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ASSESSING *ABIES NORDMANNIANA* AND *ABIES BORNMUELLERIANA* SEED QUALITY FACTORS.

A Thesis in

Horticulture

by

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ABSTRACT

A lack of Christmas tree species diversity in the United States has led to the rise of several pest and disease problems. Fraser fir (*Abies fraseri)* is one of the most popular tree species, due to its superior postharvest characteristics and overall general appearance. Unfortunately, it is highly susceptible to Phytophthora root rot and has a mortality rate considered too high for many Christmas tree growers. Likewise, Douglas-fir (*Pseudotsuga menziesii*) is in production regularly, but is prone to Rhabdocline Needlecast (*Rhabdocline pseudotsugae)*. Several control management strategies exist. However, overall species care and general decline in popularity is making maintenance expensive. Growers are interested in introducing exotic Mediterranean firs, particularly Nordmann (*Abies nordmanniana*) and Turkish (*Abies bornmuelleriana)*, due to their resistance to Phytophthora root rot and marketable Christmas tree characteristics. However, many problems occur with storage practices, as seed viability only lasts two years at most. The goal of this research project was to examine possible reasons for decline of seed viability, and to identify potential solutions for improving germination in individual seedlots. Standard germination tests were performed on seed of Nordmann fir from seedlots collected in 2011, 2013 and 2015. Germination test results suggest that Nordmann fir seed quickly lose viability using conventional *Abies* storage methods. Seed moisture content in Turkish fir seed collected in 2015 was altered prior to stratification to determine if the moisture content percentage influenced seed germination. Results indicated that a change in seed moisture was not significant in germination rate. However, seed moisture fluctuated during prechilling. Thus, by determining a solution to stabilize seed moisture during the stratification protocol, experiment results may differ. Furthermore, changes in seed moisture content were monitored after a 12-week period to determine adequate methods for seed storage. Seed was stored in three types of media; a refrigerator, freezer, and an airtight container containing zeolite beads. Results indicated that seed in the refrigerator had absorbed the

most moisture, while the seed stored in the freezer and zeolite drying beads had insignificant changes in moisture content. Seed stratification method in Nordmann, Turkish and Fraser fir was also tested to determine any differences in seed germination. While seed that underwent the stratification-redry method had slightly higher total germination by the end of the experiment, they were not statistically significant. Nevertheless, seed moisture content changed during the stratification period. Finally, wasp larvae from the genus *Megastigmus* is making importing Nordmann and Turkish firs seed a challenge for industry leaders. An incubation treatment exists for Douglas-fir (*Pseudotsuga menziesii)* that successfully kills the insects inside seed while not affecting germination. The objective was to determine if the same treatment had influence on overall seed germination for Nordmann and Turkish fir. Results concluded that seed was significantly affected by the incubation, meaning that seed that underwent the prolonged heat treatment had a lower germination rate compared to that of the control. These results suggest that Nordmann and Turkish fir seed have higher sensitivities to traditional storage and germination methods compared to other true fir species.

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Chapter 1

Introduction

United States and Pennsylvania Christmas Tree Industries

The live Christmas tree industry began in 1850, and has since expanded with evergreen trees being planted, grown, and harvested in all 50 states. Approximately 350,000 acres are dedicated to Christmas tree cultivation, and 25-30 million live trees are sold yearly across the country (Chastagner & Benson, 2000; USDA Agricultural Statistics, 2013; University of Illinois Extension, 2015). In 2013, 33 million trees were sold reaching an estimated total of 1.16 billion dollars in value (NCTA, 2013). For every harvested tree, 1 to 3 seedlings are planted the following season to ensure enough trees will be made available for future seasons. An average Christmas tree takes approximately seven years to grow to a height of six to seven feet. However, depending on tree variety, collection source, and environmental growing conditions it can take between six and fifteen years to grow a sellable tree. The most abundant and well-known species grown in the United States include Balsam fir, Douglas-fir, Fraser fir, Noble fir, Scotch pine, White pine, and Colorado spruce. The states with the largest live Christmas tree industries include Oregon, North Carolina, Michigan, Pennsylvania, Wisconsin, and Washington (Chastagner & Benson, 2000; USDA Agricultural Statistics, 2013; University of Illinois Extension, 2015).

Pennsylvania is one of the top Christmas tree producing states with approximately 45,000 acres dedicated to Christmas tree cultivation (Kuhns, 2004; USDA Agricultural Statistics, 2013; University of Illinois Extension, 2015). Based on attendance at extension education programs, mailing lists, and Christmas tree market research the estimated number of tree farms in the state is

between 1500 and 2000 (Hoover & Bates, 2013a). In the 1950s and 60s, the most abundant Christmas tree being produced in PA was Scotch pine with Colorado and Norway spruce, as well as White pine prevalent throughout the state. In the 1970's, many pest and disease problems arose because of a lack of species diversity in the region. They had a tremendous negative effect on Scotch pines, making them incredibly expensive to produce and maintain. Around this time, Douglas-fir trees were introduced to the area. Consumers preferred Douglas-fir over Scotch pine because of their soft needles and upright branching structure. Time was dedicated to improving Scotch pine through breeding programs, but industry leaders still had a hard time selling even the best of the species. Likewise, the market for Norway spruce and White pine decreased due to poor needle retention (Norway spruce) and weak branches (White pine). Douglas-fir continued to gain popularity and is now produced in every county of Pennsylvania (Kuhns, 2004). Growers have appreciated their lack of pest and disease problems and have ease of shearing due to their straight, upright form (Chastagner & Benson, 2000; Kuhns, 2004). Fraser fir has grown in popularity across the region because of its excellent postharvest characteristics and fast growth (Chastagner & Benson, 2000; Hoover & Bates, 2013a). It is 15% more valuable than Douglas-fir on a per tree basis, but is planted less in the state because of its sensitivity to poorly drained soils (Hoover & Bates, 2013a; Hoover & Bates, 2013b). As of 2004, the main species being planted across the state included Douglas fir (40%), Fraser fir (35%), Colorado spruce (8%), Balsam (Canaan) fir (3%), Eastern White Pine (3%) and others (3%) (Kuhns, 2004). Since 2010, Fraser fir has been approximately 26% of the PA Christmas tree crops second to Douglas fir at 42% (Hoover & Bates, 2013a; USDA Agricultural Statistics, 2013).

Christmas Tree Disease and Pest Impact in Pennsylvania

Phytophthora root rot in Fraser fir

Native strands on Fraser fir occur in rich organic soils about 1525 meters above sea level in the Appalachian Mountains of Virginia, Tennessee and North Carolina (Chastagner & Benson, 2000; Benson et al., 2006). Production normally occurs below their native elevation, around 1400 meters on mineral soils with poor drainage characteristics ((Hinesley & Frampton, 2002; Benson et al., 2006; Hoover & Bates, 2013b). High mortality of Fraser fir has been studied, and proven to be directly related to a disease known as Phytophthora Root Rot (Chastagner & Benson, 2000; Hinesley & Frampton, 2002; Frampton & Benson, 2004; Benson et al., 2006; Richter et al., 2011; Hoover & Bates, 2013a; Hoover & Bates, 2013b). Fraser fir in Pennsylvania is known to be affected by three species of Phytophthora *(P. cactorum, P. cryptogea* and *P. drechsleri*) (Hoover & Bates, 2013a). Disease is often introduced onto new sites through infested transplants. Other means of disease spread include movement of infested soil, pathogen spread through water runoff, and tree to tree spread (Benson et al., 2006). Phytophthora is a problem during all stages of plant production. Trees infected with Phytophthora will often have reddish brown roots, chlorosis of needles and tree death (Chastagner & Benson, 2000). Once it is recognized on a site, mortality progresses over time making the site unfit for future Christmas tree production (Hinesley & Frampton, 2002; Richter et al., 2011). Growers have attempted using fungicides as a way to manage disease, however they are most effective as a preventative at plant seedling stages. Long term, it is not an economically viable solution (Benson et al., 2006; Hoover & Bates 2013a). Thus, the best way to control disease is through proper site selection and sanitizing equipment (Hoover & Bates, 2013a). Studies have been performed to determine the mortality rate and Phytophthora resistance of Fraser fir from seed collected in North Carolina. After 122 days, the final mortality rate of Fraser fir was tested to be 90.5% with significance differences between seed collection sites (Frampton & Benson, 2004). A survey taken by North Carolina Christmas Tree Pest Management in 2001 indicated that 60% of

Christmas tree growers reported field mortality due to Phytophthora. In 2006, 72% of farmers reported field mortality (Richter et al, 2011). Consequently, industry leaders are considering introducing alternative species with higher resistance to Phytophthora root rot.

Rhabdocline Needlecast on Douglas-fir

In Pennsylvania, the most abundant tree planted in 2010 was Douglas-fir, estimated at 42% of the crop (Hoover & Bates, 2013a). However, a widespread disease commonly affecting Douglas-fir is Rhabdocline Needlecast (*Rhabdocline pseudotsugae*). It was first recognized in the state in the early 1950's but has become progressively more prevalent (McDowell & Merril, 1985). The main symptoms include needle chlorosis, premature needle loss and reduced tree growth. Additionally, 95% of Douglas-fir seed is collected from the Lincoln National Forest in New Mexico. Unfortunately, Douglas fir from Lincoln National Forest is highly susceptible to Rhabdocline Needlecast (Chastagner, 2001; Bates, 2009). The disease is spread in the springtime, when ascospores are released from previous years diseased needles. The disease causes reduced tree growth and needle chlorosis, this decreasing market value and tree quality (Catal et al., 2010; Morgenstern et al., 2014). Controlling Needlecast on Douglas-fir requires 3-4 fungicide sprays during bud break, which is not economically viable for many growers (Bates, 2009). Moreover, the depressed market prices have led to a decline in popularity of Douglas-fir in recent years.

Nordmann fir/Turkish fir as a Potential Alternative Species in the United States

Role in Europe as Leading Christmas Tree Species

Nordmann fir (*Abies nordmanniana* (Stev.) Spach.) is one of the most popular Christmas trees species in Europe, known for their exceptional form, and glossy foliage. Denmark is the leading producer of Nordmann fir, typically producing 8 to 9 million trees and 6,000 tons of boughs every year (Find et al., 2005). Visually, Turkish fir (*Abies* x. *bornmuelleriana)* and Nordmann fir

appear very similar, however distinct morphological and genetic characteristics categorize them as different *Abies* species (Cregg, 2007; Kurt et al., 2016). Both Nordmann and Turkish fir are known to have prime postharvest moisture and needle retention, with problems hardly ever arising (Nielsen & Chastagner, 2005). Their needles are well liked by consumers due to their glossy dark green appearance. Needles face forward on the branch making the tree appear soft and layered. Tree form is very symmetrical with open branching; an aspect highly desired by many European Christmas tree consumers (Chastagner & Benson, 2000; Cregg, 2007). Unlike Americans, Europeans prefer unsheared, natural looking trees. Thus, their 8-12 year growing cycling is much longer compared to the other species (Chastagner & Benson, 2000; Chastagner & Riley, 2003; Find et al., 2005; Cregg 2007). Multiple studies have been conducted on Nordmann and Turkish fir, to examine genetic variation among European provenances, as well as physical characteristics such as growth rates, foliage quality, and number of branches per whorl (Nielsen & Chastagner, 2005). While Europeans tend to display their trees for very short periods of time, Americans tend to display their trees as early as late November, and extend their display past the new year (Nielson & Chastagner, 2005; Frampton 2009). Studies focusing on needle retention are ongoing, but have shown that when Nordmann fir is displayed dry, needle abscission and shedding is a common occurrence. In a previous study, Nordmann fir displayed under dry conditions lost a significant amount of xylem pressure potential in branches, as well as many needles only 3-5 days into the experiment. Nordmann fir displayed in water held most of its needles, and maintained its xylem pressure potential. In comparison, Noble fir displayed both dry and wet showed similar, excellent needle retention (Chastagner & Benson, 2000; Chastagner & Riley, 2003). Needles drying out could have further repercussions on trees harvested early in the season, sold in warm climates, shipped long distances, and displayed for long periods of time such as in the United States (Nielsen & Chastagner, 2005).

Early Efforts to Evaluate Nordmann and Turkish fir in the United States

When evaluating a potential new Christmas tree species, several factors need to be identified and evaluated over several years to assure appropriate selection. Tree adaptability is one of the main concerns including soils, cold and heat tolerance, as well as pest tolerance. Growth characteristics (symmetry, bud distribution, branch angles) as well as ornamental quality (needle characteristics, needle retention, aroma) also need to be evaluated, as these relate to Christmas tree consumer preferences (Bates, 2014). Market studies on the subject indicate that Christmas tree consumers would consider purchasing Turkish fir grown in the United States (Kurt et al., 2016). While Nordmann and Turkish fir hold promise of success in the United States, limited evaluations have been conducted. By evaluating how exotic species behave in the US, scientists and growers can identify provenances of Nordmann and Turkish fir that perform satisfactorily (Bates, 2009). Sources often state that Nordmann fir is classified under hardiness zones 4-6/7. In small trials in Michigan Nordmann fir has showed promise in performing well (Cregg, 2007). In 2010, the Collaborative Fir Germplasm Evaluation (COFirGE) project began with the overall goal of evaluating Mediterranean firs over multiple geographic regions in the United States. Seed was collected from multiple sources to account for genetic variation from seed provenances. John Frampton of North Carolina State University traveled to Turkey and collected 100 pounds and 85 pounds of Turkish and Trojan fir seed, respectively. Species selection was based on desirable Christmas tree characteristics and previous experimentation confirming resistance to Phytophthora root rot. Over 30,000 trees are now planted in Pennsylvania, North Carolina, Connecticut, Michigan, Oregon, Washington and Denmark with data being collected on establishment and survival, bud break, growth rate, needle retention, and eventual marketability. (Bustard & Bates, 2013).

Mediterranean Firs and Phytophthora Root Rot Resistance

One of the main reasons Christmas tree growers and industry leaders are considering growing exotic species in the United States, is due to the prevalence and severity of Phytophthora root rot. Research has been conducted to test true firs and other desirable Christmas tree species to determine the degree of disease resistance. Momi fir of Japan has proven to be the most resistant to Phytophthora root rot, but does not have the desirable outward appearance of traditional Christmas tree species. While grafting Fraser fir onto Momi fir rootstock is a potential strategy; developing a species that is both resistant to disease and has desirable qualities is necessary to use properties containing Phytopthora root rot in a cost-effective manner (Hinesley & Frampton, 2002; Frampton, 2009). In 2009 inoculation tests were performed in greenhouse conditions with seedlings and transplants assessing the susceptibility of Fraser, Canaan and Nordmann fir to Phytophthora. Additionally, testing was performed in both flooded and non-flooded conditions. This was done to simulate environmental conditions of trees in the field. Results concluded that after being exposed to *P. cactorum,* Canaan and Nordmann fir both had lower foliar symptoms compared to Fraser fir. In flooded environments, Fraser and Canaan fir exposed to *P. drechsleri* had higher rates of disease compared to Nordmann fir. Overall, Nordmann fir maintained healthier ratings in both non-flooded and flooded treatments. Fraser fir had the highest mortality in flooded treatments followed by Canaan and Nordmann (Hoover & Bates, 2013b). Additionally, in another experiment, Fraser fir was collected from three different seed sources in North Carolina. They were contaminated with the Phytophthora strand *P. cinnamomi*. The overall mortality after 122 days of experimentation was 90.5%. There were significant differences among seed sources, with the lowest being 83.2% mortality (Frampton & Benson, 2004). Turkish fir being closely related to Nordmann fir, also exhibits some root rot resistance (Hoover & Bates, 2013b).

Provenances of Nordmann fir

Nordmann fir seed is often collected from different provenances in its native country of the Republic of Georgia as well as northern Turkey (Find et al., 2005). The country is generally very mountainous with the Caucasus Mountains occupying the northern third of the state. The Minor Caucuses mountain range dominates the remaining 2/3 of the country, as it spreads through the central and southern landscapes. Nordmann fir typically grows in high elevations (between 915 and 2134 M) where they receive over 100+ cm of rain yearly. Nordmann fir grows in calcareous soils, thus it can be adaptable to soils with a wide range of pH. However, they still require soils with sufficient drainage (Cregg, 2007).

Nordmann fir is very closely related to both Turkish and Trojan fir (Cregg, 2007). Provenance testing for these species began in Europe in the 1960s. Since then, experimentation has been conducted to evaluate adaptability, growth and postharvest qualities. Ambrolauri is one of the most desirable Georgian provenances for trees well suited to the European market. Ambrolauri trees are slow growing and late flushing (Nielsen et al., 2011). Although it has been introduced as an acceptable seed source in the United States, it is not well matched to American tree characteristic preferences. Instead, it is more suitable to the European preference for slow growing and natural looking trees (Cregg, 2007). Most of the Nordmann fir currently grown in the United States, including Pennsylvania, came from the Ambrolauri forests in the Republic of Georgia. Thus, it takes a while to establish and grow. Additionally, trees from this region show foliar bronzing which is indicative of marginal hardiness issues (Bates, personal communication). In 2015 and 2016, Nordmann fir seed were collected from four provenances in the Republic of Georgia, including Bakhmaro, Bakuriani, Tazrisi, Okriba and Ambrolauri (Figure 1-1).

Figure 1-1. The Republic of Georgia with locations of four provenances where Nordmann fir seed was collected in 2015 and 2016.

Nordmann and Turkish fir Seed

Abies Seed Characteristics

True fir (*Abies)* all have cone scales that dislodge from the center of the cone. All true fir seeds contain resin vesicles that form among the epidermal layer of seed during development. Seed handled roughly can result in the loss of the outer seed coat, and wing tissue, thus exposing resin vesicles to potentially damaging conditions. The damaging of seed resin vesicles leads to decreased germination potential (Kolotelo, 1997a; Kolotelo, 1997b). Cones should be collected when seed is mature. This can be indicated by cone, bract, scale, and wing color (Bonner & Karrfalt, 2008).

Any collection of seed before maturation can result in slow germination, low seed vigor, smaller seedlings and higher disease suseptibility (Tanaka 1984; Kolotelo 1997b). Immature seed

can be artificially ripened during cone storage processes in certain species; however, this method is not prefered due to a higher risk of low germination and crop yields. Cone collection practices have been improved by moving cones to storage immediately after collection. This contributes to decreasing the moisture content percentage of seed and artificial ripening (Tanaka, 1984; Kolotelo 1997b).

Conifer Seed Storage and Processing

After cones are stored and dried, seed is further processed for sowing and/or long term storage. With natural drying, cones typically open up and release seed naturally. However, cool and damp conditions are typically found in storage facilities. Therefore, for most conifers the cones are usually dried with the assistance of rotating and progressive kilns set at temperatures between 32 and 60˚C (Tanaka, 1984). While kilning is said to cause disentigration of cones in *Abies* species, many industry leaders still use the kilning procedure (Kolotelo, 1997b; Rockis, Personal Communication). Rotating kilns are typically used for smaller cone loads and have set drying temperatures/humidity, whereas progressive kilns are larger loads of cones placed in trays. The trays are adjusted at given times to expose cones to interval, increasing temperatures (Tanaka, 1984).

Abies cones are dried until they can be easily broken apart by hand, or in a process called cone tumbling (Kolotelo, 1997a). Cone tumbling occurs simutaneously with kiln drying as the seed are dried and dislodged from their conal structures. In rotating kilns, seed is dropped through small gaps in the drum structure. Unlike rotating kilns, cones placed in progressive kilns are shaken to release seed. Also known as scapling, seed seperated in this manner needs to be filtered further to remove excess debris (e.g., cone fragments, dust, and other foliage). This process is completed using vibrating screens of different sized mesh. Larger materials like cone fragments and other twigs are separated from seed that fall through the mesh holes, and are collected in bins.

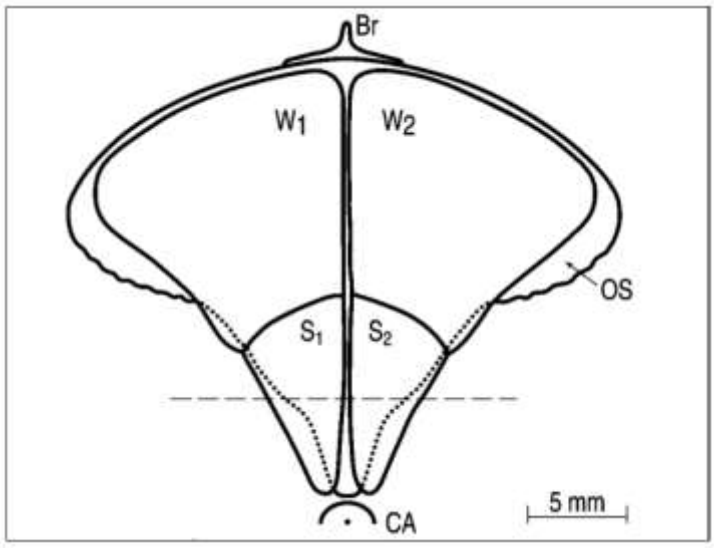

Figure 1-2. *Abies alba*, European silver fir: View of adaxial surfaces of a pair of seeds on scale. CA = cone axis; OS = ovuliferous scale; Br = bract; S1,S2 = seeds; W1,W2 = wings.

The next step in seed processing is displacing the seed from the wing structure (Figure 1-2). This is usually completed by either dry or wet winging. Dry winging uses brushes and applies rubbing motions to remove the wing from dry seed. *Abies* seed is typically dewinged using this method. Often, the seed is handled too agressively during this stage of seed processing, thus damaging seed and decreasing seed quality and germination (Tanaka, 1984; Kolotelo 1997b). Many industry leaders prefer wet dewinging, which involves wetting the wing so it is removed cleanly and with ease. Often a small rotating cement mixer containing soft brushes is used for wet dewinging (Rockis, Personal Communication). However, with this process the seed absorbs moisture. After which the seed needs to be redried before storage (Tanaka, 1984).

The last step in seed processing involves using a machine such as a fanning mill, gravity table and/or pneumatic separator to improve seed quality, and separate viable seed from non-viable (Kolotelo, 1997a). Seed sorting can also be completed using IDS (incubation-drying-separation)

which separates non-viable and empty seed from good seed. In this case, the seed is incubated for a short period then gradually dried and separated by specific-gravity processes. Since non-viable seed lose their moisture content at a faster rate than viable seed, gravity methods work to sufficiently separate seed. During final stages, cutting tests are often performed to determine if machine settings are adjusted appropriately. These processes are generally complex and often need to be adjusted based upon the qualities and attributes of each individual seedlot. To ensure seed is homogeneous, purity must be 97%+ with moisture content dried to between 4.9% and 9.9% for storage. For short term storage, temperatures slightly above freezing have been shown to be sufficient without deterioration. For long-term storage, temperatures between -15˚C and -18˚C can be effectivelty used to store seed for up to seven years. Conifer seed is normally stored in dry conditions. Moisture content is regulated by placing seed in closable storage containers and monitoring humidity in the storage unit. Polyethylene bags and fiberboard drums are also used since they are relatively inexpensive (Tanaka, 1984).

There is minimal data noting the best method of storing Nordmann and Turkish fir seed for long periods. This Project will investigate Nordmann and Turkish fir seed quality and seek to better understand factors affecting seed viability and germination.

Objectives:

I. To evaluate the effect of storage time on Nordmann fir seed germination.
II. To evaluate the impact of moisture content on Turkish fir seed germination.
III. To evaluate the change in seed moisture content of Turkish fir by storage medium.
IV. To evaluate the influence of seed stratification protocols on Nordmann and Turkish fir seed.
V. To investigate the influence of *Megastigmus* larvae heat treatment on Nordmann and Turkish fir seed germination.

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Chapter 2

Evaluating the Effect of Seed Age on Nordmann Fir Seed Germination

Seed production varies from year to year, as seed quality is often dependent on seasonal conditions. It is necessary to learn seed species characteristics, and other factors that can influence storage (Tanaka, 1984; Edwards, 2000). Seed viability during storage varies by species. There are three standard classifications of seed- microbiotic, mesobiotic and macrobiotic. In ambient conditions, microbiotic seed do not live past three years of age, while mesobiotic seed live 3-15 years. Macrobiotic seed live the longest, from 15 to over 100 years. Coniferous seed often falls under the microbiotic category. However, manipulating storage conditions may increase lifespan of seed. Conifer seed is orthodox in behavior, meaning that they store well under low temperatures and moisture contents (Tanaka 1984; Jensen 2015; Bonner & Karrfalt, 2008). Therefore, conifer seed can be stored several years with moisture content (MC) of 4-8% at temperatures between 0-5˚C. Storage length can be increased further, without losing seed viability, by lowering temperatures to ⁻15 - ⁻20˚C (Jensen, 2015; Tanaka, 1984; Edwards, 2000). Current research indicates that if seed is harvested at physiological maturity while being properly handled, it can be stored up to 50 years (Edwards, 2000). True fir *(Abies)* species are typically considered sub-orthodox, thus being stored in the same conditions as true orthodox seed, but having a shorter lifespan (Jensen, 2015). This may be from inconsistencies in seed source, time of collection and post-harvest handling (Edwards, 2000).

Martin Jensen of the University of Aarslev Horticulture Dept. has worked to evaluate the effects of storage tolerance on previously stratified Nordmann fir seed. He selected several combinations of seed MC, and storage temperature prior to experimentation. Seed germination was

measured one week into storage, and after 12 months. After 12 months, he found that the best seed survival rate was stored between 25 and 30% MC, and stored at -5˚C. Seed stored at higher MC between 34 and 42% MC lost all vigor. It was concluded that seed viability decreases by a minimum of 15-25%. Thus, Jensen concluded that the Nordmann fir seed were more sensitive to storage practices compared to other coniferous species (Jensen, 2015).

Seed quality, moisture content, storage temperature and methodology are factors to consider when researching seed storage improvement. However, optimal conditions vary by seed species, and have yet to be determined for many due to lack of research (Tanaka, 1984; Gosling 2007). Evidence suggests that Nordmann and Turkish fir seed quickly lose viability after years in storage, compared to other *Abies* species (Bates, Personal Communication). The purpose of this study was to evaluate the effect of storage time on germination potential of Nordmann fir seed.

Materials & Methods

Nordmann fir seed was collected in 2011 and 2013 from the Georgian provenance of Bakhmaro. Seed from 2015 was collected from the Georgian provenance of Ambrolauri. A total of 12 replicates were used, with 4 replicates per year. On 16 January 2017, 15 grams of seed (approx. 15 seed per gram) were soaked in polystyrene petri dishes overnight for 24 hours, in 45 ml of water. After 24 hours, seed was drained using a strainer with Double Fine Mesh (Winco), and surface dried using paper towels. Seed was then placed into a single layer in unzipped 5" x 7" plastic bags (Plymor). The bags were placed on moist Versa-Pak germination paper in plastic seed containers to maintain MC (Seedburo). Each container was labeled with seed type and year. Containers were placed into a mini refrigerator (GE®) at 2˚C for 4 weeks. On 15 February 2017, seeds were taken out of stratification and prepped for germination. Amidst stratification, fungi were observed growing on seed, which prompted a 10% Clorox bleach treatment applied to 50% of the replicates

at random for ten minutes. There were an equal number of bleach and control (no bleach) treatments for each seed year. Seed was removed from their respective plastic bags, and surface dried with paper towels. Approximately ¼ inch of Metro Mix 360 with Sun-Coir (Sun Gro), was placed on the bottom of each Seedburo plastic container, and saturated using 100 mL of water. The metro mix was compacted for seed placement. One hundred seed were chosen as the working sample using the AOSA rules for seed testing methods (AOSA Rules for Seed Testing, 2014). Seed was placed in a 10x10 arrangement, per container. Containers were relocated to a growth chamber. The growth chamber was set to 6 hr. light at 30˚C and 18 hr. dark at 20˚C. To monitor temperatures, a HOBO datalogger was placed inside the growth chamber. Soil conditions were monitored regularly and moisture was provided as necessary to maintain moisture. Germination status of the seed was evaluated after 10, 20 and 30 days, with germinated seed being removed after being counted. Seed was considered germinated for any visible length radicle emergence per seed.

Data were recorded, and analyzed at the end of the 30-day measurement period. Germination data was analyzed using an ANOVA Mixed Model (Type III) procedure in SAS (version 9.4; SAS Institute, Cary, NC). Reps were included in the random statement to account for naturally occurring differences among seed replicates. Residual plots were generated and to determine normality. Data were normally distributed. Additionally, variances of the response variable were equal. After running the ANOVA procedure, a generalized linear mixed model procedure (PROC GLIMMIX) using a LSmeans statement, adjusted to Tukey's Studentized range test was produced to analyze multiple comparisons, and categorize differences by LSmeans. The significance level (α) was 0.05 for all tests.

Results & Discussion:

Over the 30-day measurement period, Nordmann fir seed collected in 2011 and 2013 did not germinate. In contrast, 23.75% of Nordmann fir seed collected in 2015 germinated after 10 days. By day 20, germination percentage increased to 37.5%. On the final day of measuring seed germination, Nordmann fir seed collected in 2015 germinated an average of 47.5% (Figure 2-1).

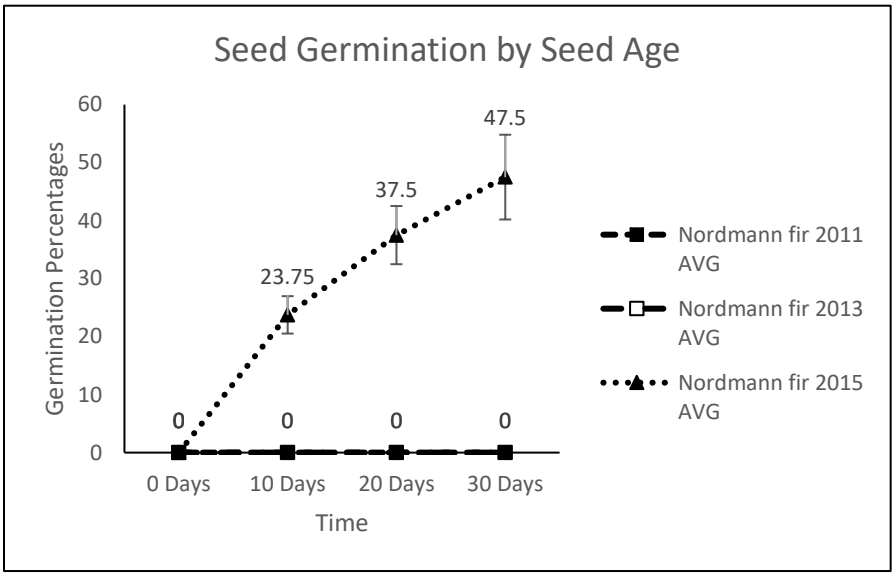

Figure 2-1. Seed germination percentages for Nordmann fir seed collected in 2011, 2013 and 2015. Nordmann fir seed from 2015 was the only seed that germinated over the 30-day period.

Results agreed with existing anecdotal evidence (Bates, Personal Communication). Seed germination varied significantly by seed age. Seed germination of the 10% bleach solution and the control were not significantly different.

Table 2-1. Analysis of Variance for seed germination by year and bleach treatment.

Effect	Num DF	Den DF	F Value	Pr > F
Year	2	27	94.15	<.0001
Bleach Treatment	1	27	2.82	0.1047
Year*Bleach Treatment	2	27	2.82	0.0773

Seed from seedlots harvested in 2011 and 2013 showed no significant differences in mean germination, and were categorized under letter B (Table 2-2). Nordmann fir seed from 2015, had a significantly higher germination percentage, and were categorized under letter A (Table 2-2). There were no mean differences between year and bleach solution, showing that the bleach solution had no significant effect on overall germination of Nordmann fir seed by year.

Table 2-2. Seed Germination Percentages Least Square Means after concluding seed germination by seed age.

Germination Percentages (LS Means Alpha =0.05)			
Bleach Treatment	NF 2011	NF 2013	NF 2015
Yes	0.0 B	0.0 B	30.7 A
No	0.0 B	0.0 B	43.5 A
[Y] LS Means with the same uppercase letter indicate no difference according to Tukey's Studentized range test with alpha =0.05			

The results from objective I, agree with evidence suggesting Nordmann fir seed viability does not last for more than several years. This suggests that Nordmann fir seed may have a higher sensitivity to warmer storage temperatures. In theory, storage temperature has greater effect on seed viability when seed MC% is higher (Tanaka, 1984; Bonner & Karrfalt, 2008). *Abies* seed are orthodox in behavior, meaning they store best under low MC% and low temperatures. *Abies* seed, including the seed used for experimentation, is traditionally stored at or below 10% MC (Jim Rockis, Personal Communication). Seed collected in 2011, 2013 and 2015 was stored in freezer

conditions slightly above freezing at 6 ± 5˚C (Bates, Personal Communication). While storing seed at these temperatures is sufficient in preventing seed deterioration, it lowers seed viability after short term storage (Tanaka, 1984). Storage temperatures slightly above freezing, could be a factor in the significant drop of seed germination by seed collection year. Future experiments with Nordmann fir should focus on the effect of seed storage temperature on seed viability. Statistical analysis between seed treated with a 10% bleach solution, and the control (no bleach treatment) was insignificant. Consequently, using a bleach solution to treat seed fungi is an effective way to kill the pathogens, without negatively affecting germination. Other factors to consider include seed composition of Nordmann fir compared to other *Abies* species, variances in species dormancy breakage or different levels of oil and resin in the seed coat that become toxic to the seed embryo (Bonner & Karrfalt, 2008; Jensen 2015; Kurt et al., 2016).

With future studies, manipulating seed storage methods, temperatures and moisture content may result in improved seed viability of Nordmann fir. This can be beneficial to industry leaders who collect Nordmann and Turkish fir. Since quality of seed varies under changing environmental conditions and on a year to year basis, seed storage methods are very important in the Christmas tree industry.

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Chapter 3

Evaluating Impact of Moisture Content during Stratification on Turkish Fir Seed Germination

Successful harvest and storage survivability is a priority for Mediterranean firs. Researchers are working to improve seed germination by seedlot (Kolotelo, 1997b; Edwards 2000; Edwards 2003). Due to the varying states of dormancy associated with Nordmann and Turkish fir seed, stratification is often employed to break dormancy. Cold stratification requires proper seed moisture content percent, low temperatures, free air movement and appropriate storage periods (Tanaka, 1984). Recent research proposes that by controlling seed MC during stratification, seed germination is improved by both speed and total percent (Edwards, 2000).

Seed moisture content and mechanical damage on legume germination were tested at the New York State Agricultural Experiment Station in Geneva, NY. Seed was dried down to two different MCs (8% and 12%), and underwent simulated mechanical damage. Results demonstrated that seed with higher MC% were less inclined to mechanical damage while seed at lower moisture content had lower germination because of seed injuries (Taylor & Prusinski, 1990). Likewise, experiments were performed at the University College Dublin to observe the germination response of *Alnus glutinosa* (L.) Gaertn. and *Betula pubescens* Ehrh. seed following a prechill treatment with varying moisture contents. Seed were either stratified in a fully imbibed state or a lower target MC of 30% and 35%. Seed was germinated over interval temperatures ranging from 7.5 to 30˚C. Overall, results of the seed dried to target MC% germinated well across all temperature intervals. In contrast, seed chilled at their fully imbibed state had an optimal germination temperature of 22-23˚C. Total germination in fully imbibed seed and seed dried to respective target MC% was insignificant, and did not vary between methods (De Atrip et al., 2007).

Martin Jensen from the Department of Horticulture at Aarhus University tested the impact of dormancy breakage in *Abies nordmanniana* seed by altering MC%. The standard protocol of dormancy breakage of Nordmann fir seed in Denmark is prechilling fully imbibed seed at 4˚C for 3-16 weeks. Previous research proposes that seed that underwent this method germinate sufficiently at 4˚C with significant differences by seedlot. However, seed germinated during extended dormancy breakage treatments and would not survive normal sowing procedures. Prechilling for a lengthier period was therefore, deemed inadequate (Jensen, 1997). Jensen concluded that very low MCs did not allow seed to break dormancy. The critical low MC of 23% was best for dormancy breakage in Nordmann fir seed. These results and conclusions indicate that a prechilling method that offers improved seed germination and predictability would be desirable. Prechilling seeds at a reduced MC appear to offer some of these benefits (Jensen, 1997).

Due to low seed inventory, Turkish fir seed was used in placed of Nordmann fir for this experiment. The objective of this study was to determine if seed stored at various MCs during stratification will affect overall germination of Turkish fir seed.

Materials & Methods

To evaluate the impact of MC% on germination, Turkish fir seed collected in 2015 from Marion, New York (43.1432° N, 77.1891° W) was used. On 18 January 2017, MC% ranges of seed dried at four set time intervals (30 min, 1 hr., 2 hr., 4 hr.) to assure seeds representing a range of MC% were created. Ten replicates of 15 grams of Turkish fir seed were soaked for 24 hours in polystyrene petri dishes with 50 ml of water. Seed was drained using a strainer (Winco) and surface dried using paper towels. Two replicates of 15 grams were placed in 2 oz. metal steel tin flat containers (Juvitus). The containers were placed in a drying oven (Precision™) at 103˚C for 17 hours. The remaining eight replicates were used to calculate MC at set time intervals. Two

replicates per interval were dried on paper towels in ambient laboratory conditions with seed being rotated every half hour. After each time interval had past, the weight of all seed was recorded and averaged. This would be considered the seed "wet weight." After 17 hours, the seed in the oven was placed into a mini desiccator cabinet (Bel-Art™ SP Scienceware™ Secador™) with indicating mesh desiccant (Drierite) to cool for a half hour, while regaining no outside moisture. The seed from the desiccator was then weighed and averaged. This was considered the seed "dry weight." The MC range of seed at each time interval was calculated using the following equation:

$$\frac{\text{Weight of Wet Seeds} - \text{Weight of Dry Seeds} * 100}{\text{Weight of Wet Seeds}} = \text{MC\%}$$

The resulting MC% ranges were calculated as follows:

30 min: 33-34% MC
1 hour: 31-32% MC
2 hours: 27-29% MC
4 hours: 23-24% MC

On 7 February 2017, 12 total replicates of Turkish fir, with three replicates being dried per each MC interval. Fifteen grams of seed were soaked overnight for 24 hours in polystyrene petri dishes with 50 ml of water. After drying each replicate at room temperature for the designated time, seed was placed in sealed 5" x 7", plastic bags (Plymor) in a single layer. The bag was placed into plastic seed containers (Seedburo) on top of moist Versa-Pak germination paper (Seedburo) to assist in maintaining seed MC. Containers were labeled with seed type and MC% intervals. The seed were placed into cold storage (GE®) at 2˚C for a total of 28 days (4 weeks). On 8 March 2017 seed was removed from stratification and prepped for germination. Seed was removed from the plastic bags, and surface dried with paper towels. About ¼ inch of Metro Mix (Sun Gro) was placed on the bottom of each container, and moistened using 100 mL of water. It was pressed down and compacted for seed placement. One hundred seed were chosen at random following the AOSA rules for seed testing methods (AOSA Rules for Seed Testing, 2014). Seed

was placed in a 10x10 arrangement, per container. Containers were then relocated to a germination growth chamber. The growth chamber was set to 6 hr. light at 30˚C and 18 hr. dark at 20˚C. Temperatures were monitored for consistency using a HOBO datalogger. Soil moisture conditions were monitored regularly, and provided moisture when needed. Seed germination was measured after 10, 20 and 30 days. Seed was considered successfully germinated for any visible length of radical emergence per seed. Germinated seed was removed from each container after being counted.

Data were recorded and analyzed after the 30-day measurement period. Germination data were analyzed using an ANOVA Mixed Model (Type III) procedure in SAS (version 9.4; SAS Institute, Cary, NC). The "reps" variable was included in the random statement to account for variation between seed samples. Residual plots were created to assure normality in data and that data met all ANOVA assumptions. A generalized linear mixed model procedure (PROC GLIMMIX) using a LSmeans statement, adjusted to Tukey's Studentized range test was performed and analyzed to categorize significant differences by data means. The significance level (α) was 0.05 for all tests.

Results & Discussion-

Final seed germination was different between seed stratified at various MC%. After 10 days, Turkish fir seed between 27-29% MC had the highest germination total at 11.3%. After 20 days, Turkish fir seed stratified at 23-24% had the highest germination, averaging at 27% (Figure 3-1). By day 30, seed between 23-24% MC, germinated just under 30%, at 29.3%. The seed stratified at 27-29% MC averaged slightly higher at 31%. Turkish fir seed between 31-32% MC

had 29% total germination after 30 days. Finally, seed stratified at 33-34% MC had the lowest germination, at 20% by the end of experimentation (Figure 3-2).

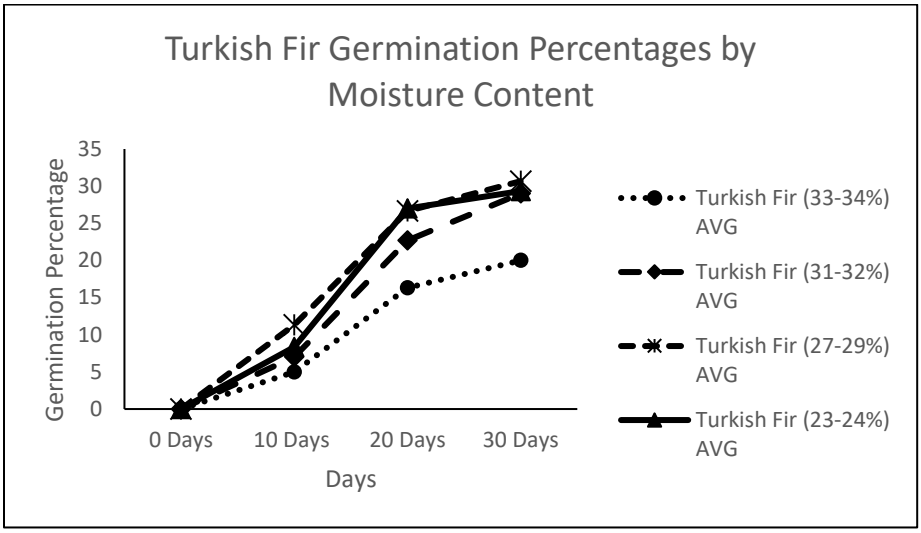

Figure 3-1. Changes in Turkish fir seed germination over a 30-day measurement period. Each line represents a seed MC% interval prior to stratification.

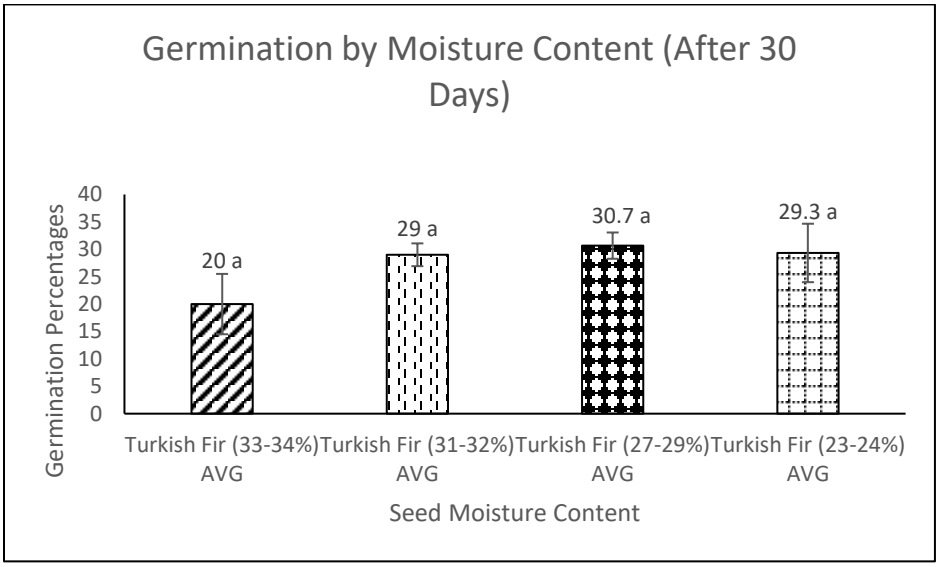

Figure 3-2. The final germination percentages based on MC after 30 days in the growth chamber. The error bars indicate the variability of data based on means.

As seen in table 3-1, there was no significant difference between each MC level based on the ANOVA output in SAS. The P-value of MC was 0.3044.

Table 3-1. SAS Output for the Analysis of Variance procedure based on seed MC% after 30 days of the experiment.

Effect	Num DF	Den DF	F Value	Pr > F
MC	3	8	1.26	0.3144

After running the analysis of variance, the means were grouped and categorized using Tukey's Studentized range test (Table 3-2).

Table 3-2. Seed Germination Percentages by LS Means, after conducting germination experiments by seed moisture content percentages.

Seed Germination Percentages by LS Means (Alpha =0.05)				
MC%	23-24%	27-29%	31-32%	33-34%
	29.3 A[Y]	30.7 A	29.0 A	20.0 A
[Y] LS Means with the same uppercase letter indicate no difference according to Tukey's Studentized range test with alpha =0.05				

A potential reason there was no significant difference in germination between MC% intervals was due to experimental errors during prechilling. This could be because of stratification taking place in a small, non-humidity regulated refrigerator. After investigating the impact of stratification protocol on seed germination (Chapter 4), the weight of the fully imbibed seed dropped from the start of prechilling. Therefore, evidence suggests that seed being stratified in the laboratory refrigerator also lost weight, during stratification. Additionally, seed is often stored by placement in closable storage containers and monitoring humidity through the storage unit (Tanaka, 1984). The compact mini refridgerator (GE®) did not allow for humidity regulation. Likewise, multiple people were using the refrigerator for experiments, thus exposing the stratifying seed to warm and dry ambient conditions of the laboratory.

Another reason results may have been statistically insignificant was due to potential seed damage. Since seed was dried down to about 10% MC prior to storage (Jim Rockis, Personal Communication), the possibility for damage through transport and other rough handling was increased. (Taylor & Prusinski, 1990). Likewise, the varying degrees of Turkish fir seed dormancy could be a contributing factor. Previous experiments suggest that the standard stratification protocol is often inadequate for breaking species dormancy in *Abies* seed (Edwards, 2000; Edwards, 2003). This evidence suggests that the stratification protocol used for determining differences in germination by seed MC% was ineffective in breaking seed dormancy. If the experiment were to be repeated, the stratification-redry method should be more effective in breaking Turkish fir seed dormancy.

Those working in the Christmas tree industry, should strive to focus on delicate handling of seed, and stratifying seed in a humidity regulated refrigerator to avoid any changes in MC%. Furthermore, determining a better way to break seed dormancy in *Abies* seed used for this experiment could alter future results.

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Chapter 4

Effect of Storage Unit on Moisture Content Change in Turkish fir Seed

Temperatures as low as -17˚C have been used successfully in storage of coniferous seed. Previous research shows that storage temperature has greater effect on seeds when their MC is higher, and vice versa (Bonner & Karrfalt, 2008). Depending on type and size, seed can be stored using a wet or dry method. Moisture content of seed is often regulated by storing in tightly closed containers or regulating humidity in storage facilities. Humidity regulated seed storage facilities are typically used for agricultural seed storage, and are expensive. However, they are excellent in preventing seed from absorbing outside moisture, particularly in humid climates. Storage containers range from polyethylene bags to fiberboard drum, or a combination of both (Tanaka, 1984). For *Abies nordmanniana,* the Woody Seed Plant manual recommends storing seed between 9-11% MC with a temperature +4˚C. However, this is only for short term storage, as seed viability will remain viable for two years at most (Bonner & Karrfalt, 2008, pg. 178).

Zeolite drying beads are a new desiccant technology developed to improve seed storage, especially in the high temperatures and humid climates of developing countries. It is a relatively inexpensive and reusable technology, only requiring airtight containers and the beads themselves. It helps seed maintain low MC, as well as keep out pests, mold, and other pathogens. The beads are made of aluminum silicates that absorb outside moisture. Once they are saturated, they can be reused multiple times by heating at a temperature >200˚C for approximately two hours. This benefits farmers who do not have access to a direct energy source. Initial costs range from $10-$20 USD per kilogram. However, the beads are made to be reused, so they are a worthwhile long-term investment (Bradford, 2015).

The goal of this experiment was to monitor MC changes after seed was stored in varying storage units for a 12-week period. This was to determine the best means of seed storage, for locations with variable financial resources and climates.

Materials and Methods

For Objective III, three replicates of 100 seeds of Turkish fir, collected in 2015 Marion, New York (43.1432° N, 77.1891° W) were used for each storage media type. Two additional replicates were used for determining the dry weight and MC% prior to storing. On 24 January 2017, all five replicates were weighed and placed in 2 oz. Metal Steel Tin Flat containers (Juvitus), and into a drying oven (Precision™) at 103˚C for 17 hours. Seed was then relocated to a mini desiccator cabinet (Bel-Art™ SP Scienceware™ Secador™) with indicating mesh desiccant (Drierite) to cool for a half hour without regaining outside moisture. Seed going into storage was reweighed, and placed in unzipped 5" x 7" plastic bags (Plymor). They were then moved to designated storage types of either a compact refrigerator (GE®), freezer (Magic Chef®), or drybeads container (Zeolite®). The two remaining replicates were placed back in the oven for an additional 17 hours. Afterwards the dry seed was weighed and averaged with the MC of the wet seed calculated using the following equation:

$$\frac{\text{Weight of Wet Seeds} - \text{Weight of Dry Seeds} * 100\%}{\text{Weight of Wet Seeds}} = \text{MC}$$

Seed was left in their respective storage units for approximately 12 weeks. On 12 April 2017, the labeled bags were removed from storage with the new wet weight being measured and recorded. Seed was placed back in the oven for 17 ± 1 hour. The change in MC after 12 weeks of storage was calculated using the same equation as listed above. The difference of MC before and after storage was calculated and recorded.

Data were recorded in and analyzed at the end of the 12-week period. MC% changes were analyzed using PROC GLM procedure in SAS (version 9.4; SAS Institute, Cary, NC). Residual plots were made to assure normal distribution in data and that all assumptions were met. In the PROC GLM statement, Tukey's Studentized Range test was used to group means by significance. The significance level (α) was 0.05.

Results & Discussion

It can be concluded that the refrigeration treatment had the largest change in MC% over the 12-week storage period (Table 4-1). Seed that was stored in the freezer and Zeolite had small changes in MC%. Refrigerator storage showed a MC% change mean value of 2.8%. Whereas, the freezer and zeolite storage units had mean changes of 0.3% and 0.0% (Table 4-1). The Zeolite and freezer storage units are both effective in maintaining seed for long term storage. Zeolite beads have great potential to be efficient for seed storage in environments where access to energy sources is scarce.

Table 4-1. Tukey's Studentized Range test was used to determine the differences between moisture content change and storage unit.

Moisture Content Changes by Storage (Means Alpha=0.05)	
Storage	Means
Refrigerator	2.8 A[Y]
Freezer	0.3 B
Zeolite	0.0 B

[Y] Means with the same uppercase letter indicate no difference according to Tukey's Studentized range test with alpha =0.05

Previous research suggests that storage temperature has greater effect on seed when MC is higher, and vice versa (Bonner & Karrfalt, 2008). *Abies* seed is typically stored at or below

10% MC (Jim Rockis, Personal Communication). Zeolite drybeads absorb moisture, keeping MC% of seeds relatively low. This suggests that by maintaining the low seed MC, the storage temperature of seed will have less of an effect on overall vigor and viability.

Industry leaders should avoid refrigerator storage due to the significant change in MC% over a 12-week period. Freezer storage is a suitable unit for storage of seed for those with access to a direct energy source. Likewise, Zeolite drybeads are shown to be successful in maintaining MC of seed through storage. Zeolite beads make a great alternative to freezer storage for industries in developing countries, hot and humid climates, and those without access to sufficient energy.

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Chapter 5

Investigating Stratification Protocol on Nordmann fir Seed Germination

Seed dormancy is an evolutionary trait that delays germination until environmental conditions are suitable for the start of seedling growth (Edwards, 1996). Coniferous seed matures and sheds in autumn as winter approaches. Without sufficient dormancy, seed is unlikely to survive until spring. In seed testing laboratories, scientists often imitate these natural environmental conditions in a prechilling process called stratification (Tanaka 1984; Edwards, 2003). Seed of true firs (*Abies*) species often display a wide range of dormancy states (non-dormant, slightly dormant, somewhat dormant, very dormant) that can vary between seed crop, source, and even among individual seed within a single cone (Bonner & Karrfalt 2008; Edwards, 2003). To establish the degree of seedlot dormancy, two germination tests need to be performed; one with unstratified seed, and the other with stratified seed. Responses to these tests can assist industry leaders in determining the degree of seed dormancy on a per lot basis, since more dormant seedlots germinate faster than less dormant seedlots (Bonner & Karrfalt, 2008). It is believed that the standard stratification protocol for *Abies* seed is often inadequate for breaking dormancy in seed that is in a deep dormancy state. As a result, many Christmas tree growers and industry leaders often fail to get seed to fully germinate.

Many different methods of stratification have been examined to better understand the processes that occur during dormancy breakage for *Abies* seed. In laboratories and nursery settings, stratification is often done by putting rehydrated seed in plastic bags, or other containers. This is formally known as the “naked stratification method.” However, stratification is also done by placing dry seed on a moist medium such as sand, filter paper or vermiculite in a refrigerated

setting. This is done so the seed imbibes moisture at a slower and potentially less damaging rate. It is also believed that the imbibition of moisture allows seed to be rehydrated to a higher MC% (Bonner & Karrfalt 2008). In the 1980's a new stratification method was researched to examine and ideally solve the issue regarding dormancy breakage in conifer species. Rather than removing seed from cold refrigeration for sowing after a period of 4 to 8 weeks, they were removed and dried down to a moisture content range between 30-35%. Seed is then placed back into prechilling for up to an additional 12 weeks. By reducing the moisture content, the seed is prevented from germinating for longer periods of time (Edwards, 2003). This relatively new technique is referred to as the redry method. A stratification dry-back method exists and is similar, except seed is placed back into stratification for only 8 additional weeks (Leadem & Clark, 1992).

During traditional stratification seed is soaked for 24-48 hours at room temperature. Moisture content should be 45% or higher and remain that way during stratification. Seed is then drained and chilled at 2˚C for a period of 4 to 8 weeks. With stratification-redry, seed are soaked for 24-48 hours, drained and chilled at 2˚C for 4 weeks. After 4 weeks, the seed is removed from the refrigerator and dried to 30-35% moisture content. Seed goes back into storage anywhere for one to three months. This allows seed to germinate at the fastest and most complete rate (Edwards, 2003; Bonner & Karrfalt, 2008).

Edwards used rehydrated seed of grand fir and stratified them for 4 weeks at 2˚C. Seed was removed from refrigeration and kept at full MC (45%), or dried to 35%, 25% and 15% MC. They were placed back into cold storage for 12 more weeks. Seeds dried to 35% germinated earlier and at a faster rate compared to the fully imbibed seed and seed dried to 25% and 15%. However, seed at 25% and 15% MC germinated higher than seed that wasn't dried at all. Replicates that remained at 45% were either placed into germination after 4 weeks or remained in cold storage for an additional 12 weeks. Seed that remained in stratification the longest germinated quickly, but had the lowest overall germination percentage due to seed deterioration from microbial activity in the

damp conditions. Since seed underwent both drying and additional storage it was necessary to distinguish the effect of the extra stratification period. Seed samples were dried down to 35%, 25%, 15%, while the control sample remained at 45%. Results showed that the dried seed had little effect on final germination percent, but did influence initial germination speed. Seed that was dried down germinated about 5-6% higher than seed at the midpoint of testing, but the differences were insignificant by the conclusion of the experiment (Edwards, 1996).

Leadem and Clark of the British Columbia Ministry of Forest Research Laboratory performed a similar experiment using seedlots from Pacific Silver fir (*Abies amabilis)*. Four replicates received the stratification-redry and dry-back method, while the control treatment received no stratification at all. After the experiment period was over, it was concluded that the stratification-redry method resulted in faster germination. In contrast, the total germination did not differ between the stratification methods. This could be due to a difference in moisture contents chosen to initiate the stratification-redry method per both experiments. While Edwards dried seed samples down to 35% in between stratification periods, Leadem and Clark dried their seed to 30%. Additionally, Leadem and Clark used no stratification method as their control method, while Edwards used a standard 4-week stratification period for *Abies* seed after they were fully imbibed with water for 24-48 hours (Leadem & Clark, 1992; Edwards 1996).

Overall, strong evidence exists that the stratification-redry method has significant improvement on germination speed and totality compared to a traditional stratification method. The goal of this experiment was to examine the effect that stratification method had on total seed germination on Nordmann and Turkish fir seed.

Materials & Methods

To examine the effect of seed stratification on overall germination, Fraser fir seed from 2015 collected in Mt. Rogers, Virginia (36.6598° N, 81.5446° W) was used as the control. Meanwhile, Turkish fir seed collected in 2015 from Marion New York (43.1432° N, 77.1891° W), and Nordmann fir seed collected in 2015 from the Georgian provenance of Ambrolauri were the seed variables. Two replications of 15 grams of Nordmann and Turkish fir, and 5 grams of Fraser seed were measured out prior to initial stratification. After soaking the seed in 50 mL of water in polystyrene petri dishes for 24 hours, seed was drained using a strainer with double fine mesh (Winco). 2 oz. metal steel tin containers (Juvitus) were labeled and weighed, with the tin weight recorded. The wet seed was poured into each container, with total weight being documented. The total seed weight was subtracted from the tin weight to give the wet seed weight. Seed containers were closed and transferred to a forced air-drying oven (Precision™) at approximately 103˚C for 17 hours. Seed containers were opened and moved to a mini desiccator cabinet (Bel-Art™ SP Scienceware™ Secador™) with indicating mesh desiccant (Drierite) for 30 minutes. After drying, seed and containers were weighed together, with the total weight being recorded. Dry seed weight was calculated by subtracting the new total weight from the individual tin weight. Moisture content of each replicate was calculated using the following equation:

$$\frac{\text{Weight of Wet Seeds} - \text{Weight of Dry Seeds}}{\text{Weight of Wet Seeds}} * 100\% = MC$$

After rehydrating two additional replicates of the Nordmann, Turkish and Fraser fir seed for 24 hours, seed fresh weight was measured and recorded. The goal fresh weight was calculated using the following equation:

$$\text{The goal FW} = \frac{\text{(Average Dry Weight (DW)*100)}}{\text{100- target M.C\%}}$$

Seed was placed on moisture absorbing paper towels, and dried to the calculated fresh weight, being turned and reweighed every half hour. Fifteen grams of Nordmann and Turkish fir seed were measured into four replicates for both traditional stratification and stratification-redry. Four replicates of five grams of Fraser fir seed were used for each method. This was done to assure at least 100 seeds were in each sample. Seeds were soaked in polystyrene petri dishes for 24 hours to regain a moisture content between 40-45% or greater. Seeds designated for traditional stratification were placed in a single layer inside unzipped 5" x 7" plastic bags. The bags were laid flat inside plastic seed containers (Seedburo) on top of Versa-Pak germination paper (Seedburo). Containers were labeled with seed type and stratification protocol. On 13 December 2016 containers were placed in cold storage (2˚C) for 28 days. After four weeks, On 10 January 2017, seeds undergoing traditional stratification were taken out of cold storage to begin germination. During stratification, fungi was observed growing on seed. A 10% Clorox bleach treatment was applied randomly to 50% of the replicates prior to germination for ten minutes. About ¼ inch of Metro Mix (Sun Gro) was placed on the bottom of each container, moistened using 100 mL of water, and compacted for seed placement. One hundred seed were chosen for the working sample using the AOSA rules for seed testing (AOSA Rules for Seed Testing, 2014). Seed was placed in a 10 x 10 arrangement, per container. Seeds designated for stratification-redry were removed from the refrigerator, dried to the designated weight (as previously calculated), and returned to cold stratification on new moist Versa-pak for an additional 28 days. On 8 February 2017, seeds were taken out of prechilling for germination. Containers were relocated to a growth chamber located on at Penn State University Park. The growth chamber was set to 6 hr. light at 30˚C and 18 hr. dark at 20˚C. Temperatures were monitored using a HOBO datalogger. Soil moisture conditions were checked regularly, and seed treatments were provided moisture when necessary. Germination status of the seed was measured after 10, 20 and 30 days, with germinated seed being removed after being counted. Germination was to be considered for any length radicle emergence per seed.

Data were recorded and analyzed at the end of the 30-day measurement period. Germination data was analyzed using an ANOVA Mixed Model (Type III) procedure in SAS (version 9.4; SAS Institute, Cary, NC). “Reps” were included in the random statement of the ANOVA procedure, to account for natural variations between each sample. Residual plots were created to assure normality in data, and that data met ANOVA assumptions. A generalized linear mixed model procedure (PROC GLIMMIX) using a LSmeans statement, adjusted to Tukey’s Studentized range test was performed and analyzed. The significance level (α) used was 0.05.

Results & Discussion

For the stratification-redry method, the objective was to dry the seed down to newly calculated seed weights. However, during stratification, the seed weight dropped below the calculated fresh weight, suggesting that the humidity in the refrigerator was less than the MC in the seed samples. The stratification-redry method was to be in cold refrigeration for 48 consecutive days, thus the seed was repackaged in new polyethylene bags along with fresh, moist versa pak. Seeds remained in stratification for an additional 28 days. While seed did not endure a traditional stratification-redry method, it is referred to as a “modified stratification.” For the traditional stratification method, after 10 days of germination, Fraser fir produced the highest average germination percent at 29.3%. Turkish fir was lowest at 2.8%. After 30 days, the final germination average for Nordmann fir was 56.8%. Turkish fir was much lower at 16%, and Fraser fir was 53.3% (Figure 5-1). Nordmann fir had a better performance in seed germination, compared to that of Turkish fir seed collected from the same year, with total seed germination from Nordmann fir comparable to Fraser fir (Figure 5-2). Modified stratification had faster germination by day 10, at 34.5%, 8% and 42% for Nordmann, Turkish and Fraser fir, respectively. After 20 days, Nordmann fir germinated an average of 48.3%. Turkish fir, averaged 13.3%, while Fraser fir was slightly

higher than Nordmann at 54.5% (Figure 5-1). For the modified stratification method, Nordmann and Fraser fir had similar total germination tallying at 58% and 57%, respectively. Turkish fir germination increased slightly after 30 days, coming in at approximately 20% germination (Figure 5-2).

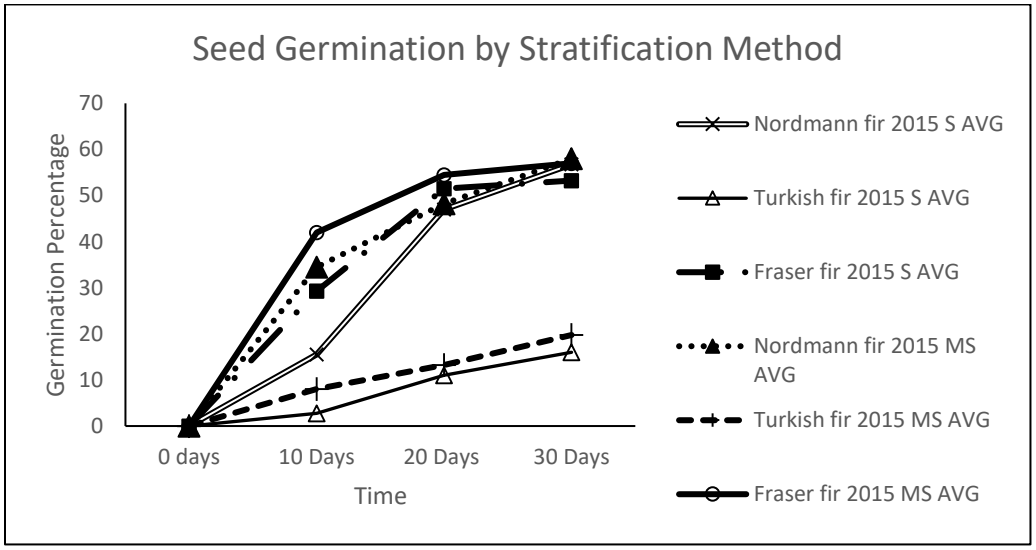

Figure 5-1. Seed germination observations were measured over a course of a thirty-day period. "S" stands for stratification treatment while "MS" is the Modified Stratification method. Turkish fir had the lowest germination compared to both Nordmann and Fraser fir.

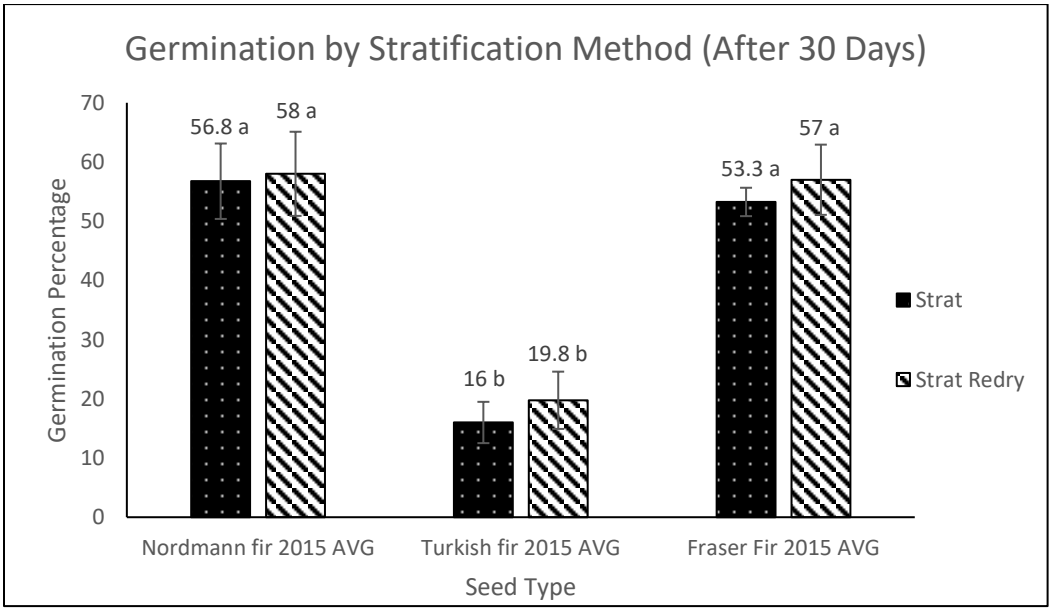

Figure 5-2. Average germination after 30 days was calculated and separated by stratification, and stratification-redry method. Tukey's lettering indicated significant differences between the means. Stratification-redry had higher overall germination by day 30, but was not significantly different between the same seed types.

There were significant differences in seed germination between seed type (Table 5-1). Seed germination was not influenced by bleach treatment. There were also no differences between final germination between stratification and stratification-redry.

Table 5-1. A mixed model (Type III) ANOVA Test was performed on the interactions of Seed, Bleach, Stratification Method (Treatment) and their interactions.

Effect	Num DF	Den DF	F Value	Pr > F
Seed	2	9	38.18	<.0001
Bleach	1	9	3.68	0.0873
Treatment	1	9	0.49	0.4995
Seed*Bleach	2	9	0.03	0.9702
Seed*Treatment	2	9	0.04	0.9606
Bleach*Treatment	1	9	3.69	0.0778
Seed*Treatment*Bleach	2	9	1.46	0.2825

Fraser fir and Nordmann fir seed that went through both stratification and stratification-redry methods did not differ in final germination percent. Turkish fir seed germination was significantly lower compared to that of Nordmann and Fraser fir (Table 5-2).

Table 5-2. Seed germination based on stratification method Least Square Means with alpha set at 0.05.

Germination Percentages (LS Means Alpha =0.05)			
Stratification	Fraser fir	Turkish Fir	Nordmann fir
	44.6667 A[Y]	9.9167 B	39.7500 A
Stratification-Redry			
	51.1667 A	13.6667 B	46.9167 A
[Y] LS Means with the same uppercase letter groupings indicate no difference according to Tukey's Studentized range test with alpha =0.05			

The seed that underwent additional time in refrigeration did have slightly higher germination percentages compared to that of the 28-day storage (Figure 5-2). For future experimentation, another analysis should be performed with seed weight being monitored and maintained to ensure accurate results.

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Chapter 6

Investigating the Impact of *Megastigmus* Larvae Heat Treatment on Nordmann and Turkish Fir Seed Germination

Exchange of tree products across the globe has resulted in biological species invasions, as this allows insects and other pests to move past natural barriers between ecosystems. For example, the European gypsy moth (*Lymantria dispar* [L.]), balsam woolly adelgid (*Adelges piceae* [Ratzeburg]), and Chestnut blight (*Chryphonectria parasitica* [Murr.] Barr.) etc. have all done extensive damage in North America after being introduced into the region. While insects can be transported through many ways, seed movement has often been ignored due to an insect's cryptic nature, and our inability to easily assess the seed due to the lack of exterior seed damage (Fabre et al., 2004; Ruth & Hedlin, 1974). When looking to import exotic Christmas tree species for use in the United States, it is crucial to assess any potential issues commonly associated with the species. Seed is regularly imported from successful cone crops overseas. This situation creates the potential for seed to become infested with pests or disease, causing USDA to condemn the shipment (Bates, Personal Communication, 2016). One pest of concern is *Megastigmus* sp.

There are roughly 126 species of *Megastigmus* with only 10% located in the North American region. With such little species variation in the western hemisphere, the USDA strictly regulates the importation of infested seed (Galindo-Gonzalez, 2012). Insects from the genus *Megastigmus* can colonize on seed of several types of *Abies* species. *Megastigmus rafni and Megastigmus pinus* are known to affect Nordmann fir. Between May and July, adult female wasps from *M. rafni* and *M. pinus* will scout out suitable trees and developing cones to deposit their eggs (Luik et al., 1999). The insects develop inside the seed, destroying seed viability and growth potential (Fig 6-1). Douglas-fir seed is often affected by *Megastigmus spermotrophus* Wachtl,

which is common throughout North America, Britain, New Zealand and many countries of Western Europe (Ruth & Hedlin, 1974; Sweeney et al., 1991). Losses due to the insect can exceed 20% in North America, while it can reach up to 100% in Europe, since the insect is well established (Sweeney et al., 1991). To examine if seed contains insect larvae, they either need to be cut or x-rayed.

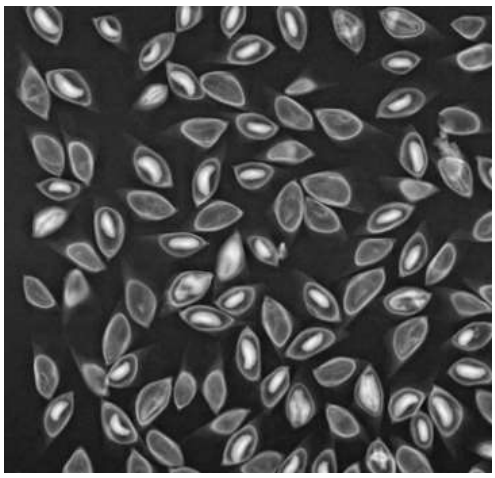

Figure 6-1. X-ray image of *Megastigmus* Larvae infestation, taken by D. Kolotelo.

Fumigants such as hydrocyanic acid and carbon disulfide have been used to kill insect larvae inside seed (Ruth & Hedlin, 1974; Sweeney et al., 1991). However, while these chemicals get rid of the insects, they greatly impact seed germination. Thus, this method is not considered practical. Consequently, infested seed were exposed to a range of temperatures to determine which temperature range killed the insects without affecting overall seed germination (Ruth & Hedlin, 1974). Results indicated high temperatures were more effective in killing the insects compared to low and high/low temperature combinations. The conclusion of the experiment indicated that in order for the insects to be killed, while maintaining germination potential, Douglas-fir seed should be treated at 45˚C for a period of 40 hours with seed > 1-year-old. Seed less than a year in age should only go through treatment for 35 hours (Ruth & Hedlin, 1974). Additionally, the Incubation

Drying Separation (IDS) method was developed to test if seed infested with *Megastigmus* larvae could be easily separated from non-infested seed. The IDS method is based on the idea that healthy seed lose moisture content at a much slower rate compared to dead seed. After soaking seed in water for 24 hours, seed was dried for 0.5, 1 and 2 hours at 25˚C. Results indicated roughly 97% of infested seed floated despite different drying lengths (Sweeney et al., 1991).

The goal of this experiment was to examine the effect of *Megastigmus* larvae heat treatment on total germination of Nordmann and Turkish fir seed after 30 days.

Materials and Methods

Turkish fir seed collected in 2015 from Marion New York (43.1432° N, 77.1891° W), and 2016 Nordmann fir seed collected in Georgian provenance of Bakhmaro were used for this experiment. Testing was conducted in the spring of 2017.

Four replicates of each seed type were used for both the control (no heat) and heat treatment variable. For the heat treatment, fifteen grams of Nordmann and Turkish fir seed were measured out for each replication, and placed in 2 oz. metal tin containers (Juvitus). They were placed in a forced air-drying oven (Precision™) at 45˚C (113˚F) for 33 hours. The seed was removed and cooled in a mini desiccator cabinet (Bel-Art™ SP Scienceware™ Secador™) with indicating mesh desiccant (Drierite) for 1 hour. Seeds were then removed from the desiccator and soaked in 35 mL of water in polystyrene petri dishes for 24 hours. Additionally, four replicates of fifteen grams of Nordmann and Turkish fir control seed were measured out and placed in petri dishes containing 35 mL of water for 24 hours.

After 24 hours, seed was drained in a strainer with double fine mesh (Winco) and surfaced dried using paper towels. Seed was then prepared for stratification. Seed were evenly distributed in a single layer in unsealed 5" x 7" plastic bags containing a moist paper towel. Versa-Pak

germination paper (Seedburo) soaked in 50 mL water was positioned at the bottom of plastic seed containers (Seedburo). The bagged seed were placed on top of the Versa-Pak, placed in a refrigerator at 2˚C on 21 March 2017 for 28 days.

On 18 April 2017, following stratification, the containers were removed from refrigeration and prepared for germination. One hundred seeds of each replicate were chosen for the working sample using the guidelines of AOSA rules of seed testing (AOSA Rules for Seed Testing, 2014). Approximately ¼ inch of Metro Mix (Sun Gro), was placed on the bottom of each plastic container, saturated using 100 mL of water, and compacted for seed placement. Seed was placed in a 10 x10 arrangement, per container. Containers were then relocated to a germination growth chamber located at Penn State University Park. The growth chamber was set to 6 hr. light at 30˚C and 18 hr. dark at 20˚C. Temperatures were monitored using a HOBO datalogger. Soil moisture conditions were checked regularly, and seed treatments were provided moisture when necessary. Germination status of the seed was measured after 10, 20 and 30 days, with germinated seed being removed after being counted. Germination was to be considered for any length radicle emergence per seed.

Data were recorded and analyzed after the 30-day measurement period. Germination data was analyzed using an ANOVA Mixed Model (Type III) procedure in SAS (version 9.4; SAS Institute, Cary, NC). “Reps” were included in the random statement of the ANOVA procedure, to account for individual differences among each seed sample. Residual plots were created to assure normal distribution in data, and that data met ANOVA assumptions. A generalized linear mixed model procedure (PROC GLIMMIX) used a LSmeans statement, adjusted to Tukey’s Studentized range test was performed and analyzed to compare mean differences.

Results & Discussion

There were significant differences in germination between Nordmann and Turkish fir seed. In contrast, no significant differences between seed treatment were observed. Nordmann fir control seed germinated quickly at 62% by day 10. Seed exposed to heat for 33 hours, averaged a 36% germination. Turkish fir seed without treatment produced an average of 8.7% germination at day 10, whereas heated seed germinated an average of 2.3%. After 20 days, Nordmann fir control seed germination increased to 66%, while treated seed averaged 42.3% germination. Turkish fir control seed increased to 14% germination, while the seed exposed to heat averaged 5.7% after 20 days (Figure 6-2). Turkish fir control seed averaged around 16% germination after 30 days. Turkish fir that was exposed to heat for 33 hours, germinated 7% by day 30. Nordmann fir not exposed to heat averaged 66% germination by day 30. Nordmann fir seed that underwent 33 hours of heat germinated at approximately 43% (Figure 6-2). After 30 days, there were differences between Nordmann and Turkish fir seed, but not with treatment (Figure 6-3).

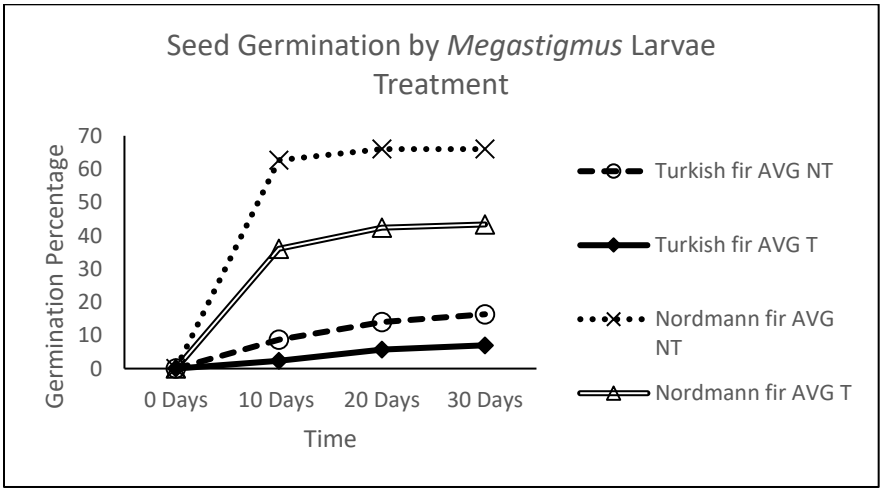

Figure 6-2. Seed germination outcome of the *Megastigmus* Larvae Treatment/No treatment for Turkish and Nordmann fir over the course of 30 days. "NT" is the control, meaning no heat treatment was applied. "T" seed underwent the treatment.

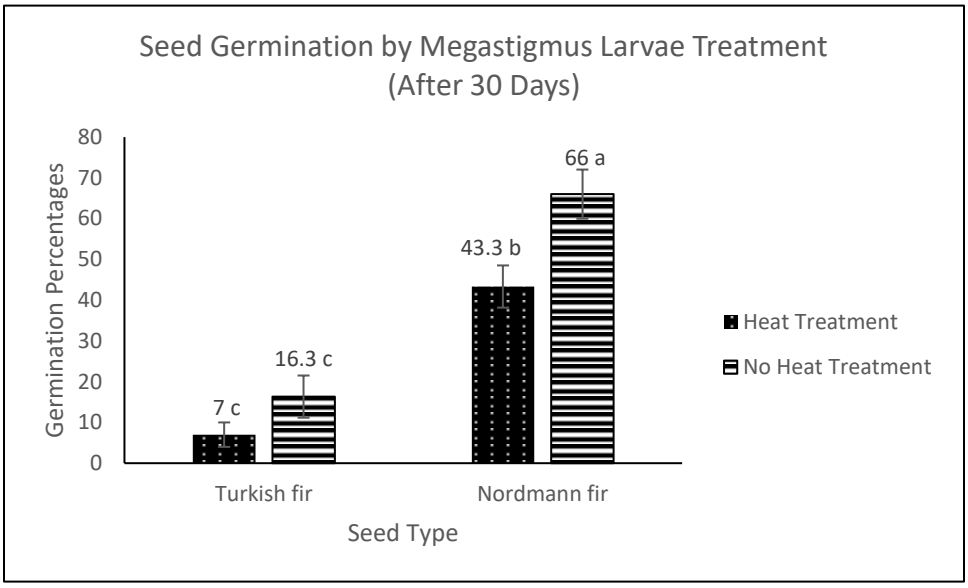

Figure 6-3. Seed germination after 30 days of both Nordmann and Turkish fir that underwent either the *Megastigmus* Larvae heat treatment, or no treatment (control). Tukey's Studentized Range data is also included.

Both seed and treatment were differences. The interaction between seed and treatment was also significant. These results indicate that there are significant differences in seed germination based on seed type, treatment and their interaction (Table 6-1).

Table 6-1. A mixed model (Type III) ANOVA Test was performed on the interactions of Seed, and *Megastigmus* Treatment/No Treatment and their interactions.

Effect	Num DF	Den DF	F Value	Pr > F
Seed	1	8	73.3	<.0001
Treatment	1	8	10.2	<.0129
Seed*treatment	1	8	1.8	0.2210

Nordmann fir seed exposed to heat had a germination mean value of 43.3, while Nordmann fir control seed had a higher germination mean value of 66.0. Turkish fir seed exposed to heat had a germination mean value of 7.0, while the control seed had a germination mean of 16.3 (Table 6-2).

Table 6-2. Seed germination based on LS Means data, and adjusted to Tukey's Studentized Range test after 30 days.

Germination Percentages (LS Means Alpha =0.05)		
Seed	Nordmann Fir	Turkish Fir
Treatment	43.3 B[Y]	7.0 C
No Treatment	66.0 A	16.3 C

[Y] LS Means with the same uppercase letter groupings indicate no difference according to Tukey's Studentized range test with alpha =0.05

These results suggest that Nordmann and Turkish fir seed do not have a significant sensitivity to heat treatment. Likewise, Douglas-fir germination had no significant response to the *Megastigmus* heat treatment (Ruth & Hedlin, 1974). By using this heat treatment on imported seed of Nordmann and Turkish fir, any larvae living inside the seed can be destroyed successfully, without effecting overall germination. Industry leaders should work with United States Department of Agriculture, APHIS personnel and create a protocol that allows for easier transport of Nordmann and Turkish fir seed into US territories.

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Chapter 7

Conclusions

This research focused on documenting seed storage and germination of Nordmann and Turkish fir. As expected, seed germination quickly diminishes for Nordmann fir seed the longer it stays in storage. Seed collected in 2011, and 2013 did not germinate at all by 2017, while seed from 2015 germinated just under 50%. Based on this information, we can expect a significant decrease in the 2015 seed germination the longer they remain in storage. Poor seed viability is a concern for industry leaders, since seed crop quality if variable by year and importing seed is already challenging. After adjusting moisture content percent prior to stratification, germination was insignificant comparing each treatment level. However, seed weight changed during prechilling thus altering end results. When storing seed long term, it is important to look for what storage media maintains consistent seed MC. After comparing changes in MC over a 12-week period using refrigerator, freezer and zeolite storage, freezer and zeolite were the most effective. Zeolite beads are a promising technology, ideal for developing countries if access to refrigeration is limited. Previous research suggests that the traditional stratification method is often ineffective for dormancy breakage and maximizing germination potential in true fir seed. The traditional stratification method with the stratification-redry procedure, had insignificant results. Again, seed lost MC while in prechilling. Finally, after testing a *Megastigmus* larvae heat treatment on both Nordmann and Turkish fir, there were insignificant results meaning that the heat treatment did not effected total germination in both seed.

Results from these experiments can greatly benefit those working in the Christmas tree industry as they work towards introducing Nordmann and Turkish fir into the United States Christmas tree market. Finding a way to keep seed from losing weight during stratification

protocols will likely change the germination total of seed with different MC% prior to prechilling, as well as seed undergoing different stratification methods. Additionally, researching dormancy breakage is necessary, since multiple factors effect seed dormancy, particularly in *Abies* species. Likewise, performing germination tests after altering seed storage conditions should also be considered. Further expansion of research topics include identifying Nordmann and Turkish fir trees from overseas provenances that resemble, and will successfully perform in different climates locations in the US.