

STRATEGIES FOR SUCCESSFUL GERMINATION OF
ROCKY MOUNTAIN JUNIPER (*JUNIPERUS*
SCOPULORUM SARG.) IN AN OKLAHOMA
NURSERY

By

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CHAPTER I

INTRODUCTION

Rocky Mountain juniper (*Juniperus scopulorum* Sarg.), which is very similar in appearance to eastern redcedar (*Juniperus virginiana* L.), is one of more than 13 juniper species native to North America (Little 1979; Fowells 1965; Zanoni and Adams 1975). *Juniperus scopulorum* is a valuable and hardy species useful for windbreaks and shelterbelts and also provides benefits for wildlife food, shelter and nesting. The bark and berries of this species have been used for medicinal purposes (Kindscher 1992).

Previously, *J. virginiana* was planted in windbreaks across the Great Plains. However, the invasive nature of this juniper in Oklahoma and the Great Plains prompted Oklahoma Secretary of Agriculture, Dennis Howard, and Oklahoma Secretary of Environment, Brian Griffin, to initiate the Redcedar Task Force. This group determined that the invasion of *J. virginiana* is having devastating effects within Oklahoma by decreasing forage, increasing water use and decreasing land available for livestock (Drake et al. 2002). The findings from this group led to a verbal mandate restricting the Oklahoma Forest Regeneration Center (state run nursery) from growing *J. virginiana* for sale within the state (personal communication, George L. Geissler, Oklahoma Forestry Services, 2009). This mandate and the invasive nature of *J. virginiana* led to the search for alternative evergreen tree species, such as *J. scopulorum*, which are essential to establish new shelterbelts and/or windbreaks to reduce soil erosion, conserve energy,

serve as visual barriers, provide wildlife habitat, and to provide aesthetic value to the landscape. *Juniperus scopulorum* should not become an invasive juniper species in Oklahoma like *J. virginiana*. While *J. virginiana* is adapted to and reproduces quite profusely given the short, cold Oklahoma winters *J. scopulorum* seeds need a much longer and colder stratification period to germinate (Allen, et al. 2008). Also, *J. scopulorum* produces less seed compared to the much larger seed crops of *J. virginiana* (Allen, et al. 2008). Optimum age for seed production of *J. scopulorum* is not usually until 20 years of age or more under favorable conditions, whereas *J. virginiana* produces large amounts of seed by about 10 years of age (Scianna 2000; Noble 1990).

Germination success of *J. scopulorum* in Oklahoma and other Great Plains nurseries has been very sporadic (Scianna 2000; Noble 1990). Successful propagation of *J. scopulorum* depends on more effective stratification techniques, which will in turn provide more consistent, uniform germination and seedling establishment in nurseries. *Juniperus scopulorum* seed are difficult to germinate, in large part because of seed coat impermeability and multiple dormancies (Djavanshir and Fechner 1976). The seed coat and prophylactic sheath surrounding the embryo and megagametophyte impede water absorption. Following water imbibition, seeds then require an after-ripening warm period followed by cold stratification to break dormancy of the hypocotyls (Djavanshir and Fechner 1976). Because of the multiple dormancies with this species, finding an effective and consistent stratification treatment to achieve uniform germination in a nursery seedbed is difficult. In fact, successful seed germination of *J. scopulorum* is one of the major problems facing Great Plains tree seedling nurseries (Rietveld 1989).

The recommended stratification treatment for *J. scopulorum* is 45-90 days of warm 20°C night/ 30°C day (68°F night/ 86°F day), moist conditions followed by 30-120 days of cool 5°C (41°F), moist conditions (Johnsen and Alexander 1974). At present, the Oklahoma Forest Regeneration Center (OFRC) nursery primarily uses the stratification method of 94 days or longer of warm, moist stratification followed by fall planting (Porterfield 1995). The OFRC also has had some success with placing *J. scopulorum* seed in cold, moist stratification for about 120 days and then sowing in the summer (Porterfield 1995). However, the germination percentages varied among years. This might be due to interannual weather variation, planting of different seed sources, or to differences in seed viability between years.

There has been considerable interest in finding more effective stratification treatments. For instance, Barbour (2009) and Benson (1973) used various scarification and stratification treatments including liquid nitrogen soaks, hydrogen peroxide soaks, citric acid soaks, aerated water soaks, and varying lengths of time for cold and warm stratification. The process of burying the seed for a particular length of time (13 weeks or longer depending on the geographical location) in the winter is also an example of pregermination treatments that have been used for this tree species. In addition to scarification or stratification treatments, other factors may affect germination success in the nursery. Planting depth is related to temperature fluctuations experienced by seeds and there is potential for solar radiation to stimulate or inhibit germination with shallow planting. Timing of planting also may be important especially since seeds with a double dormancy, like *J. scopulorum*, are sensitive to temperature regime and can enter

secondary dormancy if they get too warm following cold stratification (Hartmann, et al. 2002).

Juniperus scopulorum is native to the extreme northwest corner of the Oklahoma panhandle, but not the rest of the state (Little 1971). Consequently there are no local seed sources near the OFRC. Therefore, screening several different seed sources is important to find a source with reliable germination and establishment success in Oklahoma. Typically, the OFRC in Goldsby, OK, where this study took place, uses seed sources from South Dakota and Colorado. A seed source from Utah was used once as well. In addition to these seed sources, other commercial sources of *J. scopulorum* seed are available (personal communication, Porterfield 2006).

Objectives

The objectives of this project were to 1) determine suitable protocols for successful germination and establishment of *J. scopulorum* at the Oklahoma state nursery (OFRC) by evaluating the effects of a series of stratification treatments, two planting depths, and two planting dates and 2) test the best stratification treatments on a number of different seed sources from across the *J. scopulorum* natural range.

CHAPTER II

REVIEW OF LITERATURE

Literature Review

Species biology

Juniperus scopulorum is in the Division Coniferophyta (conifers), Class Pinopsida, Family *Cupressaceae* (Cypress), and Genus *Juniperus L.* (juniper). *Juniperus scopulorum* is the most widely distributed of the eleven junipers native to the United States (Little 1971). The geographic range of *J. scopulorum* is very scattered, but the heaviest concentrations occur from central British Columbia and southern Alberta, Canada through northwestern Montana to southeastern Idaho and south into Colorado and New Mexico, primarily following the Rocky Mountain range (Zanoni and Adams 1975). This species grows near sea level at its northern extremes to more than 2,440 m (8,000 ft) above sea level in the southern portion of its range. Across this scattered distribution, *J. scopulorum* often is found growing on exposed and severe sites, such as in alkaline soils on ridges and rocky slopes, but is not usually a dominant forest component in these situations (Oosting 1956; Society of American Foresters 1980).

Many environmental and physiological factors influence where *J. scopulorum* grows. These include in elevation, temperature, precipitation, soil type, soil moisture, and soil nutrient concentration (Noble 1990). *Juniperus scopulorum* occurs in pure

stands only in northern portions of its range at the middle and lower elevations (Herman 1958; Leonard, et al. 1987; Wright, et al. 1979). Throughout its range this species is found in mixed stands with Ponderosa pine (*Pinus ponderosa* Douglas ex C. Lawson) on south and west facing exposures and with Douglas-fir (*Pseudotsuga menziesii* var. *glauca* Beissn.) on north and east facing exposures. *Juniperus scopulorum* is quite abundant where it co-occurs with Douglas-fir. At higher elevations, throughout the Rocky Mountains, *J. scopulorum* occurs with Engelmann spruce (*Picea engelmannii* Parry ex Engelm.), lodgepole pine (*Pinus contorta* Douglas) and limber pine (*Pinus flexilis* E. James) (Noble 1990). At even higher elevations, around 2900 to 3700 m (9500 to 12000 ft) it can occasionally be found growing with bristlecone pine (*Pinus aristata* Engelm.). At lower elevations, around 900 to 2500 m (2952 to 8202 ft), primarily in its central and southern range, *J. scopulorum* grows with white fir (*Abies concolor* Lindley ex Hildebrand), aspen (*Populus tremuloides* Michx.) and sometimes narrowleaf cottonwood (*Populus angustifolia* James) (Society of American Foresters 1980; Fowells 1965). *Juniperus scopulorum* is generally found throughout the western portions of the Great Plains and western states except California and Kansas (Figure 1). It only occurs naturally in the western edge of Cimarron County in the Oklahoma Panhandle (Noble 1990).

The size of *J. scopulorum* is variable ranging from a shrub to a small tree. Height growth is very slow, usually 3-30 cm (6-12 in) or less per year in dry areas just east of the Continental Divide (Scianna 2000). However, *J. scopulorum* growth varies considerably across locations and sites. In Canada, most *J. scopulorum* reaches 30 cm (12 in) in basal diameter and 3 to 4 m (10 to 12 ft) in height; however, some trees reach 9 m (30 ft) in

height. Some trees of this species have been measured on the north rim of the Grand Canyon with heights of 5 to 6 m (15 to 20 ft) and 30 to 46 cm (12 to 18 in) in basal diameter. The *J. scopulorum* trees that grow at lower elevations in the Southwest are reported to achieve heights of 6 to 15 m (20 to 50 ft) and basal diameters of 46 cm (18 in) (Herman 1958; Fowells 1965).

This species is usually very long lived (Figure 2). There is a specimen in western South Dakota that is approximately 750 years old and another in Logan County, Utah estimated to be over 3,000 years old (Chase 1970; Fowells 1965).

Juniperus scopulorum grows from Hardiness Zone 3 sites -40°C to -34°C (-40°F to -30°F) to Zone 7 sites -18°C to -12°C (0°F to 10°F) (Scianna 2000) and can grow under a wide range of soil and climate conditions. It can grow on numerous soil types, such as soils derived from basalt, limestone and shale including poorly developed, stony, shallow and erodible soils with low moisture-holding capacity. This species performs best, however, on fertile, well-drained sites with a pH of approximately 6.5 to 7.0 (Scianna 2000). *Juniperus scopulorum* favors average minimum air temperatures above -23 to -21°C (-10 to -5°F), but does well in Montana and Wyoming on Zone 4 sites with minimum air temperatures of -34 to -29°C (-30 to -20°F). Over much of its range, *J. scopulorum* grows in areas with an annual precipitation of 38 to 46 cm (15 to 18 in). It can tolerate as little as 28 to 30 cm (11 to 12 in) and as much as 66 cm (26 in) of precipitation (Scianna 2000). This juniper is a drought-enduring species and is hardier than the closely-related species *J. virginiana*; however, *J. scopulorum* shows more productive growth and overall vigor in areas of higher precipitation. Paleobotanical (ancient vegetation) studies have indicated that the macroclimate of the *J. scopulorum*

range has changed from a mesic (a medium supply of moisture) to a more xeric (less water or drier) condition and thus this climate change has not favored *J. scopulorum* regeneration and growth (Noble 1990).

A morphological description of the species includes the following: the bark is reddish-brown, with a slightly rough and scaly covering in the sapling stage and becoming more fibrous with age (Figure 3). The bark of *J. scopulorum* seems to “shred” as the tree ages, but does not exfoliate or peel away as much as the bark on *J. virginiana* (Scianna 2000). In more mature trees, the leaves are overlapping scales and in the juvenile stage, the leaves have an awl-like shape. The flower (strobilus) of *J. scopulorum* is “inconspicuous”; meaning that the outer flower parts are usually less than 0.6 cm (0.25 in) in height. This makes viewing the flowers difficult unless using a magnifying glass (www.skyways.org/orgs/fordco/wildflowers/ci/ci.html).

Seed production

Individual trees of *J. scopulorum* have either male cones or female cones (dioecious). The staminate (male cones) are usually located at the tips of the branchlets with a yellowish-brown coloring. The ovulate (female cones) are about 0.9 cm (0.3 in) in diameter and are usually round with a greenish-yellow color before pollination occurs. After pollination, the female cone develops a round, berry shape with a bluish-purple coloring on the coat (Figure 4). The seeds mature two years after pollination, usually around September to December (Scianna 2000). *Juniperus scopulorum* may begin bearing seeds between 10 and 20 years of age, but optimum production occurs between 50 and 200 years (Noble 1990). Few seeds are found in the cones, usually one or two,

occasionally three. The seeds are very small; about 2 to 4 mm (0.08 to 0.16 in) in diameter with a light, brownish color and ridged near the seed base (Johnsen and Alexander 1974) (Figure 5). A single seed usually contains one embryo, but sometimes one seed may contain two embryos that are separated by a thin tissue wall. If there are two embryos in one seed, they can be separated by gently tearing away and eliminating the thin tissue wall (Scianna 2000). About 100 kg (220 lbs) of *J. scopulorum* cones will produce about 11 to 28 kg (25 to 62 lbs) of seed containing about 8,000 to 43,000 seeds per pound depending on seed source (Johnsen and Alexander 1974). *Juniperus scopulorum* seeds are disseminated primarily by birds. When other food sources are scarce, mammals such as deer, domesticated sheep and bighorn sheep also disseminate the seeds by eating the cones and foliage (Randles 1949).

Root System

Juniperus scopulorum has shallow, but extensive lateral root system. This kind of root growth usually occurs where the species is growing on rocky outcroppings and root penetration is difficult. The species can develop a much deeper root system if grown in deeper, more fertile soils (Johnsen and Alexander 1974). If the species' root system is undercut at the three-year seedling stage in nurseries, a more extensive and stronger lateral root system will develop (Scianna 2000).

Pests and Problems

Juniperus scopulorum is host to several disease organisms. Juniper blight (*Cercospora sequoiae* Ell. & Ev.) is a serious disease that infects this species. Many shelterbelts in the Great Plains have been devastated by this disease (Hepting 1971).

Juniper blight causes discoloration on the lower branches and, if left untreated, can cause serious defoliation. Once this is noted, immediate removal of infected trees should eliminate the disease from the affected windrow or planting. Another way to eliminate juniper blight is to spray the trees with Bordeaux (a mix of copper sulfate and hydrated lime used as a fungicide) mixture at 7 to 10 day interval beginning in early summer and throughout the summer season (Hodges 1962; Hodges 1975).

Juniperus scopulorum is also a host to cedar-apple rust (*Gymnosporangium juniperi-virginianae* Schwein.) which can be a serious problem to the apple industry. On *J. scopulorum* this disease causes woody galls, ‘witches’ brooms and twig excrescences or large orange-colored spheres exuding tilial columns infecting the branches of the tree (Hepting 1971; Johnson, et al. 1976) (Figure 6).

Juniper mistletoe (*Phoradendron juniperinum* Engelm. ex A. Gray) can also cause stem damage on this species (Figure 7). Phomopsis blight (*Phomopsis juniperovora* Hahn) can drastically affect *Juniperus* seedling health in the nursery setting. In the early stages, a partial blighting of the foliage occurs that can spread and kill stem tissue (Peterson and Hedges 1999). This disease is usually only seen on seedlings four years and younger (Hepting 1971; Johnson, et al. 1976). (Figure 8). Chemical control can usually prevent Phomopsis if applied at two-week intervals early in the spring when new growth is occurring. Copper hydroxide, manganese ethylene bisdithiocarbamate, propiconazole, or any other commercial fungicide will work (Punithalingam and Gibson 1973).

Juniperus scopulorum is susceptible to a variety of spider mites, insects and nematodes (Furniss and Carolin 1977; Stephens 1973). Two species of spider mites

(*Oligonychus ununguis* Jacobi) and (*Eurytetranychus admes* Prichard & Baker) (Figure 9) feed on *J. scopulorum* needles and can expand their populations to great numbers in a very short time (Smith 1978). Two species of juniper berry mites eat the developing cones of *J. scopulorum* and can drastically decrease the number of seeds in each cone. They are *Trisetacus quadrisetus juniperinus* Nalepa, and *Trisetacus quadrisetus typicus* Thomas (Smith 1978). There is also a small red false spider mite, *Pentamerismus erythreus* Ewing that can cause considerable damage in *J. scopulorum* shelterbelt plantings (Noble 1990). *Juniperus scopulorum* is also susceptible to a variety of Coleoptera (true insects) such as Lepidoptera (butterflies and moths), Diptera (flies and midges) and Psyllids (jumping plant lice). These insects can damage and eventually destroy roots, bark, twigs, foliage and cones (Noble 1990). The nematode *Pratylenchus penetrans* Cobb (Figure 10) causes large numbers of root lesions on the seedlings (Figure 11). This problem seems to be centered in nursery settings where the nematode has reached high populations (Hepting 1971). Nematicides are effective in controlling this nematode, but nematicide residues have been detected in groundwater. Hot water treatments of 45.5°C (114°F) on the affected roots for 15 to 20 minutes sometimes kills the nematode, but regulation of the water temperatures is critical (Westerdahl, et al. 2003).

Individual trees of *J. scopulorum* can die over time due to excessive erosion. Plants of this species commonly get established on exposed sites that are already eroded (Noble 1990). *Juniperus scopulorum* is also susceptible to fire damage and death because of the thin, stringy bark on both young and older trunks and because of the flammable terpenes or resins that the tree exudes from injury (Hepting 1971). This

species is a favorite “rubbing post” for many species of animals, particularly buck deer in rut. The animals rub against the trunk causing damage and exposing the inner wood to pathogens. When range conditions are poor, the animals will also browse the foliage higher in the tree, known as “high lining” which may result in a loss of vigor and growth and could ultimately cause poor flower development and seed viability (Noble 1990).

Population Genetics and Hybrids

Information on genetic variation among populations of *J. scopulorum* is scarce. Rudloff (1975) reports many phenotypic differences in growth, morphology, phenology, and resistance to heat and cold temperatures among subpopulations of *J. scopulorum*. Flake et al. (1978) believe that the overlapping populations of *J. scopulorum* and *J. virginiana* in the Missouri River Basin indicate secondary intergradation (allopatric introgression) rather than primary intergradation (allopatric divergence) is occurring. *Juniperus scopulorum* gene flow seems to be moving in an easterly direction. Hybridization data on *J. scopulorum* is complex. The Missouri River Basin population is thought to be a hybrid swarm between *J. scopulorum* and *J. virginiana* because neither parent has been found (Noble 1990). Hybridization among junipers is possible. Successful, controlled hybridization of *J. scopulorum* has been reported with both a horizontal juniper (*J. horizontalis*) and with eastern redcedar (*J. virginiana*) (Fassett 1944; Fechner 1976; Herman 1958; Van Haverbeke 1968). Natural hybridization between *J. scopulorum* and alligator juniper (*J. deppeana*) has been reported as well (Fassett 1944; Fechner 1976; Herman 1958; Van Haverbeke 1968).

There are several horticultural varieties of *J. scopulorum*. Most of these varieties derive from the hybrid (*J. scopulorum* var. *viridifolia*) which is called “Chandler Blue” and/or “Hill Silver” (Hoag 1965). There are other varieties including “Medora”, a bluish, compact type with upright branches; “Pathfinder”, a silver-blue color with a more open canopy; “Colorgreen”, a more compact, green variety and “Hillborn Globe”, which has a bluish-green foliage with a more prominent, pyramid form. All these varieties have been propagated as grafted specimens (Noble 1990).

Special Uses

Juniperus scopulorum had many uses in the past and is still useful today. Some of the earlier uses by the Native Americans were for food and decorations; for example, many tribes used the bark for incense in ritual ceremonies (Kindscher 1992). The Blackfeet Indian tribe used the berries to help stop vomiting and alleviate stomach ailments. They also boiled the juniper leaves and then mixed them with turpentine to make a mixture for treating arthritis (Kindscher 1992). Another natural remedy that the Blackfeet used was to crush the *J. scopulorum* roots and then mix in *Populus* leaves to make a liniment for backaches (McClintock 1909; Johnston 1970; Hellson 1974). Juniper berries were eaten whole or pulverized and boiled to make a tea which the Cheyenne women would drink to speed along child birth (Grinnell 1962). The Gros Ventres Indians used the juniper tea as a cure for asthma (Kroeber 1908). The bark from *J. scopulorum* was stripped and woven into baskets and cradles. The Native Americans also used the wood as firewood for heating and cooking purposes, and *J. scopulorum* is still used as firewood (Noble 1990).

The wood of *J. scopulorum* has a fine grain, with whitish sapwood and dark, red heartwood with light purple and white streaks throughout. *Juniperus scopulorum* wood has very close physical characteristics to *J. virginiana*. These similarities include color, odor, figure and strength of the wood. The wood, when cured, is resistant to decay and has been used for fence posts (Herman 1958). The smaller height patterns and rapid tapering into the crown of *J. scopulorum* does not generally provide usable lumber; however, some smaller sawn sections of *J. scopulorum* have been used as closet linings, custom-built furniture and cedar chests. The brightly colored heartwood has been carved and used for artistic novelties (Herman 1958; Randles 1949).

Juniperus scopulorum is an excellent species for use in shelterbelts, as living snow fences, in parks and native landscape settings, and on reclamation sites. (Scianna, et al. 2000). *Juniperus scopulorum* is an important food source and provides wildlife habitat and shelter for various birds and mammals, including cedar waxwings (*Bombycilla cedrorum* V.) in the fall and in early winter. Robins (*Turdus migratorius* L.), turkeys (*Meleagris gallopavo* L.) and deer (*Odocoileus virginianus* Z.) utilize this species for food and shelter (Scianna, et al. 2000).

Seed Dormancy

Dormancy in seed is a condition where the seed itself is viable (alive), but the internal structures are inactive and will not allow the radicle or cotyledons to emerge from the seed even with a favorable germination environment (Hartmann, et al. 2002). Dormancies cause seeds to avoid germinating during adverse conditions. Seed dormancy not only prevents immediate germination following seed maturity, but also often

regulates the timing, conditions and place that germination will occur (Hartmann, et al. 2002). There are many reasons why dormancy occurs in seeds including environmental conditions (temperature, light or lack thereof, scarification and stratification elements upon the seed), physiological characteristics of the seed (hard seed coat, chemical inhibitors within the seed itself), or an immature embryo (Hartmann, et al. 2002).

Two main categories of seed dormancy exist: primary dormancy and secondary dormancy. Primary dormancy relates to a condition of the seed as it is shed from the parent plant. Within primary dormancy there is either quiescent or dormant seed. The seed is said to be quiescent when it is dry, yet has the ability to germinate, but is limited by the environment, i.e. temperature or water. If the seed is dormant, it will not germinate even if the environmental conditions are suitable (Hartmann, et al. 2002).

The other category of dormancy, secondary dormancy, is a survival mechanism that prevents germination when other environmental conditions are not favorable. These unfavorable conditions might include high temperatures, prolonged light or darkness, water stress or lack of gas exchange (Hartmann, et al. 2002). For example, if a seed fails to germinate after a primary dormancy is broken because the temperature is too high or too low, or the soil moisture is too high or too low, the seed can go back into a 'secondary dormancy' and would take even longer to germinate, if it germinates. Knowing a species' secondary dormancy characteristics can help to implement successful germination treatments (Hartmann, et al. 2002).

Three types of primary dormancies occur: exogenous, endogenous and combination, or 'double' dormancy. Primary exogenous dormancy is usually due to a

factor outside the embryo such as seed coat thickness (seed coat dormancy or exogenous physical dormancy), or tissue or chemical inhibitors (exogenous chemical dormancy). The tissues (either endosperm or megagametophyte depending on the species) that surround the embryo might prevent water uptake or gas exchange to the embryo. These same tissues within a seed might also leach chemical inhibitors into the embryo or there may be inhibitors within the embryo sac that inhibit radicle protrusion from the embryo itself (Hartmann, et al. 2002). Chemicals that accumulate during fruit production and in seed-covering tissues can remain with the seed even after harvest and thus can act as germination inhibitors to the seed body itself. Sometimes prolonged leaching with water or removal of the seed coat and/or covering can help improve germination of these types of seed. Chemical inhibitors are primarily found in the endosperm and are activated at higher temperatures, so exposing the seeds to lower temperatures of 15°C (59°F) or below helps eliminate the influence of inhibitors and initiates germination (Hartmann, et al. 2002)

Primary endogenous dormancies are dormancies that are due to factors within the embryo. Primary endogenous dormancies include rudimentary embryos, linear embryos, or undifferentiated embryos (Hartmann, et al. 2002). A rudimentary embryo occurs when the seed has a “proembryo” (very tiny, not fully developed) embedded in a large amount of endosperm or megagametophyte. Chemical treatments of potassium nitrate or gibberellic acid will aid in germination of rudimentary embryo seeds (Hartmann, et al. 2002). An example of a linear embryo is one that is torpedo-shaped and only fills about half of the seed cavity. Exposure of these types of seeds to warmer temperatures greater than 20°C (68°F) will aid in germination. Application of gibberellic acid can also help in

initiating germination. Undifferentiated embryos should not really be considered dormant because they simply lack sufficient amounts of endosperm or megagametophyte to germinate and grow. These types of seed are usually germinated via micropropagation techniques (Hartmann, et al. 2002).

Endogenous physiological dormancies also fit into the primary dormancy category. These can be separated into three types based on the depth of dormancy. These are nondeep, intermediate and deep. Nondeep physiological dormancy is the most common form of dormancy found in seeds. The seeds found in this form of dormancy need light or dark to germinate (photodormancy), periods of pre-chilling stratification, or dry storage periods to help break dormancy (after-ripening) (Hartmann, et al. 2002). Seeds with intermediate physiological dormancy usually require one to three months of chilling while in an imbibed or aerated state. This is the most common type of dormancy for tree, shrub and some herbaceous plant seeds. Excised embryos from these seeds usually germinate with little difficulty. Seeds with deep physiological dormancy require a much longer period of moist, cold stratification (approximately two to five months). If embryos are excised from these seed, they usually will not germinate or will develop into abnormal seedlings (Hartmann, et al. 2002).

Primary combinational or double dormancies combine two or more dormancies such as morphophysiological dormancy (underdeveloped embryo) and physiological dormancy (seed coat dormancy). Double dormancy is used to describe seeds that usually take two years to germinate. The most common type of double dormancy is known as morphological dormancy which requires a lengthy warm stratification period greater than

15°C (59°F) followed by lengthy cold temperatures of 1-10°C (34-50°F) to allow the embryo to start developing and radicle emergence to occur (Hartmann, et al. 2002).

Double dormancies also include ‘epicotyl dormancy’ and ‘epicotyl and radicle dormancy’. In the case of epicotyl or (shoot) dormancy, the radicle or (root) will germinate, but the epicotyl remains dormant. To overcome this type of dormancy, warm stratification followed by a cold stratification is needed (Hartmann, et al. 2002). An epicotyl and radicle dormancy is one in which the radicle will germinate after the first cold treatment, but the epicotyl remains dormant until the second cold stratification has been completed (Hartmann, et al. 2002). An adequate treatment when one is dealing with an epicotyl and radicle dormancy is to initiate an initial cold stratification followed by warm, and then a second cold stratification regime (Gunn 2005). Other examples of combination or double dormancies include species with seeds that need scarification as well as stratification techniques to break the dormancy (personal communication, Janet Cole, Professor of Horticulture, Department of Horticulture and Landscape Architecture, OSU 2008).

Breaking Dormancies

Numerous methods can be used to break the different types of seed dormancies. Direct seeding is one method in which natural environmental conditions are utilized to fulfill dormancy requirements (Hartmann, et al. 2002). There are four important factors for successful germination when direct seeding. Start with good seed bed preparation; this entails making sure the nursery area has been tilled thoroughly, any organic or soil amendments have been added, and that the soil itself has been fumigated to eliminate soil

pathogens. Second, make sure to use good quality seed that have been tested for viability and will produce healthy seedlings once germination has started. Third, make sure to plant during the correct window of time which is determined by the temperature requirements of the seed. If the species' seed requires warm soil temperatures to germinate, planting the seeds too early in the season might cause "chilling" injury, uneven germination, or disease and possibly growth abnormalities. Finally, it may be helpful to use seed treatments to make sowing seeds easier and to help facilitate breaking of dormancy. For example, it is sometimes helpful to plant seeds that have been treated with a fungicide to protect against disease, and priming the seed can help initiate rapid and uniform germination (Hartmann, et al. 2002).

Scarification and stratification are other methods that can help to break dormancies of many species' seed. Scarification is a method by which the seed coat or covering is broken, scratched or altered in some way to make the seed coat more permeable to water and gases. Stratification is a method where periods of cold and warm temperatures and moist medium are used to ease or lessen the dormancy conditions in the embryonic tissue and help to initiate germination (Hartmann, et al. 2002).

Several scarification and stratification techniques can be utilized. Mechanical scarification requires the seed coat to be scratched, broken or altered in some way, either by rubbing with sandpaper, cutting with a file, using a hammer or large-scale machine scarifiers (Hartmann, et al. 2002). Acid scarification involves placing dry seeds in a container and then covering them with concentrated sulfuric acid with minimal agitation. Seeds of some species may need a ten minute or less soak while seeds of other species may require up to 6 hours or more in concentrated sulfuric acid. Following scarification,

the seeds are rinsed in water for about ten minutes and immediately planted, or are allowed to dry and then planted, or can be stored for later planting (Hartmann, et al. 2002). Hot water scarification is another scarification technique in which the seed lot is dropped into a container of hot water 77 to 100°C (170 to 212°F) for a very short time (usually five to ten seconds, depending on the seed lot). The seeds are then placed into a container of warm water and allowed to soak for 12 to 24 hours. Most seed lots should be planted immediately after the hot water treatment (Hartmann, et al. 2002).

Various stratification regimes may be used to break seed dormancies. One of the most common stratification techniques is to use a period of moist-warm or moist-cool, whichever satisfies the type of dormancy in that seed. Seeds that require a cold treatment can be planted directly outdoors in a prepared seed bed, cold frame or nursery row at the appropriate time of year to provide the needed cold conditions. The seed lot may also be placed in a moistened medium and refrigerated with temperatures ranging from 1 to 10°C (33 to 50°F) for a specific period of time to meet the cold requirements to break dormancy. If refrigeration is not available, the seeds can be placed in a controlled outdoor setting. These can be pits several feet deep or raised beds that are covered with wooden frames. The same seed preparations are used, but natural rainfall and outside winter temperatures provide the moist, cool treatment (Hartmann, et al. 2002).

Propagation of *Juniperus scopulorum*

Fruit Collection and Seed Storage

Maturation of *J. scopulorum* seed takes approximately two years. One year old as well as two year old cones will often be found on the same tree (Scianna 2001). The

mature two year old cones will be found on the older (two year old) foliage and are dark blue to nearly black in color with a whitish waxy substance on the outside of the cone. Immature cones will be greenish or light blue in color and on the current year's foliage. It is helpful to rub the outside of the cone being collected to look for the ripeness color indication (Moench 2000; Scianna 2000).

Within its natural range, *J. scopulorum* is usually a good to prolific seed producer. It is beneficial to harvest the cones (fruit) from the middle of the branches or closer to the main trunk of the tree. These cones will be the two year old cones with second-year seed. Avoid collecting from the tips of the branches; these cones will probably be immature with only first year seed. The best time for fruit collection is from late November into December (Scianna 2000). A favorable, heavy crop of seed usually occurs every two to five years, but some seed is produced annually (Scianna 2000). Avoid collecting seed from trees that have evidence of insect damage. Indicators of this will be small exit holes in the bottom of second-year cones. This type of damage is produced by the juniper seed chalcid (*Eurytoma juniperina* Macrorileya). Little information on this insect exists and no known control protocols are available (Scianna 2000).

Optimum fruit collection usually occurs in late October through early December, but timing varies annually. Some cones can hold mature seed into the early spring. Placing some animal deterrent in or around the trees and frequent observation of the trees helps to prevent fruit loss to birds or other animals. For every 2 to 4 kg (4 to 9 lb) of fruit collected, 0.5 kg (1 lb) of clean seeds can usually be extracted (Noble 1990). Noble reports that on 20 year old trees that are at least 2.4 m (8 ft) in height, 11.4 kg (25 lb) of fruit can be harvested with approximately 1.9 kg (4.2 lb) of bulk seeds extracted. To

estimate the number of viable seeds per pound, the seeds can be tested with tetrazolium (triphenyl tetrazolium chloride, TTC, a chemical used to indicate cellular respiration in seeds) (Scianna, et al. 2000). Freshly harvested cones should be processed right away, but can be stored for several months under optimum environmental conditions. It is best to store *J. scopulorum* cones in paper sacks in a cooler at 1°C (34°F) and high humidity (80 to 90%). Surface drying of the cones before storing is helpful in preventing mold. Bags should not be stacked to insure proper air circulation to the cones while in storage (Scianna 2000). *Juniperus scopulorum* cones have a rough skin and thick, resinous pulp that makes extracting seed difficult. The cones are usually placed into a macerator to help break them apart. Once the seeds have been extracted from the cones and cleaned (washed and dried to a 10 to 12% moisture content) the seeds can be placed in plastic bags and stored in sealed containers at temperatures of -6°C (20°F) to 4°C (40°F) (Scianna 2000).

Vegetative Propagation

Most *Juniperus* species can be propagated via asexual means. It is best to take cuttings from a younger, healthier tree than an older one. The asexual propagation success becomes even greater when cuttings are taken from container grown stock; the rooting system tends to be healthier overall (Dirr and Heuser 2006). The most successful asexual propagation results from cuttings taken from juvenile phase tissue, usually under five years of age (Scianna, et al. 2000). It is a rule of thumb that the cuttings should be taken after several hard freezes. The majority of growers tend to take the cutting between December and March. Growers “stick” (inserting the freshly cut stems down into the

rooting medium) cuttings anywhere from July to April, but the late fall or winter rooting success is much higher than the summer rooting success (Dirr and Heuser 2006).

Cultivars of *J. scopulorum* are rather difficult to root via cuttings, but can be done with approximately 25% success. *Juniperus scopulorum* are usually propagated more successfully via grafting (Dirr and Heuser 2006). The optimum time to collect cuttings for *J. scopulorum* propagation is late November or early December when outside temperatures are around 1.6 to 7.2°C (35 to 45°F). The cutting should be at least 7.5 cm (3 in) long with a woody base; it is preferable to make a ‘heel cut’ and remove foliage from the bottom 2.5 cm (1 in) of the cutting. Following collection, the cutting should be dipped in a fungicidal solution as well as various rooting hormones depending on the cultivar. Some examples of beneficial rooting hormones in the rooting process are Naphthalene acetic acid (NAA) solution, Indole-3-butyric acid (IBA)-talc, or IBA-solution (Dirr and Heuser 2006). After dipping, the cuttings should be placed in flats with 9:1 perlite: peat mixture. After the cuttings have been stuck, the flats should be placed on heated mats with bottom heat at about 15 to 18°C (60 to 65°F) for the first six weeks and then increased to 21 to 24°C (70 to 75°F). Bottom heat is required for *J. scopulorum* to root. Cuttings can usually be transplanted five to six months after sticking (Dirr and Heuser 2006).

Vegetative propagation of ornamental selections of *J. scopulorum* can be done via grafting, preferably using mature tissue (Scianna, et al. 2000). The grafting process is most successful from December to February. With *J. scopulorum*, it is best to use potted stock plants. They should be brought inside the greenhouse to make the grafts. The understocks should be at least 0.5 to 0.8 cm (0.2 to 0.3 in) in diameter where the cut is to

be made. Needles should be stripped from the stem at the soil surface up to at least 7.5 to 10 cm (3 to 4 in) of where the cut is to be made, and the tops of the understock should be evenly trimmed. Aggressive root growth in the 'potted' understock is indicative of the optimum time to start the grafting process (Dirr and Heuser 2006). The foliage of the understock should be sprayed with any commercial fungicidal before grafting to prevent root rot or overpopulation of fungus gnats. The scionwood should be collected from healthy stock plants and should be at least 13 to 15 cm (5 to 6 in) in length with the bottom 5 cm (2 in) stripped of needles. The fresh, soft growth at the top of the scionwood should be removed. The scionwood should be dipped in the same fungicide that was applied to the understock, after which a side graft may be made onto the understock, wrapped and allowed approximately 2 weeks to heal. It usually takes 5 to 6 weeks for all grafts to heal if they are done successfully (Dirr and Heuser 2006).

Dormancy in *Juniperus*

Germination is often delayed in junipers primarily because seed of most *Juniperus* species are dormant (Young and Young 1992). It is believed that the genus *Juniperus* has a low germination rate due to immature embryos and seedcoat impermeability, thus *Juniperus* has a double dormancy (Scianna 2000). In *Juniperus*, seed coat dormancy is a primary dormancy type where seeds fail to germinate because the seed coat is hard and impermeable to water. In addition to seed coat impermeability, *J. scopulorum* seed are difficult to germinate due to an immature embryo issue, that is an endogenous, morphological dormancy and possibly the very hard seed coat or prophylactic sheath just inside the seed coat makes radicle emergence very difficult (Barbour 1998 and Scianna 2000).

Scarification and Stratification Techniques for *Juniperus*

Juniperus scopulorum is nearly impossible to germinate without scarification and stratification. Slow or no germination is common (Noble 1990). When attempting to grow *J. scopulorum* seedlings in a nursery, germination is erratic and varies widely with the seed source, the seed lot, and standard nursery operations. These difficulties make achieving consistent *J. scopulorum* germination rates difficult (Scianna 2001).

After proper collection and storage, seeds should be floated to separate unfilled seeds from viable seeds. Various stratification treatments have been used by different nurseries. For instance, 120 days (16 weeks or 4 months) of warm, moist stratification in peat moss mix followed by 150 days (20 weeks or 5 months) of cold moist stratification is used by the Bridger Plant Materials Center in Bridger, Montana (Scianna 2001). According to Johnsen and Alexander (1974), a warm, moist stratification treatment at temperatures of 20 to 30°C (68 to 86°F) for 45 to 90 days (6 to 12 weeks or 1.5 to 3 months) followed by a cold, moist chilling treatment of 1 to 3°C (34 to 37°F) for an unspecified number of days produced germination rates approximately 40% to 50%.

The Oklahoma Forest Regeneration Center (OFRC) in Goldsby, Oklahoma tried various treatments in 1995. The first consisted of 94 days of warm stratification at 22.2°C (72°F), followed by 120 days of cold stratification at 4°C (39.2°F) which achieved 55% germination. The second treatment consisted of 48 days of warm stratification at 22.2°C (72°F), followed by 120 days of cold stratification at 4°C (39.2°F) resulting in 61% germination. The last treatment was 27 days of warm stratification at 22.2°C (72°F) followed by 120 days of chilling at 4°C (39.2°F) and resulted in 14% germination

(Porterfield 1995). Ms. Jill Barbour, Germination Specialist with the National Seed Laboratory, tried over 21 different stratification or pregermination treatments with *J. scopulorum* in a controlled, laboratory setting. Treatments included warm stratification followed by cold stratification. Liquid nitrogen as well as varying concentrations of citric acid and hydrogen peroxide soaks were used as scarification treatments. The following five treatments resulted in the best germination of those tried: 1) three days of water soak, followed by 16 weeks of cold stratification at 3°C (37°F), followed by nine weeks of warm stratification at 22.2°C (72°F), followed by a second round of 9 weeks of cold stratification at 3°C (37°F); 2) three day water soak followed by 16 weeks of warm stratification at 22.2°C (72°F), followed by 13 weeks of cold stratification at 3°C (37°F); 3) four day water soak, followed by 12 weeks of warm stratification at 22.2°C (72°F), followed by 13 weeks of cold stratification at 3°C (37°F); 4) a 3% hydrogen peroxide soak for 90 minutes, followed by 8 weeks of warm stratification at 22.2°C (72°F), followed by 13 weeks of cold stratification at 3°C (37°F); and 5) a 10,000 ppm citric acid soak for 6 days, followed by 6 weeks of warm stratification at 22.2°C (72°F), followed by a cold stratification at 3°C (37°F). These stratification techniques gave germination ranges from 22% to 55% (Barbour 2009).

Nursery and Field Establishment of *J. scopulorum*

Regeneration of *J. scopulorum* in nurseries is primarily via seed (Scianna 2000). Spring planting can substitute for the warm stratification regime and fall planting can substitute for the prechilling regime. With proper cultivation, seedlings can reach approximately six inches in height in three years. Survival is greater if seeds are planted in containers and allowed to grow in more optimum controlled settings such as

greenhouses (Scianna 2000). In nursery establishment, success of *J. scopulorum* planting is better if seeds are placed in mulched seedbeds under partial shade (Noble 1990). If seeds are planted in a nursery bed, undercutting three year old seedlings will help to stimulate lateral rooting (Scianna 2000). Field establishment via seeds is not recommended due to the long dormancy and poor germination rates of this species (Scianna 2000). Under natural conditions, *J. scopulorum* seedlings more often become established on moist sites under partial shade. This is noted in particular since the geographical sparseness of *J. scopulorum* is due partly to this species' inability to establish itself on drier sites (Noble 1990). However, the moist sites that this species favors also make it more susceptible to frost-heaving, which is fatal to seedlings (Noble 1990). Even though *J. scopulorum* is fairly shade tolerant when in seedling and sapling stages, it becomes shade intolerant with age; requiring a consistent amount of light for healthy crown and height development (Scianna 2000). It is also advisable to protect recently planted seedlings from sun and wind during dry, hot weather (Scianna 2000).

CHAPTER III

MATERIALS AND METHODS

This was a two-year study. In the first year, combinations of seven different stratification treatments were tested at two planting depths for both a fall (December) and a spring (March) planting. In the second year, the best stratification treatments from year one were tested on seeds from six seed sources from across the *J. scopulorum* natural range. Fall and spring plantings were also conducted in year two.

Year 1- Stratification Study

Various stratification treatments were tested using one seed source from Wasta, SD. The following treatments were selected based on research and suggestions from Ms. Jill Barbour with the National Seed Laboratory in Dry Branch, Georgia.

13 weeks cold/13 weeks warm (13C/13W)

13 weeks warm/13 weeks cold (13W/13C)

26 weeks warm (26W)

26 weeks cold (26C)

13 weeks warm (13W)

13 weeks cold (13C)

Control (no stratification)

Colorado buried seed (COB)

Oklahoma buried seed (OKB)

Warm stratification was at 22.2°C (72°F) and cold stratification was at 4°C (39.2°F). The Colorado and Oklahoma buried treatments were only tested during the spring planting season in 2007.

Before stratification, 5 g (about 400 seeds) of *J. scopulorum* seed from Wasta, SD (provided by David Porterfield, OFRC) was placed inside small plastic bags that had been perforated with a dissecting needle; there were about 100 small holes per bag. One bag was prepared for each treatment and replication. All seed bags, except those containing seeds for untreated control plots were placed inside a 38 liter (10-gallon) tank filled with aerated water for four days. The aeration was provided by a porous stone at the bottom of the tank connected to a pump via a plastic hose. Before soaking, the seed bags were closed via a zipper type closure. When the aerated water treatment was complete, the seed bags were placed in larger plastic bags containing moistened peat moss (moistened to the point of seeing small droplets of water when peat moss was squeezed) and labeled with corresponding treatment codes. Bags were placed inside plastic containers lined with black plastic to exclude light and then placed into their respective stratification treatments for the designated times. Holes were drilled into lids of the plastic containers to allow gas exchange for the seeds.

Following stratification, a two-way factorial experiment was installed as a randomized complete block design. The factors tested were: 1) stratification treatments (described above), and 2) planting depth 0.95 cm (0.4 in) and 0.48 cm (0.2 in). This experiment was conducted as a fall planting (planted December 11-12, 2006) and a spring planting (planted March 7-9, 2007). For the fall 2006 planting, six blocks were distributed throughout two nursery beds (7 stratification treatments at 2 depths=14 total

treatments, 14 treatments x 6 blocks = 84 plots total). For the spring 2007 planting, an Oklahoma and Colorado buried seed treatment was included. Buried bag treatments simulated natural temperature and moisture conditions. For the buried bag treatments, seeds were sent to the Oklahoma Forest Regeneration Center (OFRC) and to the Colorado State Forest Service Nursery. Once the seeds arrived, they were placed inside a tightly-woven mesh bag and buried approximately 30.5 cm (12 in) deep for about 13 weeks (3 ½ months).

For the spring planting, six blocks were distributed throughout two beds (9 stratification treatments at 2 depths=18 total treatments, 18 treatments x 6 blocks=108 plots total). Individual plots were 31 cm (1 ft) long by 152 cm (5 ft) wide with seeds planted in five rows across the bed at a density of 125 seeds per plot (five rows of 25 seeds per row in each plot). Seeds were planted by hand in plots that had been tilled with a John Deere tractor pulling a PTO-driven rototiller (Deere and Company, Moline, Illinois). After the seeds were planted, a 1.3 cm (0.5 in) deep layer of hardwood sawdust was applied with a hydraulically-driven sawdust spreader and then Hydro-Mulch was sprayed via a Bowie Hydro-Mulcher (Bowie Industries, Inc., Bowie, Texas). The beds were irrigated before and after planting and then during the growing season as needed. On April 6, 2007, an initial field count was conducted for the fall 2006 and spring 2007 field plantings. Both alive and dead germinants were counted to determine total germination that had occurred. A second field count was conducted on July 18, 2007.

Laboratory Germination Tests

One piece of 90-mm-diameter filter paper (Whatman no. 1, Whatman International, Ltd., Maidstone, England) was placed in the bottom of 90-mm-diameter plastic petri dishes. The filter paper was moistened with deionized water. At the end of the stratification treatments, uncracked seeds were selected from each treatment bag and 25 seeds were placed in each of eight petri dishes for each treatment. Seeds were placed in a germination chamber (Percival Germinator, Boone, IA) at 15°C (60°F) and a 32 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light period from 7 a.m. to 3 p.m. The chamber was dark from 3 p.m. to 7 a.m. Seeds remained in the chamber for 50 days and were checked for germination at 3- to 4-day intervals. Seeds were considered germinated when the radicle was 5 mm long. Seeds were removed from the dish after germinating.

Some seeds germinated during stratification. The number of seeds germinated during stratification was counted from two extra bags prepared for each treatment immediately before stratification was complete. Both cracked seed (seed coat swelling enough to cause cracks) and germinated seed (exposure of radicle at least 5 mm in length or longer outside the seed coat) were counted.

Year 2 –Seed source study

From the data obtained in Year 1, the treatments of 13W/13C and 13C resulted in the highest germination percentages. For the second year of the study, these treatments were applied to six different seed sources. Seeds in Year 2 were prepared and treated as described above for Year 1. The six seed sources were 1) National Seed Foundation in Moyie Springs, Idaho; 2) J. Herbert Stone Nursery in Central Point, Oregon; 3) Colorado State Forest Nursery collected at Meeker, Colorado; 4) Colorado State Forest Nursery

collected at Salida, Colorado; 5) Lawyer Nursery collected at Logan, Utah; and 6) Bessey Nursery collected at Wasta, South Dakota.

Seeds were planted December 20, 2007 (fall planting) and March 21, 2008 (spring planting). For the fall planting, only the 13C stratification treatment was tested. For the spring planting, both the 13C and 13W/13C treatments were tested. The fall planting was planted in six blocks distributed throughout two nursery beds (6 seed sources x 6 blocks = 36 plots total). Similarly, the spring planting was planted in six blocks but contained twice as many plots because it used two stratification treatments (2 stratification treatments x 6 seed sources x 6 blocks=72 plots total). Both plantings were analyzed as randomized complete block design with either seed source (fall planting) or seed source and stratification treatment (spring planting) as factors. Planting depth was 0.48 cm. Otherwise, the seed source experiment in year 2 was planted and treated as described above for the stratification experiment in year 1.

On April 21, 2008, the initial field count was conducted. Both live and dead germinants were counted to determine total germination that had occurred. A second field count was conducted on June 4, 2008. In addition to the field study, germination tests were conducted in the laboratory using eight Petri dishes from each treatment with 25 seed in each dish. Germination conditions and protocols were the same as those described above. Seeds that germinated or cracked during the stratification treatments were again counted as described above.

CHAPTER IV

RESULTS

Year 1 –Stratification study

April field totals (dead + alive) indicate the maximum number of seedlings that successfully germinated in the field after planting. The July live field counts indicate the percentage of seeds that were expected to survive to become useable seedlings. All year-one fall planting stratification treatments resulted in April total field counts above 13% (Table 1). April total field counts were similar for the two depths for the fall planting with (20.0%) for 0.48 cm and (18.8%) for 0.95 cm depths. For the fall planting, stratification treatment significantly affected germination (Table 2) such that the 13C/13W stratification treatment had a germination percent of 24.7% which was greater than the Control (15%) and the 26C treatment (13.5%) (Table1).

For July live field counts for the fall planting, there was a significant interaction between planting depth and stratification treatment effect (Table 2). While the overall means for the two planting depths were similar 14.6 % for 0.48 cm and 14.8 % for 0.95 cm, there were differences in the rank of stratification treatments among the depths. For instance, 13W/13C stratification treatment had the highest percent seedling count for the 0.48 cm planting depth (21%) and the 26W stratification treatment had the lowest seedling percent of (10.2%) (Table 1). In comparison, the 13C stratification treatment had the highest percent seedling count (23.8%) and the 26C stratification treatment had the lowest percent seedling count (7.0%) for the 0.95 cm planting depth (Table 1). When

each planting depth was analyzed separately, differences among stratification treatments were not significant ($p = 0.42$) for the 0.48 cm planting depth, but were significant for the 0.95 cm planting depth ($p = 0.04$). For the 0.95 cm planting depth, the seedling counts for the 13C stratification treatment (23.8%) was greater than the Control (10.8%), 13W/13C (10.2%), and 26C treatments (7.0%) while the seedling counts for the 13C/13W stratification treatment (20.0%) was greater than the 26C treatment (7.0%) (Table 1).

For the spring planting, stratification treatments significantly affected April total percentage of seeds that germinated (Table 3, Table 4). The 13W/13C stratification treatment had a germination percent of (22.8%) which was greater than the other treatments (Table 3). The OKB (18.6%) and COB treatment (15.4%) were next highest, and greater than all other treatments. The 13C treatment (9.2%) was greater than the 26C treatment (4.9%). The 13W, 13C/13W, 26W, and Control treatments had germination rates below 0.2% (Table 3). April total field counts for the spring planting were similar for the two depths (8.3% for 0.48 cm and 7.5% for 0.95 cm). The July live field counts were lower than the April total counts but had similar patterns in statistical significance among treatments. The 13W/13C stratification treatment had a seedling percent of (13.9%) which was greater than other treatments. The COB treatment (10.5%), OKB treatment (9.6%), and 13C treatment (8.1%) did not statistically differ among themselves but were greater than the remaining treatments with lower mean seedling counts. The 26C treatment (3.2%) was greater than the 13C/13W, 13W, 26W, and Control that all had seedling counts less than 0.1% (Table 3). For July live field counts, survival was similar for the two depths (5.5% for 0.48 cm and 4.5% for 0.95 cm).

For the laboratory germination test, stratification treatments affected germination percentage of seeds used for the fall planting ($p < 0.0001$) with treatment means ranging from 0 to 22.4% (Table 5). The 13C stratification treatment had a germination percent of 22.4%, and the 13W/13C treatment had a germination percent of 20.4% both of which were greater than those of remaining treatments. Seeds in the 26C treatment had a greater germination percentage (8.0 %) than those in 13C/13W (1.2%) and 13W (1.2%) treatments as well as the treatments that had no germination (26W, Control, Dry control). Germination tests for seeds used in the spring planting resulted in significantly different germination counts ($p < 0.0001$) that ranged from 0 to 27.6% for treatment means (Table 5). The 13W/13C and 13C stratification treatments had germination percents of 27.6% and 21.6% and these were greater than all the other treatments. The 26C stratification treatment (12.0%) was greater than the remaining treatments. The COB treatment (8.0%) was similar to the OKB treatment (5.6%), but was greater than 13C/13W (2.0%), 13W (0.4%), 26W (1.2%), Control (1.2%) and Dry Control (0%).

The number of seeds that began germination (cracked or germinated) during stratification differed ($p < 0.0001$) among treatments (Table 6). The 13W/13C stratification treatment counts for cracked seed (111.3 out of 400 seeds total) were significantly greater than the remaining treatments. The 13C treatment (67.5) and 26C treatment (64.0) had cracked seed counts significantly greater than 13C/13W (21.5), and 26W (17.5). The 26W and 13W stratification treatments were statistically similar. Germinated seed counts were different ($p < 0.0001$) and averaged from 0 to 91. The 26C stratification treatment counts for germinated seed (91.0 out of 400 total) were significantly greater than the other treatments. The 13W/13C (48.0), 13C/13W (22.0)

and 13C (13.8) were greater than the 13W and 26W, which had no seeds germinate during stratification. When cracked and germinated were combined, the 13W/13C treatment (159.3) and 26C treatment (155.0) had counts significantly greater than 13C (81.3), 13C/13W (43.5) and 26W (17.5) treatments.

Total seed activity for *J. scopulorum* seeds was defined as the percentage of seeds that were counted as germinants in the April total field count added to the seeds that germinated while in stratification (Table 7). All treatments in the year one fall planting resulted in total activity percentages above (9.5%) (Table 7). The 26C stratification treatment had the highest seed activity (36.3%) for the fall planting; however, this treatment had a large percentage of germination occurring while the seeds were in stratification and these germinants could not be planted in the field. The 13W/13C stratification treatment showed the highest seed activity (34.8%) for the spring planting. Again, the 26C treatment had germination occurring while in stratification. Average total seed activity for fall and spring resulted in the highest total seed activities for the 13W/13C stratification treatment (33.7%) and 26C (32%) treatment.

Year 2 –Seed source study

The fall planting in year two resulted in April total field counts that ranged from 0 to 18.8% for the various seed sources ($p < 0.0001$) (Table 8). The Wasta, SD seed source had a germination percentage of 18.8% and was higher than that of the other five seed sources. The fall planting resulted in June live field counts ranging from 0.2 to 16.8 % ($p < 0.0001$) (Table 8). The Wasta, SD seed source had a germination percentage of (16.8%) and was higher than the other five seed sources.

In addition to testing seed source effects, the spring planting in year two tested the difference between stratification at 13C and 13W/13C. For the spring planting, April total field counts ranged from 0 to 31.0% (Table 9). Seed source ($p < 0.0001$), stratification treatment ($p < 0.0001$), and the interaction between seed source and treatment effect were significant ($p < 0.003$). Overall, the 13W/13C treatment resulted in 13.8% germination compared to 2.2% for the 13C stratification treatment (Table 9). The interaction between seed source and germination treatment occurred because only the Wasta, SD source had germination rates above 1% for the 13C treatment while all the seed sources had germination greater than 4% for the 13W/13C treatment. When the two stratification treatments were analyzed separately, germination of the Wasta, SD source was greater than the other seed sources for the 13C planting. For the 13W/13C stratification treatment, the Wasta, SD source germination was greater than the other seed sources and the Moyie Springs, ID, Central Point, OR, and Salida, CO sources were greater than the Meeker, CO source.

The spring planting in year two resulted in June live field counts ranging from 0.0 to 26.2 % (Table 9). There was a significant effect of seed source effect ($p < 0.0001$) as well as a significant interaction between seed source and treatment effect ($p = 0.0004$). The June live totals were lower than the April total (6.7 vs. 8.0%) due to mortality, but statistical differences related to treatments were the same as those described above.

A subset of seeds that were uncracked were tested for germination potential under constant temperature of 15°C (60°F) and photoperiod of 32 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for 8 hours of light and 16 hours of darkness. Year-two germination test percentages ranged from 0 to 9.2% among seed sources (Table 10). The 13W/13C stratification treatment resulted in

greater germination than the 13C stratification treatment ($p = 0.006$) (3.1 vs. 1.6%). The Wasta, SD source (8.2%) had greater germination than the other seed sources (1.1%).

The number of seeds that began germination (cracked or germinated) during stratification differed ($p < 0.0001$) among treatments (Table 11). For cracked seed counts, the effects of seed source, stratification treatment, and their interaction were significant ($p < 0.0001$) because none of the seeds cracked in the 13W/13C treatment while seed of two seed sources in the 13C treatment exhibited some cracking. Within the 13C stratification treatment, the Wasta, SD source had 11.5 cracked seeds (out of 400 total), the Moyie Springs, ID source had 0.5 cracked, and the other seed sources had no cracking. For germinated seeds, the effects of seed source, stratification treatment, and their interaction were significant ($p < 0.0001$). In contrast to cracked seeds, germination occurred almost entirely in the 13W/13C treatment. Within the 13W/13C treatment, the Wasta, SD, Salida, CO, and Moyie Springs, ID had greater germination than the other seed sources and the Central Point, OR had higher germination than the Logan, UT and Meeker, CO sources.

Total seed activity for *J. scopulorum* seeds is defined as the percentage of seeds that were counted as germinants in the April total field count combined with the seeds that germinated while in stratification (Table 12). The only sizable seed activity noted in the fall or spring using the 13C stratification treatment was from the Wasta, SD seed source. For the 13W/13C stratification treatment, all six seed sources exhibited seed activity. The Wasta, SD seed source had the highest amount of seed activity of 56%. The Moyie Springs, ID seed source was next with 35% seed activity and the Salida, CO

seed source had 33% seed activity. The rest of the seed sources were below 30% seed activity.

CHAPTER V

DISCUSSION AND RECOMMENDATIONS

Year 1 –Stratification study

Seed of *J. scopulorum* has multiple dormancies. According to Djavanshir and Fechner (1976), the seed coat and the sheath surrounding the embryo prevent water absorption. Without water imbibition, the seeds will not swell and thus will not begin to germinate. In this study, the *J. scopulorum* seed were exposed to four days of an aerated water soak. According to a moisture content study by Barbour (1998), the initial moisture content of dried *J. scopulorum* seeds was 9.9%. After four days of aerated water soak, the moisture content increased to 37% and did not increase any further with longer soaking.

After the seeds have imbibed a sufficient amount of water, an after ripening warm period followed by a cold period has been shown to break the dormancy of the hypocotyls (Djavanshir and Fechner 1976). Even though warm stratification followed by cold stratification had previous documented success, other studies have shown conflicting results regarding different stratification and pregermination treatments. The Woody Plant Seed Manual indicates that the most common treatment for overcoming *J. scopulorum* dormancies is a long moist stratification at 3 to 5°C (37.4 to 41.0°F) (Allen, et al. 2008). Another report suggests freezing the seed, but this method is generally unsuccessful (Johnsen and Alexander 1974). Similar to Djavanshir and Fechner, another study found

that *J. scopulorum* usually responds well to warm temperatures of 25°C (77°F) for about 45 to 240 days followed by cold temperatures at 3 to 5°C (37.4 to 41.0°F) (Johnsen and Alexander 1974). However, Young and Young (1988) reported no germination using this two-temperature stratification protocol.

Barbour (2009), suggested three possible stratification treatments. One was 16 weeks of warm temperatures followed by 13 weeks of cold temperatures (germination 55%). The second treatment had 12 weeks of warm temperatures followed by 13 weeks of cold temperatures (germination 49%). The third treatment had a 90 minute, 3% hydrogen peroxide soak with 8 weeks of warm temperatures and then 13 weeks of cold temperatures (germination 45%).

A strength of this study is that germination was tested under field conditions rather than solely in the laboratory. In support of Djavanshir and Fechner, 13W/13C in the current study resulted in a higher germination percentage than the other treatments when combining the results of a fall and spring planting. Based on the results from the year one stratification study and the generally good responses of multiple seed sources to the 13W/13C treatment as well as the general suitability of a warm/cold stratification treatment from previous laboratory tests, a warm/cold stratification treatment is recommended. This treatment provided the most uniform and successful germination rate for a nursery setting.

Seeds planted in the fall germinated in all treatments and with greater success than those in the spring planting. This was likely due to the naturally occurring cold temperatures and/or temperature fluctuations in the field after planting in December. In

contrast, only stratification treatments that ended with cold exposure produced any seedlings for the spring planting, i.e., 13C, 13W/13C, COB, and OKB. Because planting in spring (March) occurs when temperatures are already warm, i.e., average air temperatures of 9.3°C (48.8°F), average soil temperatures of 7.9°C (46.3°F) and maximum soil temperatures at 11.0°C (52.0°F) (Oklahoma Mesonet data). It appears that naturally occurring field conditions are too warm to stimulate germination. While a period of warm stratification is necessary to help break radicle dormancy (Djavanshir and Fechner 1976), the natural variation in temperature after fall planting appeared to be adequate to fulfill dormancy requirements of some seeds in all treatments including the untreated Control. In addition to requiring cold stratification, the cold temperature appears to be needed at the end of the stratification treatments. For the spring planting the 13C/13W treatment did not produce any germinants. The OKB and the COB treatments were planted to see how the *J. scopulorum* seeds would respond to natural 'stratification' conditions. Because the natural stratification occurred during the winter, the buried bag treatments could only be tested with spring planting. The natural fluctuations in temperature of the buried bag treatments did result in germination, although it was less than that of the 13W/13C treatment. The average soil temperatures for the buried bag in Oklahoma were: 7.3°C (45.2°F) for December 2006, 4.6°C (40.2°F) for January 2007, 5.0°C (41.0°F) for February 2007 and 13.4°C (56.2°F) for March 2007. The temperatures experienced by the COB were probably several degrees lower than the OKB, but above freezing.

The two least successful treatments in the fall planting were the 26C and untreated Control. The 26 week cold stratification was too long since a large number of

seeds germinated while in stratification exhibiting both elongated radicles and some cotyledon developments. These seedlings could not be planted mechanically or by hand. Germination in the 26C stratification treatment decreased field planting success since many of the seeds with potential activity could not be planted. In contrast to 26 weeks of cold stratification exposure, seeds that were exposed to 13 weeks of cold had the greatest number of cracked seeds that were active and could be planted. Warm stratification resulted in less seed germination during the stratification period than cold stratification.

Seeds in the Control treatment were not stratified other than the natural temperature variation experienced once planted. Lower germination was probably due to a shorter window for stratification than other seeds that experienced at least 13 weeks of either warm or cool moist conditions. Additionally, low germination in the fall planting could be due to the lack of aerated soak since the Control seeds were planted dry.

For all treatments averaged across the fall and spring planting, the number of seedlings alive in July (9.9%) was lower than the number alive in April (12.2%). Additionally, 3.1 % of seedlings died sometime before counting in April. Seedlings appeared to die from various factors including erosion of beds from flooding or overwatering, animal impact, and perhaps by Phomopsis blight (*Phomopsis juniperovora*) (see Pests and Problems). However, 81 % of those seedlings alive in April survived to July and appeared to be growing into eventually saleable seedlings.

Seed viability is an important variable that determines the number of seeds that need to be planted to produce a desired seedling density in the nursery beds. For the April total field percentages in the fall planting of year one, the 13C, 13C/13W, and

13W/13C stratification treatments were similar in germination percentage and all greater than 20%. Given a cut test of 77% (OFRC) and a TZ test of 65%, a germination rate of 20% would indicate that approximately one third of the potentially viable seeds produced seedlings.

The importance of cold exposure at the end of stratification was reinforced by the laboratory germination results. Uncracked seed were immediately removed from the respective cold or warm stratification treatments and placed in a germinator at 15°C (59°F). Only treatments that ended with cold temperature, i.e., 13C, 13W/13C, and 26C exhibited germination rates above 2.0%. As with the field data, germination was reduced in the 26C treatment because many of the viable seed had already germinated during stratification.

The OKB and COB treatments had moderate laboratory germination rates that were lower than their field germination rates. This could be explained by the fact that most of the seeds when removed from these stratification treatments were covered with fungal growth. The moist, warm environment in the germinator may have accelerated fungal growth and contributed to the lower relative success of the OKB and COB in the laboratory germination tests. The soil temperatures and moisture were more variable, so the fungus may not have grown as fast when planted in the field.

Total seed activity was the sum of germinated seeds during stratification and seeds that successfully produced seedlings in the field (both cracked and uncracked were planted). For the fall planting, the 26C treatment had the greatest activity, but most of these seeds could not be planted.

There was no significant difference when comparing the planting depths for either the fall or spring planting in year one. The difference in these two depths was not large and apparently not enough to cause differences in germination and establishment. The comparison of the planting depths was chosen based on the operational planting depth used by OFRC (0.48 cm) and what might be possible on an operational scale (0.95 cm). Obviously, there is a planting depth that affects germination, but it is deeper than 0.95 cm.

The results from year one suggest that the stratification treatment of 13W/13C is the best treatment of those tested for either fall or spring planting in an Oklahoma nursery. However, since some germination occurred while the seeds were in the 13W/13C stratification treatment, the ending 13 weeks of cold temperature might be too long. There was more flexibility when planting in the fall. Ending with cold stratification or planting early enough in the fall to have natural exposure to cold temperatures is critical for successful seed germination of *J. scopulorum*. The buried bag stratification treatment might be acceptable, but 13W/13C was more successful and more easily employed to ensure consistent and repeatable results. Planting deeper (0.95 cm) did not affect germination or establishment success of this seed.

Year 2 –Seed source study

Based on the results from the year one-stratification study, 13C had the second highest success (23.4% for 13C vs. 24.7% 13C/13W) for the fall planting. Given the relative flexibility related to stratification treatments before the fall planting and the simplicity of the 13C treatment, only the 13C treatment was used for the fall planting in

year two. For the spring planting in year two, 13W/13C also was included because 13W/13C was the best stratification treatment for the spring planting.

For the fall planting in year two, the only seed source with significant field germination success was the Wasta, SD source which was the only seed source used in year one. For spring planting with seeds treated with 13C, once again, the only successful seed source was Wasta, SD. However, the 13W/13C treatment for the spring planting resulted in all seed sources exhibiting some success with the Wasta, SD source once again having the highest success rate.

The lack of germination in the 13C treatment for all seed sources except the Wasta, SD source suggests that results for the fall planting from year one may be specific to the Wasta, SD seed source. While natural temperature fluctuations in Oklahoma following fall planting apparently fulfill any warm stratification requirement of the Wasta, SD source, the other seed sources apparently require a period of warm stratification before cold stratification or some other treatment for success. This warm requirement fits with the multiple dormancies reported for *J. scopulorum* seed (Allen, et al. 2008; Djavanshir and Fechner 1976).

One factor that may cause variation in seed source stratification requirements is that *J. scopulorum* in western South Dakota reportedly will hybridize with *J. virginiana*. Van Haverbeke (1968) reported that some western South Dakota seed were F₁ hybrids, i.e. 50% *J. scopulorum* genes and 50% *J. virginiana* genes. If the Wasta, SD seed do contain *J. virginiana* genes, it is reasonable to expect a higher percent germination in the field planting, since *J. virginiana* seed requires only 30 to 120 days of cold stratification

to germinate (Allen, et al. 2008). The lack of a warm requirement for *J. virginiana* seed suggests that *J. virginiana* does not have multiple dormancies, which may contribute to greater success in germinating hybrid seed.

Using the 13W/13C spring planting for comparison, differences in success due to seed source occurred. Little information exists on population and genetic variability of *J. scopulorum* (Noble 1990). With the species' scattered distribution which includes a wide elevation and latitudinal range, there are differences among populations in various physical attributes such as growth, morphology, and resistance to heat and cold (Noble 1999). Therefore, it is logical that there may be differences in germination rates and/or stratification requirements among populations. Whether differences in germination success among the seed sources is due to seed source differences in germination rate or due to an untested interaction between stratification treatment and seed source is unknown. Additional research involving multiple stratification treatments and the various seed sources is necessary to determine whether all seed sources respond to stratification treatments in the same way. In the seed source study, laboratory germination rates were only 18% of field success. This might indicate the seeds underwent an additional ripening period once in the field. These results suggest that the 13W/13C stratification treatment is a more effective stratification treatment than the 13C treatment on different sources of *J. scopulorum* seed. To better understand the potential interaction between seed source and stratification treatment more research on this subject is needed.

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Table 1: Percentage of *J. scopulorum* seedlings counted alive or dead associated with planting depths and stratification treatments for fall 2006 planting in an Oklahoma nursery. Dead represents seedlings that germinated and then died. S.E. denotes standard error. A total of 125 seeds were planted per plot. Treatment designations indicate stratification under warm (22.2°C) or cold (4°C) conditions for the indicated number of weeks (13 or 26). CONTROL seeds were planted without any stratification. (n = 6).

Depth (cm)	Treatment	April 2007 Live		April 2007 Dead		April 2007 Total		July 2007 Live	
		%	S.E.	%	S.E.	%	S.E.	%	S.E.
0.48	13C	17.0	± 3.2	5.0	± 2.2	22.0	± 2.4	14.2	± 2.6
0.48	13C/13W	18.8	± 4.6	3.4	± 1.4	22.2	± 4.0	14.4	± 2.2
0.48	13W	18.6	± 4.6	1.4	± 1.0	20.0	± 4.4	16.8	± 3.4
0.48	13W/13C	26.0	± 5.1	1.8	± 1.3	27.8	± 4.5	21.0	± 4.1
0.48	26C	16.2	± 4.0	2.2	± 1.1	18.4	± 4.2	14.8	± 4.2
0.48	26W	11.8	± 3.7	2.8	± 1.1	14.6	± 4.7	10.2	± 3.5
0.48	CONTROL	11.8	± 3.1	3.6	± 1.2	15.4	± 2.8	11.4	± 2.6
0.95	13C	23.4	± 3.0	1.4	± 0.7	24.8	± 2.6	23.8	± 2.1
0.95	13C/13W	22.8	± 4.3	4.4	± 2.6	27.2	± 3.1	20.0	± 4.1
0.95	13W	19.0	± 4.8	2.2	± 2.2	21.2	± 4.7	15.8	± 4.8
0.95	13W/13C	12.4	± 4.4	1.2	± 0.6	13.6	± 4.4	10.2	± 2.9
0.95	26C	8.0	± 4.2	0.6	± 0.2	8.6	± 4.2	7.0	± 3.4
0.95	26W	18.4	± 5.1	3.4	± 2.0	21.8	± 4.6	16.4	± 4.3
0.95	CONTROL	11.0	± 2.1	3.6	± 1.8	14.6	± 2.8	10.8	± 3.4

Table 2: P-values associated with planting depths and stratification treatments for fall 2006 planting of *J. scopulorum* in an Oklahoma nursery.

Source	df	April 2007 Live	April 2007 Dead	April 2007 Total	July 2007 Live
		P > F			
Depth	1	0.74	0.63	0.58	0.93
Trt	6	0.13	0.54	0.045	0.19
Depth x Trt	6	0.14	0.70	0.067	0.048

Table 3: Percentage of *J. scopulorum* seedlings counted alive or dead associated with planting depths and stratification treatments for spring 2007 planting in an Oklahoma nursery. Dead represents seedlings that germinated and then died. S.E. denotes standard error. A total of 125 seeds per plot were planted. Treatment designations indicate stratification under warm (22.2°C) or cold (4°C) conditions for the indicated number of weeks (13 or 26). CONTROL seeds were planted without any stratification. OKB and COB represent Oklahoma and Colorado ‘natural stratification’ treatments where bags of seeds were buried in the ground at the specified locations and then planted out in the field at the Oklahoma nursery. (n = 6).

Depth (cm)	Treatment	April 2007 Live		April 2007 Dead		April 2007 Total		July 2007 Live	
		%	S.E.	%	S.E.	%	S.E.	%	S.E.
0.48	13C	9.6	± 1.7	0.2	± 0.2	9.8	± 1.8	10.0	± 1.2
0.48	13C/13W	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0
0.48	13W	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0
0.48	13W/13C	24.4	± 2.5	0.2	± 0.2	24.6	± 2.5	14.2	± 2.8
0.48	26C	5.0	± 1.4	0.2	± 0.2	5.2	± 1.6	3.8	± 1.1
0.48	26W	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0
0.48	CONTROL	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0
0.48	COB	15.8	± 2.9	1.0	± 0.4	16.8	± 3.1	11.8	± 2.9
0.48	OKB	18.0	± 3.1	0.6	± 0.3	18.6	± 3.1	10.2	± 2.2
0.95	13C	8.2	± 1.8	0.4	± 0.2	8.6	± 1.9	6.2	± 1.5
0.95	13C/13W	0.2	± 0.2	0.0	± 0.0	0.2	± 0.2	0.2	± 0.2
0.95	13W	0.2	± 0.2	0.0	± 0.0	0.2	± 0.2	0.0	± 0.0
0.95	13W/13C	20.6	± 1.7	0.4	± 0.2	21.0	± 1.7	13.6	± 1.9
0.95	26C	4.4	± 1.1	0.2	± 0.2	4.6	± 1.2	2.6	± 0.6
0.95	26W	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0
0.95	CONTROL	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0
0.95	COB	13.4	± 2.6	0.6	± 0.6	14.0	± 2.7	9.2	± 1.3
0.95	OKB	16.4	± 3.0	2.2	± 0.5	18.6	± 3.3	9.0	± 1.9

Table 4: P-values associated with planting depths and stratification treatments for spring 2007 planting of *J. scopulorum* in an Oklahoma nursery.

Source	df	April 2007 Live	April 2007 Dead	April 2007 Total	July 2007 Live
		P > F			
Depth	1	0.17	0.13	0.27	0.11
Trt	8	<.0001	<.0001	<.0001	<.0001
Depth x Trt	8	0.93	0.015	0.94	0.87

Table 5: Germination percentage of *J. scopulorum* seed for fall 2006 and spring 2007 laboratory germination test. S.E. denotes standard error. Treatment designations indicate stratification under warm (W) or cold (C) conditions for the indicated number of weeks (13 or 26). CONTROL seeds were hydrated with no stratification and DRY CONTROL were dry without stratification. COBS were Colorado buried seeds. OKB were Oklahoma buried seeds. Eight replications of 25 seed each were used except for the COB (n = 3).

Treatment	Fall 2006		Spring 2007	
	Percent (%)	S.E.	Percent (%)	S.E.
13C	22.4	± 4.0	21.6	± 3.6
13C/13W	1.2	± 0.8	2.0	± 0.8
13W	1.2	± 0.8	0.4	± 0.4
13W/13C	20.4	± 4.0	27.6	± 3.6
26C	8.0	± 2.8	12.0	± 2.8
26W	0.0	± 0.0	1.2	± 0.8
CONTROL	0.0	± 0.0	1.2	± 0.8
DRY CONTROL	0.0	± 0.0	0.0	± 0.0
COB	-		8.0	± 4.0
OKB	-		5.6	± 1.2

Table 6: The number of seeds that began germination (cracked or germinated) during stratification for *J. scopulorum* seed. These seed were counted from 4 bags pulled randomly from each treatment. There was approximately 5 gm (400 seed) in each bag. These bags were pulled out and counted before the rest of the treatment bags were taken to the field for planting. The ‘in stratification’ counts were done in conjunction with the fall planting.

Treatment	Cracked		Germinated		Total	
	Means	S.E.	Means	S.E.	Means	S.E.
13C	67.5	± 5.6	13.8	± 4.1	81.3	± 9.0
13C/13W	21.5	± 4.9	22.0	± 2.4	43.5	± 5.5
13W	3.5	± 1.2	0.0	± 0.0	3.5	± 1.2
13W/13C	111.3	± 12.2	48.0	± 9.4	159.3	± 8.3
26C	64.0	± 5.5	91.0	± 7.9	155.0	± 10.7
26W	17.5	± 3.7	0.0	± 0.0	17.5	± 3.7
CONTROL	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0

Table 7: Total seed activity (field germination plus germination during stratification) for *J. scopulorum* seeds calculated as April field counts plus seeds that germinated during stratification that had radicles too elongated to plant.

Treatment	Fall Planting Activity (%)	Spring Planting Activity (%)
13C	26.9	12.7
13C/13W	30.2	5.7
13W	20.6	0.2
13W/13C	32.7	34.8
26C	36.3	27.7
26W	18.2	0.0
CONTROL	9.5	0.0

Table 8: Percentage of *J. scopulorum* seedlings counted alive or dead associated with seed sources. All sources were stratified for 13 weeks at 4°C (13C) for fall 2007 planting in an Oklahoma nursery. Dead represents seedlings that germinated and then died. S.E. denotes standard error. A total of 125 seeds per plot were planted.

Seed Source	Treatment	April Live		April Dead		April Total		June Live	
		%	S.E.	%	S.E.	%	S.E.	%	S.E.
Moyie Springs, ID	13C	0.4	± 0.2	0.0	± 0.0	0.4	± 0.2	0.4	± 0.2
Central Point, OR	13C	1.4	± 0.4	0.2	± 0.2	1.4	± 0.5	1.4	± 0.4
Meeker, CO	13C	0.2	± 0.2	0.0	± 0.0	0.2	± 0.2	0.2	± 0.2
Salida, CO	13C	1.0	± 0.3	0.0	± 0.0	1.0	± 0.3	1.0	± 0.2
Logan, UT	13C	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0	0.2	± 0.2
Wasta, SD	13C	16.8	± 1.9	2.0	± 0.3	18.8	± 1.9	16.8	± 1.8

Table 9: Percentage of *J. scopulorum* seedlings counted alive or dead associated with seed sources. All sources were stratified for 13 weeks at 4°C (13C) and 13 weeks at 22.2°C followed by 13 weeks at 4°C (13W/13C) for spring 2008 planting in an Oklahoma nursery. Dead represents seedlings that germinated and then died. S.E. denotes standard error. A total of 125 seeds per plot were planted.

Seed Source	Treatment	April Live		April Dead		April Total		June Live	
		%	S.E.	%	S.E.	%	S.E.	%	S.E.
Moyie Springs, ID	13C	0.2	± 0.2	0.0	± 0.0	0.2	± 0.2	0.6	± 0.2
Central Point, OR	13C	0.6	± 0.3	0.0	± 0.0	0.6	± 0.3	1.2	± 0.6
Meeker, CO	13C	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0
Salida, CO	13C	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0
Logan, UT	13C	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0
Wasta, SD	13C	12.6	± 2.6	0.2	± 0.2	12.8	± 2.6	10.0	± 2.1
Moyie Springs, ID	13W/13C	13.4	± 1.4	0.6	± 0.2	14.0	± 1.4	11.2	± 0.6
Central Point, OR	13W/13C	12.0	± 1.6	0.0	± 0.0	12.0	± 1.6	10.4	± 1.7
Meeker, CO	13W/13C	4.2	± 1.2	0.2	± 0.2	4.4	± 1.1	3.4	± 1.0
Salida, CO	13W/13C	12.0	± 2.2	0.2	± 0.2	12.2	± 2.3	10.2	± 1.8
Logan, UT	13W/13C	8.6	± 1.6	0.6	± 0.2	9.2	± 1.6	7.0	± 1.6
Wasta, SD	13W/13C	29.8	± 2.8	1.0	± 0.5	31.0	± 3.0	26.2	± 2.5

Table 10: Germination percentage of *J. scopulorum* seed counted in laboratory germination test. S.E. denotes standard error. A total of 25 uncracked seeds were placed in each of eight Petri dishes for each seed source, stratified at both 13C and 13W/13C and then placed into a germinator at a constant temperature of 15°C (60°F).

Seed Source	Treatment	Total	
		%	S.E.
Moyie Springs, ID	13C	0.0	± 0.0
Central Point, OR	13C	1.2	± 0.8
Meeker, CO	13C	0.4	± 0.4
Salida, CO	13C	0.0	± 0.0
Logan, UT	13C	0.0	± 0.0
Wasta, SD	13C	8.0	± 2.0
Moyie Springs, ID	13W/13C	3.6	± 1.2
Central Point, OR	13W/13C	2.0	± 1.2
Meeker, CO	13W/13C	0.4	± 0.4
Salida, CO	13W/13C	3.2	± 1.2
Logan, UT	13W/13C	0.4	± 0.4
Wasta, SD	13W/13C	9.2	± 1.6

Table 11: The number of seeds that began germination (cracked or germinated) during stratification for *J. scopulorum* seed. These seed were counted from 4 bags pulled randomly from each treatment. There was approximately 5 gm (400 seed) in each bag. These bags were pulled out and counted before the rest of the treatment bags were taken to the field for planting. Counts were done only in conjunction with the fall planting.

Source	Treatment	Cracked		Germinated		Total	
		Means	S.E.	Means	S.E.	Means	S.E.
Moyie Springs, ID	13C	0.5	± 0.5	0.0	± 0.0	0.5	± 0.5
Central Point, OR	13C	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0
Meeker, CO	13C	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0
Salida, CO	13C	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0
Logan, UT	13C	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0
Wasta, SD	13C	11.5	± 5.5	2.0	± 1.0	13.5	± 6.5
Moyie Springs, ID	13W/13C	0.0	± 0.0	84.0	± 9.0	84.0	± 9.0
Central Point, OR	13W/13C	0.0	± 0.0	60.0	± 4.0	60.0	± 4.0
Meeker, CO	13W/13C	0.0	± 0.0	10.0	± 1.0	10.0	± 1.0
Salida, CO	13W/13C	0.0	± 0.0	83.5	± 5.5	83.5	± 5.5
Logan, UT	13W/13C	0.0	± 0.0	28.5	± 3.5	28.5	± 3.5
Wasta, SD	13W/13C	0.0	± 0.0	99.5	± 7.5	99.5	± 7.5

Table 12: Total seed activity (field germination plus germination during stratification) for *J. scopulorum* seeds calculated as April field counts plus seeds that germinated during stratification that had radicles too elongated to plant.

Source	Fall 13C	Spring 13C	Spring 13W13C
	(%)	(%)	(%)
Moyie Springs, ID	0.4	0.2	35.0
Central Point, OR	1.6	0.6	27.0
Meeker, CO	0.2	0.0	6.9
Salida, CO	1.0	0.0	33.1
Logan, UT	0.0	0.0	16.3
Wasta, SD	19.3	13.3	55.7

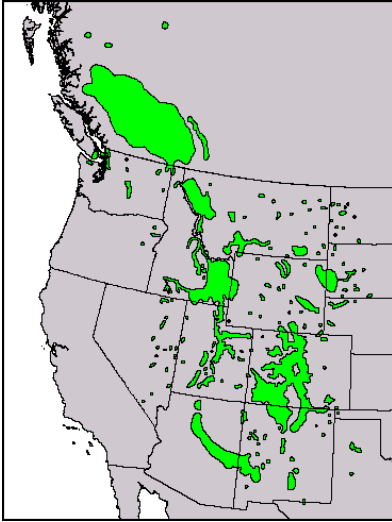


Figure 1. The natural distribution of *Juniperus scopulorum* (Noble 1990).



Figure 2. Cross-section of a *Juniperus scopulorum* 1600 years old.
(<http://web.utk.edu/~grissino/gallery.htm>)(Photo © H.D. Grissino-Mayer and R.K. Adams)



Figure 3. *Juniperus scopulorum* bark and leaves. Tom DeGomez, University of Arizona (Bugwood.org)



Figure 4. *Juniperus scopulorum* female cone. Norbert Frank, University of West Hungary (Bugwood.org)



Figure 5. *Juniperus scopulorum* seeds (2 mm diameter length) Steve Hurst ARS Botany Laboratory, USDA, NRCS. 2009. The PLANTS Database (<http://plants.usda.gov>, 18 September 2009). National Plant Data Center, Baton Rouge, LA 70874-4490 USA.



Figure 6. Cedar apple rust (*Gymnosporangium juniperi-virginianae* Schwein.) Pat Zungoli, Clemson University Forestrypests.org/images



Figure 7. Juniper mistletoe (*Phoradendron juniperinum* Engelm. ex A. Gray) William Jacobi, (Bugwood.com)



Figure 8. Phomopsis blight fungus on juniper (*Phomopsis juniperovora* Hahn) Robert L. Anderson Forestrypests.org/images



Figure 9. Admes spider mite (*Eurytetranychus admes* Prichard & Baker) (Bugwood.com). Rayanne Lehman, Pennsylvania Department of Agriculture, United States



Figure 10. (*Pratylenchus penetrans* Cobb) larva and egg. ©William Wergin, 2009.



Figure 11. Root damage caused by (*Pratylenchus penetrans* Cobb) (Bugwood.com) R.J. Reynolds Tobacco Co. Slide Set, 2002.



Figure 12. *Juniperus scopulorum* seed with emerging radicles. Pamela K. Tauer, 2007.

VITA

Pamela Kay Tauer

Candidate for the Degree of

Master of Science

Thesis: STRATEGIES FOR SUCCESSFUL GERMINATION OF ROCKY MOUNTAIN JUNIPER (*JUNIPERUS SCOPULORUM* SARG.) IN AN OKLAHOMA NURSERY

Major Field: Forestry

Biographical:

Education: Completed the requirements for the Master of Science in Forest Resources at Oklahoma State University, Stillwater, Oklahoma in May, 2010. Completed the requirements for the Bachelor of Science in Forestry at Oklahoma State University, Stillwater, Oklahoma in May 1996.

Experience: I was raised in the country and helped on a farm near Muskogee, Oklahoma. I worked for the Fort Polk Army Forestry Unit in 1997. I worked in the Crop Stress Physiology laboratory at Oklahoma State University from 1997-2002. I worked at and managed the Research Greenhouses at Oklahoma State University from 2002-2003. I also have several years of laboratory experience that I obtained while working in the Horticulture Physiology laboratory at Oklahoma State University from 2003-2010.

Professional Memberships: I am a member of good standing with the Society of American Foresters for over 16 years. I am also a member of the Xi Sigma Pi (The National Forestry Honor Society) since 2008. I received a certificate of training in the Seed Testing course from the National Seed Laboratory in 2007.

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Date of Degree: May, 2010

Institution: Oklahoma State University

Location: Stillwater, Oklahoma

Title of Study: STRATEGIES FOR SUCCESSFUL GERMINATION OF ROCKY MOUNTAIN JUNIPER (*JUNIPERUS SCOPULORUM* SARG.) IN AN OKLAHOMA NURSERY

Pages in Study: 73

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Scope and Method of Study: Low and inconsistent germination of *Juniperus scopulorum* in nurseries limits successful production of this species. The objectives of this study were to determine suitable pregermination protocols that would help to germinate and establish *J. scopulorum* at the state nursery (OFRC) so it could be grown as an alternative windrow species in the state of Oklahoma. In the first year of the study, one seed source of *J. scopulorum* was used with seven stratification treatments applied. Stratified seeds were planted in the nursery at two depths in both December and March. In April and July, the seedlings were counted to determine which stratification treatment produced the most germinants. Germination tests and 'in stratification' counts were also conducted. In the second year of the study, the best stratification treatments were applied to six seed sources of *J. scopulorum* to determine seed source variability in germination success

Findings and Conclusions: 13 weeks of warm stratification and 13 weeks of cold stratification (13W/13C) and 13 weeks of cold stratification (13C) treatments were the best treatments from year one of the study with greater overall success with the December planting than the March planting. Planting depth did not affect seedling production. The 13W/13C treatment was by far the best treatment on all six seed sources used in year two of the study. The Wasta, SD seed source of *J. scopulorum* had the highest germination rate.

ADVISER'S APPROVAL: Dr. Rodney E. Will
