2195 m, at 39.4575 N 116.99306 W in the Toiyabe National Forest. The northernmost stand evaluated is in the Raft River Mountains in Box Elder County, Utah and is one of the most northern stands of *P. monophylla* anywhere, with the sampled trees being at 1920 m elevation at 41.95278 N 113.32167 W in the Sawtooth National Forest. The southernmost stand was sampled in Hamlin Valley in Iron County, Utah, with an elevation of 2047 m at 37.99833 N 113.96944 W. The easternmost stand was sampled in Juab County near Eureka, Utah, at 39.97889 N and 112.1750 W and an elevation of 1957 m.

Six healthy trees were selected and sampled within each of the four stands. Selection was based on visual cues of high cone production such as current cones in the canopy of the tree and large numbers of cones at the base of the tree. Once selected, six branches at least 1 m in length were removed from the top half of the tree in order to evaluate the number of cone scars on each branch. Scion wood was collected concurrently with the cone scar branches. This provided scion wood from the top half of the tree, which has been shown to be more likely to produce female cones once grafted (Almqvist, 2013). Once the cone scars were counted, the three most productive trees per stand were selected for use in grafting. If three trees with significantly greater cone production did not exist within the sampled trees, then the trees that had the healthiest

bud wood as determined by visual observation were selected. For example, none of the selected trees from the Box Elder County stand were significantly more productive than the others, so the three trees for this stand were selected from those with more current season's growth as this facilitated easier grafting. The scion wood was cut to 10 cm in length, then wrapped in a damp paper towel, placed on ice in a cooler for transportation, then stored for one week in a refrigerated cooler (Table 1). With three trees per stand and four stands, this provided twelve trees as scion accessions for the grafting process. None of the evaluations identified an individual tree that had superior annual cone production.

A total of 24 grafts were completed per accession, 12 of which were wedge grafts and 12 were veneer grafts. Both grafting styles were tied with 1 x 12.7 cm grafting rubber bands. This resulted in 72 grafted seedlings from each stand and 288 grafts total. The grafted seedlings were randomized in a double-layer polyethylene covered hoophouse and covered with a layer of 1 or 2 mm plastic to help prevent desiccation. A single layer of Remay® row cover °was also placed over the plastic to reduce solar heat buildup. The plastic covering was removed once per week for four hours to reduce humidity and disease potential. An extra layer of white plastic was hung up inside the greenhouse to reduce light directly over the grafted seedlings. Six weeks after grafting, the plastic cover was removed, and the row cover was removed one week after that. This process occurred sequentially by group based on when the scion wood was collected. After the last grafting event was completed, the grafted seedlings were replaced in a random design.

On April 9, 2018, all the grafted seedlings were moved to a glass greenhouse with 60% shade to better control temperatures. Apical buds were pruned off the rootstocks on

April 13, 2018 for all but the grafts from Juab County, which were pruned on May 1, 2018. This pruning removed apical dominance and facilitated scion bud break. On May 2, 2018, the trees were moved to a full-sun greenhouse, with temperatures set at 23°C day and 18°C night temperatures. During each move, the trees were randomly placed in blocks to minimize the effect of the environment on graft success. After this point, the care and evaluation of the trees occurred simultaneously rather than sequentially.

The grafted seedlings were irrigated when randomly assigned trees (including roots and potting media) weighed 80% of their field capacity weight. The irrigation was done as a constant liquid feed at 100 parts per million of Peters Excel 21-5-20 multipurpose fertilizer. The trees were treated with FeEDDHA (6% Fe) at 10 ppm Fe on November 11, 2018 when they began to show signs of chlorosis. This was repeated on April 19, 2019 at 20 ppm Fe.

Until July 2018, graft mortality was not easily identified. In order to evaluate the grafts during May and June 2018, a bud grading scale was used to evaluate the health of the grafts. The levels of evaluation are as follows: 0 = dead or dying, 1 = healthy needles, no bud expansion, 2 = slight expansion of bud, as seen in slight green color between bud scale, 3 = expansion of bud, with needles beginning to be visible, 4 = green, expanding bud with needles shorter than ½ inch, 5 = bud with needles ½ inch long or more. As of 2 July 2018, graft development was evaluated by survival rates. The grafted seedlings that had successful scion bud break by 2 July 2018 were cut back to 1 cm above the graft union while the scions that did not have break bud were separated and monitored.

Monthly evaluations continued for all the trees until January 2019. Those that were not initially pruned on July 2, 2018 were all pruned on October 9, 2018.

The trees were inadvertently allowed to continue active growth well into the fall of the year, so they did not go dormant in time for fall planting. Rather than planting them in late fall and risking winter injury, they were hardened off slowly in the full sun greenhouse. This was done starting in September by turning off supplemental lighting, irrigating without fertilizer and lowering the temperature to 18°C day and 4.5° C night. This was continued in December by lowering the temperatures by 5°C weekly until the greenhouse set points were 5°C daytime and 4°C nighttime temperatures. On February 7, 2019, the grafted seedlings were moved to a lighted refrigerated storage unit (4° C day and night). Maintaining temperatures this low in the greenhouse would not have been possible as the season progressed towards spring, and we wanted to ensure that the trees had at least two months of cold stratification temperatures before planting them in the spring.

The seedlings were planted on April 24, 2019 at the USU Richmond Research Farm (41.89093 N, 111.8473 W, altitude of 1422 m). The holes for planting were dug with a 30.49 cm (12 in) auger, about 1.2 m (4 ft) deep. The trees were planted in twelve rows with twelve trees per row. One of each grafted scion accessions was randomly assigned to be planted in each row, with each row containing either wedge or veneer grafted trees. A buffer row of remaining trees was planted surrounding the randomized 12 rows resulting a final planting of 14 trees per row in 14 rows (Figure 2). Trees were planted with 4.57 m (15 feet) between them and 6.1 m (20 feet) between rows. The

irrigation design included a 1987.3 L (525 gal) water tank with a flexible hose for taking water to the field. A 2.54 cm (1 in) diameter polyvinyl chloride (pvc) pipe directed the water from north to south along the east edge of the trees, connecting to 1.27 cm (0.5 in) diameter Rainbird XFD500 – XF driplines that were laid out along each row. At each tree, a Rainbird FE10-25S flag emitter with a flow of 3.79 L (1 gal) per hour was placed in the dripline, with 0.635 cm was placed at the highest point of the field, allowing the trees to be watered via gravity, suppling at least 7.57 L (2 gal) of water to the root zone of each tree per each irrigation. The system will supply water as needed with drip irrigation for two growing seasons until the trees can develop root systems and become established. Trees were not irrigated until June 2019 due to mild, wet weather experienced at the farm, and the last irrigation occurred on September 24, 2019. The trees were not fertilized once planted. The survival rates and growth measurements of the trees were measured on July 3, 2019.

Results

The growing scions were evaluated at the beginning of each month from May to September 2018, then less consistently until January 2019 when they were placed in a cooler. During May and June of 2018, it was difficult to determine if scions were dormant or dying, since only a small fraction were obviously dead. Browning needles were not a reliable method of determining survival since some grafts could recover after having some needle browning. Growing buds were evaluated with a scale (Figure 2) that ranged from 0 to 5. Buds with browning to brown needles were grade 0, dormant buds with green needles were grade 1, buds that were beginning to expand and had a slight

green color between the scales were grade 2, expanded buds with needles just barely visible were graded 3, expanding buds with needles shorter than 1.27 cm (0.5 in) were graded 4, and expanding buds with needles longer than 1.27 cm (0.5 in) were graded 5.

The health of the grafts was evaluated using Welch's two sample t-test to compare the bud grades. Side-wedge grafts had higher mean bud grades in both May (side-veneer = 1.9, side-wedge = 2.3, $p = 0.0001$) and June (side-veneer = 3.6, side-wedge = 4.3, $p = 0.0001$, Figure 3). By July of 2018, the dead or dying scions were easier to distinguish and most of the buds had broken and begun to produce needles. The needle length, candle length and rootstock diameter were measured while also recording which grafts were alive or dead (Figure 4). There was no difference between the survival rate of side-wedge and side-veneer grafts (side-wedge mean = 95.14%, side-veneer mean = 88.65%). Side-wedge grafts had significantly longer needles ($p = 0.009$, side-wedge mean = 4.01 cm, side-veneer mean = 3.32 cm) and slightly longer buds than side-veneer grafts ($p = 0.09$, side-wedge mean = 3.58 cm, side-veneer mean = 3.26 cm). By January, the side-wedge survival rate was 90.9% and side-veneer was 80.7% (Figure 5).

On July 3, 2019, the growth of the planted trees was evaluated by measuring the apical candle length, needle length and the number of set buds on each scion. The survival rate of the planted trees was 99%, possibly due to the mild, wet weather during the spring of 2019. No difference between the growth of side-veneer and side-wedge grafts was found, though differences were found when compared with scion accession sources. Grafted seedlings with scions from BUT3 and BUT4 had significantly longer needles than LNV1, LNV2 and IUT3 ($p < 0.02$). Grafted seedlings with scions from

BUT3, BUT4, IUT1, IUT5, JUT3 and JUT4 had significantly more candle growth than LNV1 and LNV6 ($p < 0.04$) (Figure 6). Grafted seedlings with scions IUT5 had more set buds than all other grafted seedlings ($p < 0.003$), and JUT4 had more buds than LNV6, IUT1 and IUT5 ($p < 0.02$) (data not shown).

Discussion and Conclusion

It is no surprise that the grafting of *P. monophylla* scions onto *P. edulis* rootstocks was successful since it is done routinely in commercial production. But the scions used in this study were from wild-grown trees rather than cultivated trees and were done at what could be considered a commercial scale. In a study with *P. pinea* and the effect of irrigation, it was found that drought had a greater effect on plant growth than temperature (Loewe and Delard, 2016). This may be why the grafts with scions from the Box Elder County accessions, which had higher average precipitation, had such high success rates when compared with the grafts from the other counties. The anomaly is that the Lander County grafts had such high success rates despite having estimated annual mean precipitation rates as low as the mean precipitation rate in Iron County, which had the lowest graft survival rate of the stands. Either this indicates that precipitation within each stand does not affect the success rate of the grafted scions or that there was another factor that influenced the survival of the grafts from the two different stands. Accession source did have an effect on the survival rate of the graft types. Scions from accessions IUT4 (40%), IUT5 (58%), and LNV6 (75%) had lower survival rates with side-veneer graft types. These accessions also came from environments with lower mean precipitation rates and higher mean temperatures (Figures 2 and 3). Further study could be done to

determine if side-wedge graft types are more favorable for wild scions collected from adverse environments.

The survival rate of the side-wedge graft, which is used in commercial production of pinyon pine, was not statistically different than the side-veneer graft, which is the graft type often published as best for grafting seedling conifers (Davies et al. 2018; Nelson, 2017). Both graft types could be used with success in future propagation. These survival rates were achieved while using scions from wild-grown trees. Using scions from cultivated trees should increase the survival rates.

Further research could be completed using this grove of trees. The interaction of *P. monophylla* scion and *P. edulis* rootstock on growth rates and cone production could be evaluated. This graft pairing is used with success in ornamental production of the plants, but the effects of the species interactions on cone and nut production is yet undocumented. The scion had the greater effect on cone production when grafting *P. taeda* scions onto mature rootstocks (Jayawickrama et al. 1997). Though no research has been completed to determine the effect of grafting superior producing accessions of *P. monophylla* onto *P. edulis*, scions will likely have a greater effect than rootstocks in this situation. This orchard would provide a site to evaluate the cone production of each ramet and to determine if the effect of grafting *P. monophylla* onto *P. edulis* rootstock is beneficial for cone production on both individual ramet and orchard scales.

The effect of cultivation of the *P. monophylla* and *P. edulis* grafted trees could be researched and evaluated. Supplemental irrigation of the stand of trees could potentially increase cone production beyond what the four stands of wild-grown trees could produce,

considering that in the study with *P. pinea*, irrigation increased both diameter at breast height and cone production when compared to unirrigated trees (Loewe and Delard, 2016). Increased cone production of superior producing trees may be compelling enough to provide motivation for further stands of cultivated *P. monophylla* trees. Cultivation could provide insight to the differences between the four sampled stands.

The grafting process of this project was long with many steps. Other than the six weeks for the grafts to heal, much of the maintenance can be simplified or streamlined. Moving the grafted seedlings from one greenhouse to another could be simplified by keeping the trees in a greenhouse until the grafts are healed and moved outside after danger of frost damage. This would ensure that the trees would harden off throughout the summer and fall and be ready for planting in the fall, rather than needing maintenance through the winter. Adding FeEDDHA to the fertilizer through the growing season would reduce the chance of chlorosis.

Scions from the Box Elder and Lander counties had higher survival rates than the Iron and Juab counties (Figure 5). Specific accessions within each stand had differing levels of survival success, with BUT1, BUT4 and LNV1 having 100% survival rate (Figure 5). Further evaluation would be needed to determine if these differences were due to genetics or environmental factors within each stand.

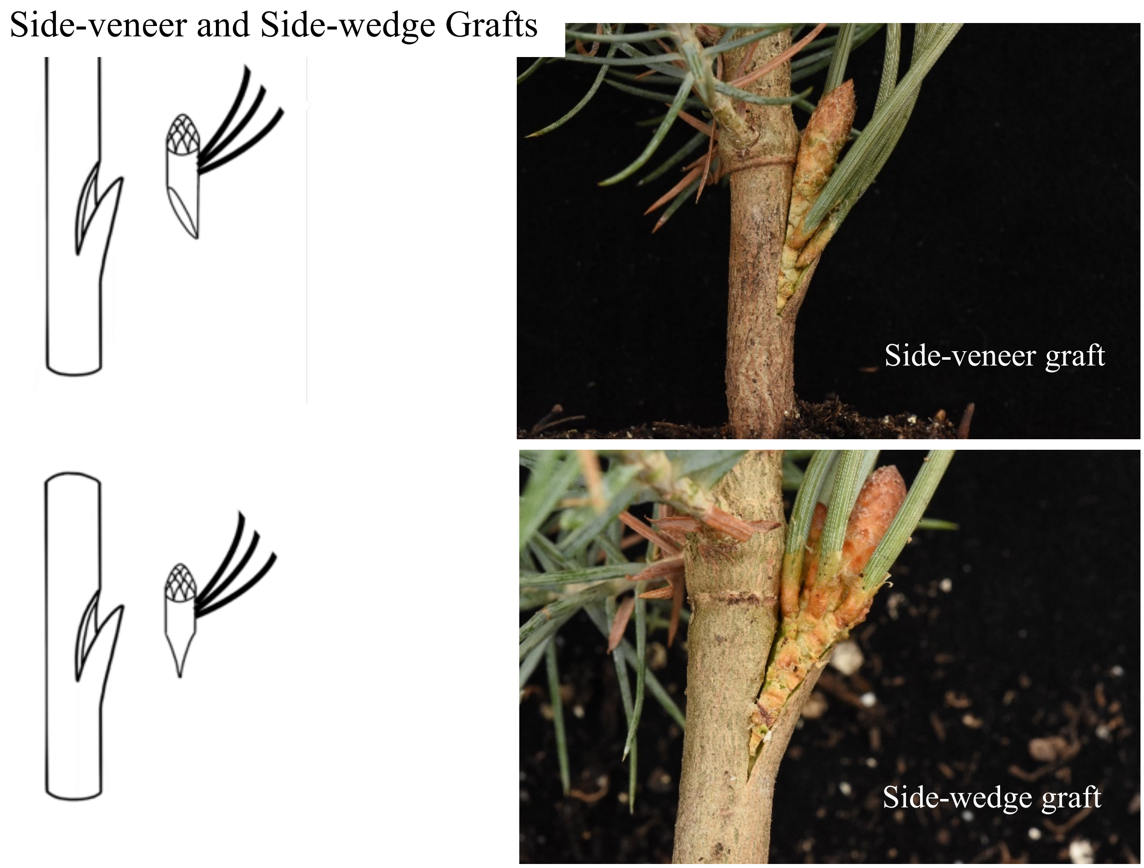

Figure 1. Line images and photographs of the two graft types. Top, a depiction and photograph of a side-veneer graft. Bottom, a depiction and photograph of a side-wedge graph.

Table 1

Sequential Collection, Grafting, and Aftercare of Scions

Location	Scion Collection	Grafting	Plastic removed	Row Cover Removed
Lander County, NV	2-Feb-18	9-Feb-18	23-Mar-18	30-Mar-18
Box Elder County, UT	12-Feb-18	20-Feb-18	3-Apr-18	10-Apr-18
Iron County, UT	23-Feb-18	2-Mar-18	13-Apr-18	20-Apr-18
Juab County, UT	5-Mar-18	12-Mar-18	23-Apr-18	30-Apr-18

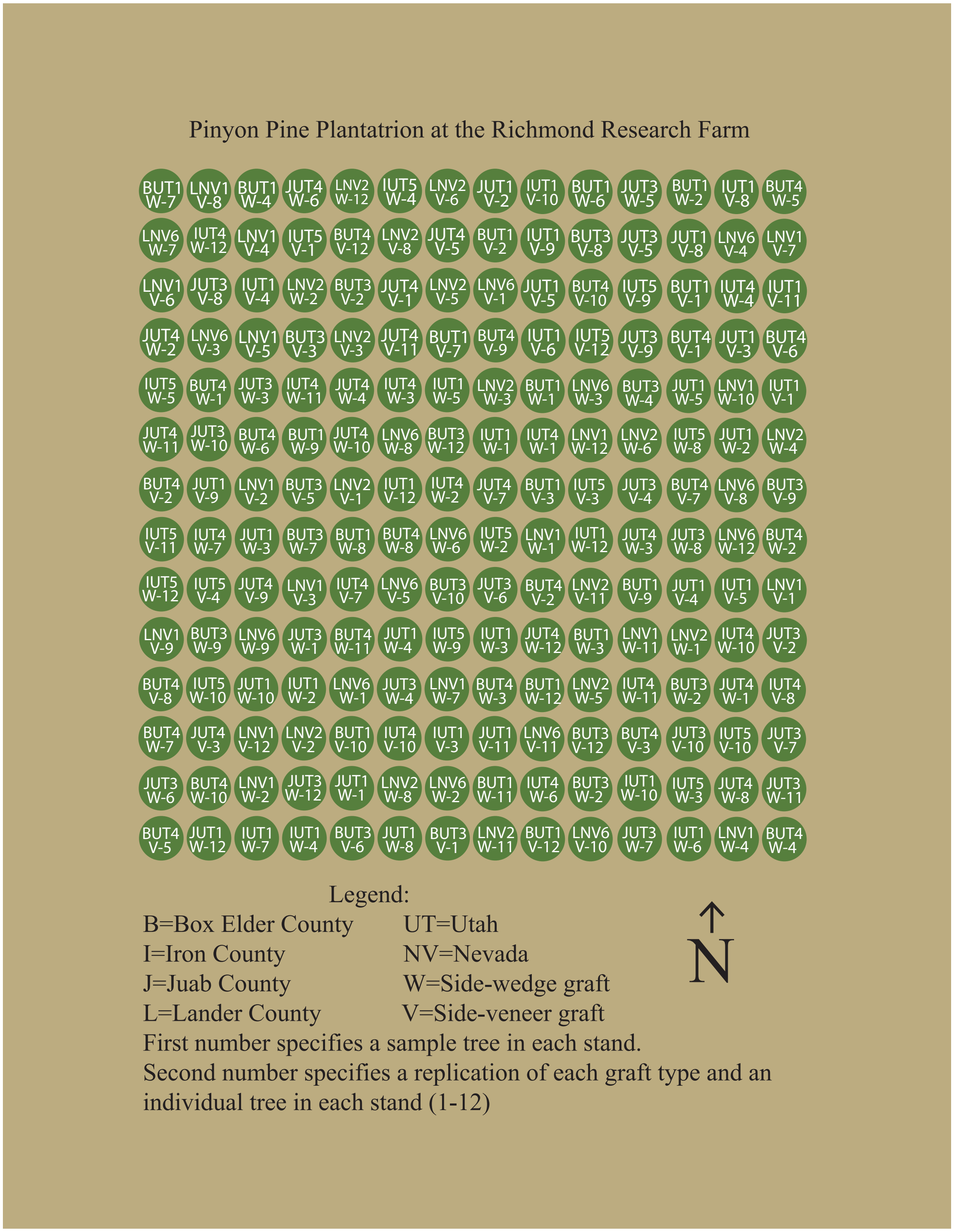

Figure 2. This map shows the planting plan of the grafted seedlings. The border trees provide a buffer so that all internal plants have similar surroundings. The inner twelve columns are grouped based on what graft type was used, with one scion from each accession source included in each column.

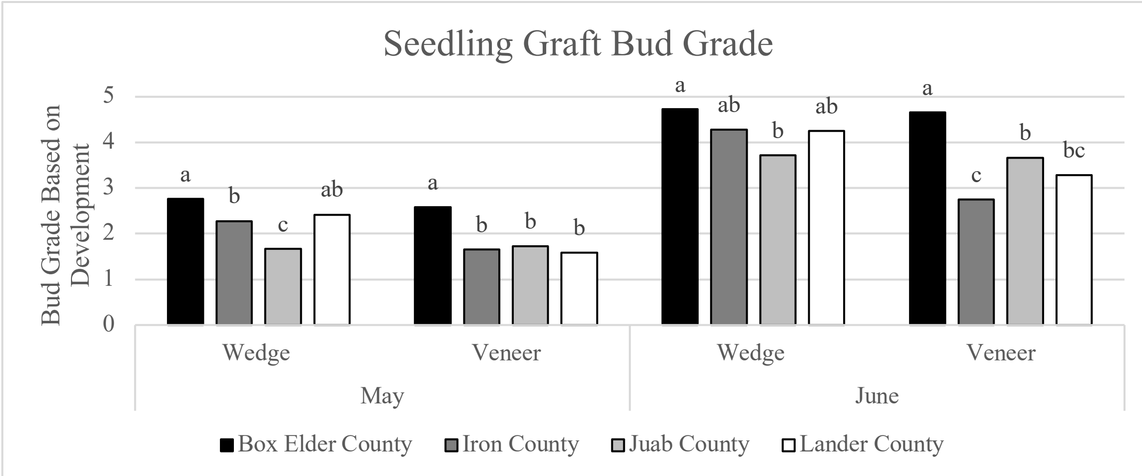

Figure 3: The grafted scion health was evaluated on a scale from 0-5 at the beginning of May and June, since some of the buds were breaking but determining scion morbidity was not yet possible. (If means between the bud grades of counties are different within each month and graft type are followed by the same letter, they are not significantly different at $p < 0.05$.)

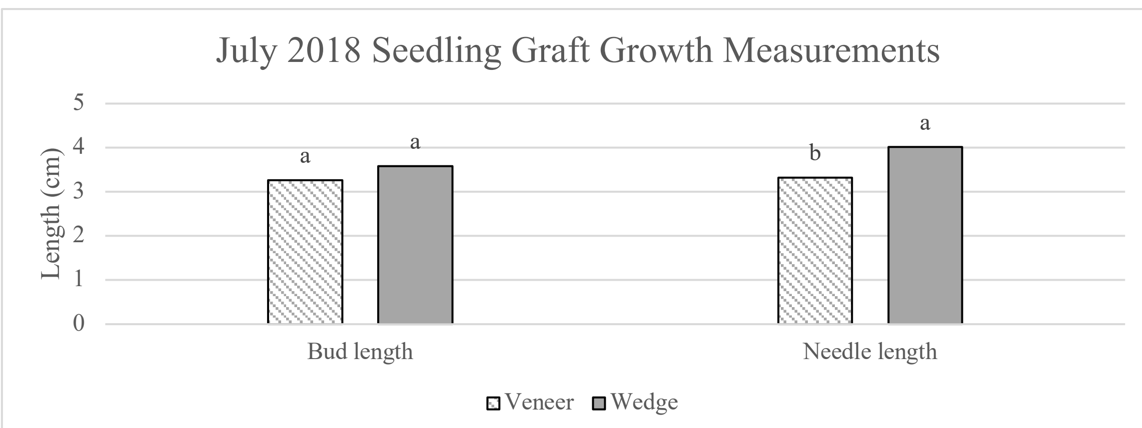

Figure 4. The development of scions as measured by bud length and needle length. The difference between bud lengths of side-veneer and side-wedge grafts is not significant, though the needle lengths of side-wedge grafts are longer than the side-veneer grafts (If means from each group are followed by the same letter, they are not significantly different at $p < 0.05$).

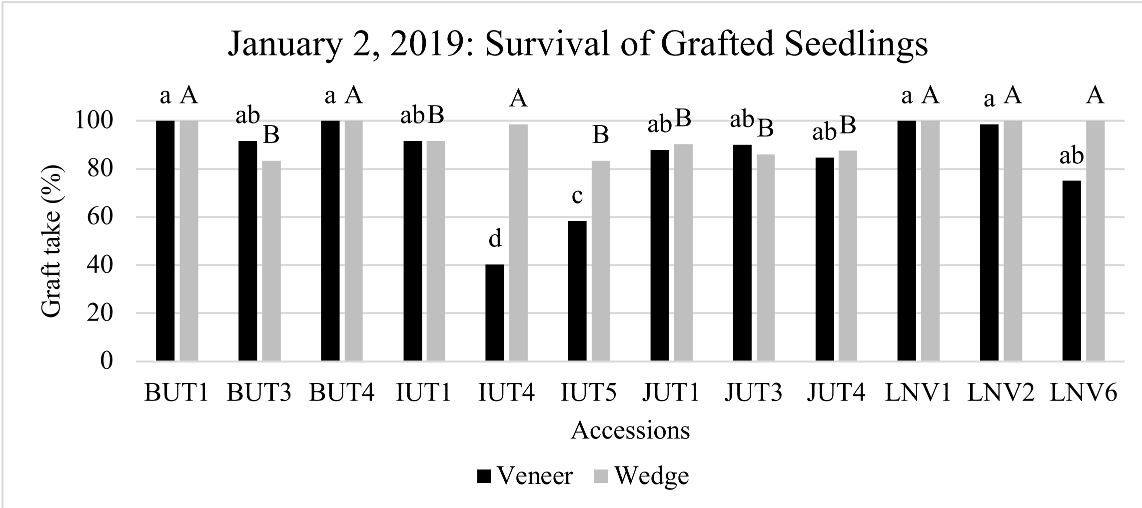

Figure 5. Grafted seedling success rates for scions from the four sampled Great Basin stands. These evaluations were done almost a year after the grafts were completed. Accessions from the Box Elder County and the Lander County stands had higher graft survival rates than the accessions from the Iron County and Juab County stands ($p < 0.025$). Overall success was 90.0% for side-wedge grafts and 80.7% for side-veneer grafts ($p = 0.014$)

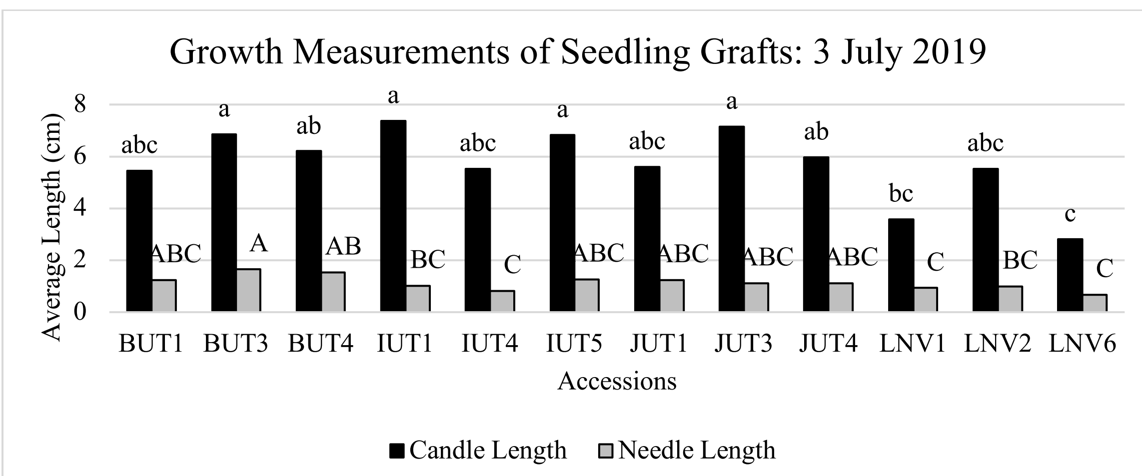

Figure 6. The candle length and the needle length were measured and counted for the planted grafted seedlings on July 3, 2019. No difference existed between side-veneer and side-wedge growth measurements. Means with same lowercase letters for candle length or capital letters for needle length are not significantly different among accessions by Tukey’s method for multiplicity ($p < 0.05$).

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APPENDICES

APPENDIX 1

DATA FOR SAMPLED TREES

Table A1

GPS Coordinates and Initial Names of Sampled Trees

Tree Name	Latitude and Longitude	Initial Tree Name
BUT1	41.9526, -113.3219	Raft River 1 (RR1)
BUT2	41.9254, -113.3217	Raft River 2 (RR2)
BUT3	41.9513, -113.3212	Raft River 3 (RR3)
BUT 4	41.9527, -113.3222	Raft River 4 (RR4)
BUT5	41.9519, -113.3282	Raft River 5 (RR5)
BUT6	41.9516, -113.3300	Raft River 9 (RR9)
IUT1	37.9941, -113.9707	Hamlin Collection 1 (HC1)
IUT2	37.9952, -113.9706	Hamlin Collection 2 (HC2)
IUT3	37.9953, -113.9703	Hamlin Collection 3 (HC3)
IUT4	37.9950, -113.9709	Hamlin 1 (H1)
IUT5	37.9956, -113.9712	Hamlin 4 (H4)
IUT6	37.9952, -113.9701	Hamlin 9 (H9)
JUT1	39.9795, -112.1728	Eureka 201 (E201)
JUT2	39.9810, -112.1727	Eureka 202 (E202)
JUT3	39.9807, -112.1752	Eureka 203 (E203)
JUT4	39.9806, -112.1755	Eureka 204 (E204)

Tree Name	Latitude and Longitude	Initial Tree Name
JUT5	39.9808, -112.1752	Eureka 205 (E205)
JUT6	39.9798, -112.1760	Eureka 206 (E206)
LNV1	39.4585, -116.9930	Austin 1 (A1)
LNV2	39.4586, -116.9923	Austin 2 (A2)
LNV3	39.4587, -116.9927	Austin 3 (A3)
LNV4	39.4589, -116.9927	Austin 4 (A4)
LNV5	39.4591, -116.9929	Austin 7 (A7)
LNV6	39.4586, -116.9933	Austin 8 (A8)

Table 2A

Chosen names of sampled stands with initial names used when sampling stands

Stand Name	Original Stand Name
Box Elder County	Raft River Stand
Iron County	Hamlin Valley Stand
Juab County	Eureka Stand
Lander County	Austin Stand