

Rocky Mountain Juniper Study: Preliminary Results

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Introduction

Rocky Mountain juniper (*Juniperus scopulorum* Sarg.) seed are hard to germinate in the nursery, containers, or laboratory because they have multiple dormancies. The seedcoat and prophylactic sheath surrounding the embryo impede water absorption. The embryo's epicotyl is not dormant; only the hypocotyl displays dormancy and requires cold stratification (Djavanshir and Fechner 1976).

Stratification of dormant seed involves three stages (Nikolaeva 1969). During the first stage an initial vigorous swelling of seed take place. Following full imbibition the second stage ensues. Enzymatic activity and hydrolysis of proteins and fats begins. Products from the hydrolytic process prompt the beginning of embryo growth, which constitutes the third stage. Growth activation occurs during stratification in two periods. Mitoses occurs at the end of warm stratification when seed open; whereas, the second activation of growth happens at the end of cold stratification, prior to germination.

This leads to a perennial nursery problem because germination cannot always be predicted. Weather patterns are not identical in every nursery that grows Rocky Mountain juniper. Southernmost nurseries may not receive enough cold weather to provide a sufficient cold stratification period for seed germination. In years when snowfall is not adequate to cover the beds, the seed dry out due to cold, dry, winter winds. Generally, outdoor stratification is not as efficient as constant temperature and moist laboratory stratification, except under conditions of continuous snow cover (Young and Evans 1976). When the seed dry out, they go into secondary dormancy, thereby inhibiting germination.

Multiple stratification regimes make it difficult to manage the seed sown in nursery beds. Past experience among nursery managers has shown that Rocky Mountain juniper seed germinate most uniformly when a warm stratification period precedes the cold stratification period (Meines 1965). Most nurseries plant the seed in July to provide for the

warm stratification period. USDA Forest Service Bessey Nursery in Halsey, NE provides the seed an additional 6 months of cold stratification prior to summer sowing (Benson 1976). Summer sowing is not consistent or predictable, because so many things can go wrong before the seed germinate next spring.

At present, there is not a repeatable germination test that can be used to sow seed. An official laboratory germination procedure has not been established. Many of the seed have been found to be empty or to have low viability, which yields inconsistent laboratory results. A tetrazolium test is used as a substitute for germination tests, because it can be performed on the seed in a couple of days. The test measures seed viability but does not predict germination.

Hypothesis

Interest has been shown in applying weight separation to Rocky Mountain juniper seed to improve germinability (Lee, Cregg, Fleege, 1995). Lee (Masters' thesis 1996) used sodium chloride solutions to separate seed by density and significantly increased germination over all density classes when compared to a control. In this study, mechanical weight separation was done with a gravity table.

Weight and size are confounded in seed, so seed must be sized before weight separation can occur. The gravity table is not designed to separate size and weight simultaneously. Separating low viability seed from high viability seed by using a gravity table increases germination potential. The general rule of thumb is that heavy seed germinate better than light seed. High viability seedlots have less variability and yield more consistent results in testing.

After the high viability seed are isolated, then a method to germinate the seed in the laboratory needs to be determined. Much research has been done on Rocky Mountain juniper; several laboratory treatments appear to work, but they are hard to compare in separate studies. Plus, many seedlots have low viability, which leads to inconsistencies in the data. When all germination treatments showing promise were incorporated into this study, a comparison could be made for optimum treatment selection. The next step would be to repeat the best laboratory treatments in the nursery for test correlation.

Seedlot information

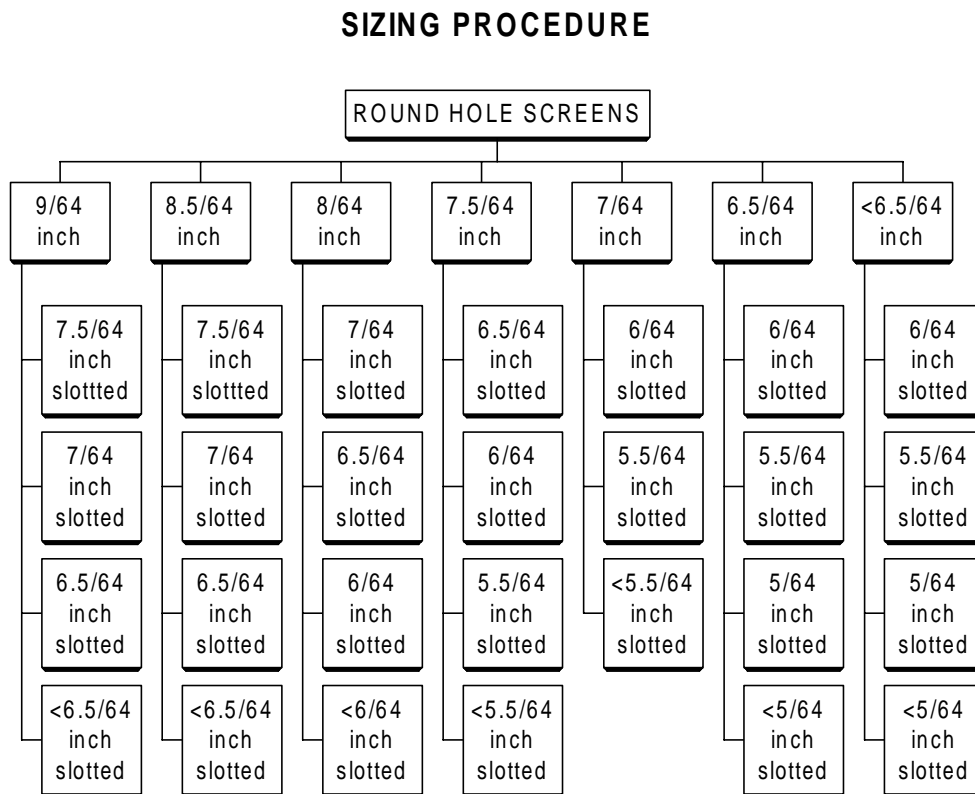
The Forest Service Bessey Nursery supplied a 30-pound lot of Rocky Mountain juniper seed for the study. The seed source was from western South Dakota and commonly used in the Great Plains. It has proved reliable, and it is relatively easy to obtain a steady seed supply. According to Van Haverbeke (1968), the Rocky Mountain juniper in western South Dakota is a hybrid of 50% Rocky Mountain juniper genes and 50% eastern redcedar (*Juniperus virginiana* L.) genes. Van Haverbeke (1968) postulates that the *Juniperus virginiana* population in eastern and central North America may be derived from the western juniper complex in the Rocky Mountains, especially from *Juniperus scopulorum*. The main migration pathway of pines in North America is southward and eastward (Mirov 1967). A similar migration pattern may equally apply to junipers, because they are associated with pine in the most xeric habitats of the western United States (VanHaverbeke and Read 1976).

Seed sizing

The sizing procedure developed by Bob Karrfalt, director of the National Tree Seed Laboratory in Dry Branch, Georgia resulted in 27 sizes (figure 1). The seed were first divided into 8 sizes using round hole screens ranging from $< 6.5/64$ -inch to $9/64$ -inch in diameter. The eight sizes were then further subdivided with slotted whole screens ranging from $< 5/64$ -inch to $7.5/64$ -inches in diameter. The round and slotted whole screens were increased in half size increments.

Following the sizing procedure, seed from the 27 sublots were tested for viability with a chemical staining test using 2,3,5-triphenyltetrazolium chloride. Live tissue stains red. The viability ranged from 61% to 79%, an 18% difference.

Figure 1



Gravity table procedure

The Oliver® 30 DC gravity table was used to separate heavy weight seed from light weight seed. The Oliver® gravity table has a rectangular deck covered with screen that can be inclined and moved eccentrically, with air forced through the holes in the deck. The air creates a vertical stratification of the seed and eccentric shaking creates a horizontal stratification as the seed move down from the feed end to the discharge

end of the deck. Heaviest seed are concentrated at the high deck side, light seed at the low side, and medium seed in the middle of the deck. There are three discharge chutes the seed fall through at the end of the deck creating three weight classes.

Seed samples, from each weight class, were x-rayed to check the table's operation. The light seed fraction was rerun over the table by itself and the seed was subdivided into medium, light seed and trash (figure 2). The medium and lightweight seed were combined and labeled lightweight seed. The final result was four weight classes: heavy, medium, light, and trash for each of the 27 sizes (figure 2). The procedure yielded 93 subsamples. Seed from each of the 93 sizes were tested for viability with a tetrazolium test. Table 1 is the average percent viability of subsamples by weight. The best seed was used to reduce variation and inconsistencies in the data.

Figure 2

GRAVITY TABLE PROCEDURE

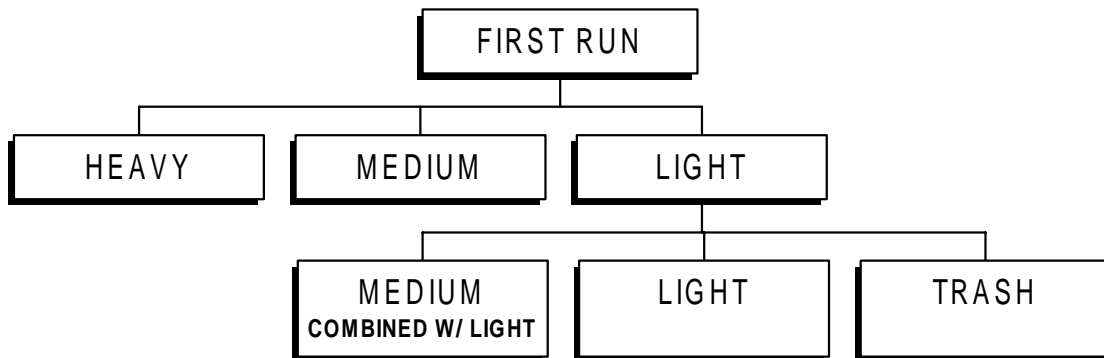


Table 1

Effect of Weight Separation

Before Pregermination Treatments

SEED WEIGHT	% VIABILITY
HEAVY	80
MEDIUM	77

LIGHT	65
TRASH	53

Seedlots selected

Following viability testing, seed from 5 out of the 93 subsamples, were selected and subjected to pregermination treatments (table 2). The seed sizes ranged from large to small, all had high viability (above 80%), and all were in the heavy weight class.

Table 2

SEEDLOTS SELECTED

SCREEN SIZES			
ROUND	SLOTTED	WEIGHT	% VIABILITY
8.0/64	< 6.0/64	HEAVY	81
7.5/64	6.0/64	HEAVY	92
7.0/64	5.5/64	HEAVY	94
6.5/64	5.5/64	HEAVY	97
< 6.5/64	5.0/64	HEAVY	92

Pregermination treatments

Before seed treatments were applied, full imbibition was determined by selecting a seed sample from the seedlot. The initial moisture content of the seedlot was 9.9%. After a 4 day water soak at room temperature, the moisture content increased to 37% and did not increase further with longer soaking periods.

The five samples (table 2) were subjected to 21 pregermination treatments (Table 3). Two hundred seed per treatment were used. The seed were placed in a plastic bag punctured with holes before initiating any treatment. In addition, seed from each of the five samples were allowed to imbibe room temperature water in an aerated, 5-gallon fish tank for up to 4 days before treatment (Pack 1921; Van Haverbeke and

Comer 1985). The one exception was dry seed were immersed into the liquid nitrogen treatments. Seed receiving warm or cold stratification were placed into a plastic container filled with dampened peat moss. Warm stratification was performed at 72°F (22°C) and cold stratification at 37°F (3°C). Seed placed in a freezer were subjected to 20°F (-7°C). In the liquid nitrogen treatments, seed were subjected to the liquid, not the vapor form of nitrogen at -321°F (-196°C). The accelerated aging chamber was set at 104°F (40°C). Chemical treatments included a 3% hydrogen peroxide soak and a 10,000-ppm citric acid soak (Van Haverbeke and Comer 1985).

Table 3

Pregermination Treatments

Number	Treatment
1	3 day water soak, 26 weeks cold, 3 weeks warm
2	3 day water soak, 90 days in freezer, 13 weeks cold, 13 weeks warm, 5 weeks cold, 4 weeks warm (Afanasiev and Cress 1942; Rietveld 1989)
3	1 day liquid nitrogen soak, 4 day water soak, 6 months cold
4	1 day liquid nitrogen soak, 4 day water soak, 1 month cold, 1 day liquid nitrogen soak, 5 months cold, 14 weeks warm, 4 weeks cold, 4 weeks warm
5	1 day liquid nitrogen soak performed 3 times with 4 weeks cold between, followed by 21 weeks cold, 4 weeks warm, 8 weeks cold, 4 weeks warm, cold ongoing
6	1 day liquid nitrogen soak performed 4 times with 4 weeks cold between, followed by 16 weeks cold, 9 weeks warm, 4 weeks warm, cold ongoing
7	1 day liquid nitrogen soak performed 5 times with 4 weeks cold between, followed by 12 weeks cold, 4 weeks warm, 4 weeks cold, 10 weeks warm, cold ongoing
8	1 day liquid nitrogen soak performed 6 times with 4 weeks cold between, followed by 7 weeks cold, 9 weeks warm, 9 weeks cold, 4 weeks warm, cold ongoing
9	Liquid nitrogen soak for 6 months, 9 weeks cold, 17 weeks warm
10	Liquid nitrogen soak for 2 months, 33 weeks cold, 9 weeks warm
11	Accelerated aging chamber 7 days, 25 weeks cold, 13 weeks

	warm, 4 weeks cold
12	3 day water soak, 14 weeks cold, accelerated aging 7 days, 12 weeks cold, 12 weeks warm, 9 weeks cold
13	6 day 10,000 ppm citric acid soak, 6 weeks warm, 10 weeks cold (Van Haverbeke and Comer 1985; Cotrufo 1963)
14	4 day soak, 12 weeks warm, 13 weeks cold
15	A 90 minute, 3% hydrogen peroxide soak, 8 weeks warm, 13 weeks cold (Van Haverbeke and Comer 1985)
17	3 day soak, 16 weeks cold, 9 weeks warm, 9 weeks cold (Rietveld 1989)
18	3 day soak, 16 weeks cold, 10 weeks freezer, 14 weeks warm, 4 weeks cold, 4 weeks warm (Afanasiev 1955; Rietveld 1989)
19	90 minute sulfuric acid soak, 27 weeks cold, 9 weeks warm, 9 weeks cold, 4 weeks warm, cold ongoing (Djavanshir and Fechner 1976)
21	3 day soak, 16 weeks warm, 13 weeks cold (Clark Fleege's recommendation)
23	3 liquid nitrogen dunks each for 24 hours, followed by a 4 day soak, 24 weeks cold, 6 weeks warm for 2 seedlots, 15 weeks warm for 3 seedlots
24	5 day soak, used Georgia soil instead of peat moss, 24 weeks cold, 15 weeks warm, 4 weeks cold

Treatments 16, 20, & 22 were deemed unnecessary so they were never performed.

Clarke Fleege was the Bessey Nursery's nursery manager at time of experiment.

Treatments 5, 6, 7, 8, 19 abandoned because seed did not show signs of germinating

Georgia soil used to test affect soil microorganisms may have on germination.

Germination procedures

Seed were examined periodically and considered ready for germination testing when a few of the radicles emerged: a majority of the seed were swollen and had cracked seedcoats. Seed were removed from the plastic bag, rinsed, and allowed to surface dry before planting in germination dishes. Eight germination dishes with 25 seed each were placed in a walk-in germination room at a constant 59°F (15°C) with 8 hours of light/16 hours darkness. The fluorescent lights emitted 750 to 1250 lux or 75 to 125 foot candles which was in accordance with the rules of the Association of Official Seed Analysts, 1999 section 4.9.e. Pack (1921) noted that after-ripened seed should be planted at temperatures below 59°F (15°C). Chadwick (1946) found that germination following stratification

was best at 50°F (10°C) to 55°F (13°C) and germination was considerably retarded at temperatures above 65°F (18°C) to 70°F (21°C).

The clear, plastic germination dishes measured 4 ½-inch wide by 6 ½-inch long by 2 ½-inches tall. Kimpak® was used as the growing medium, and each dish received 80 milliliters of water before planting the seed. Germination was considered complete after the radicle, hypocotyl, and cotyledons emerged. The dishes were examined weekly and germination recorded. Weekly and total cumulative germination percentages were calculated.

Table 4

Best Treatments

Number	Treatment
17	3 day water soak, 16 weeks cold, 9 weeks warm, 9 weeks cold - seed germinated in stratification during the second cold period
21	3 day water soak, 16 weeks warm, 13 weeks cold
14	4 day soak, 12 weeks warm, 13 weeks cold
15	90 minute, 3% hydrogen peroxide soak, 8 weeks warm, 13 weeks cold
13	6 day 10,000 ppm citric acid soak, 6 weeks warm, 10 weeks cold

Table 5

Germination Results

Subsample	Treatment Number							Viability
	21	14	15	13	3	9	24	
8.0 x < 6.0H	55	55	48	8	4.5	7	0	81
7.5 x 6.0H	52	46	45	27	13	15	0	92
7.0 x 5.5H	54	64	46	30	13	9	0	94
6.5 x 5.5H	57	66	45	31	12	9	26	97
< 6.5 x 5.0 H	59	12	43	14	6	6	0	92
AVERAGE	55	49	45	22	10	9	6	91

H signifies heavy seed

Results

The mechanical weight separation successfully stratified the Rocky Mountain juniper seed by weight. The seed, with the greatest germination potential, were separated from low potential germination seed, thereby improving the seedlot. The average viability by weight was 80% heavy, 77% medium, 65% light, and 53% trash (table 1). There was a 27% viability difference between heavy and trash seed when averaged over size. The viability among all 93 sizes ranged from 36% to 97%, a 61% difference.

Five treatments produced the best germination (Table 4). Seed in treatment 17 germinated in cold stratification; this was the only treatment where cold stratification preceded warm stratification. Repeating the treatment may not be necessary, since it was Rietveld's (1989) best treatment on Rocky Mountain juniper seed. Rietveld's seed sources included collections from Meade County and Cheyenne River South Dakota, which is the same general area as this study's seed source. As further validation, the Forest Service Bessey Nursery uses a cold/warm/cold stratification regime.

The other four treatments had a warm stratification period followed by a cold stratification period. Van Haverbeke and Comer's (1985) recommended treatment (citric acid soak) was the fifth best treatment in the study. Treatment 21 (16 weeks warm, 13 weeks cold), proposed by Bessey Nursery's manager Clark Fleege, had the best germination of all treatments. Treatment 15 (3% hydrogen peroxide soak) was similar to Van Haverbeke and Comer's (1985) study, except they used 30% hydrogen peroxide. Their best hydrogen peroxide treatment (13 minute water soak, 30 minute hydrogen peroxide soak, 8 weeks warm stratification, 12 weeks cold stratification) yielded a 78.7% germination rate. Three of the five best treatments in this study were also the best treatments in other experiments.

The best treatments are not difficult to perform at the nursery. They can be used to augment the summer sowing program as insurance against a failed crop. In the event the summer sown seed do not come up, then the spring planted seed can be used to produce seedlings.

Several treatments (5, 6, 7, 8, 19) were abandoned when the seed was not ready to germinate after a year of pretreatment. Even if the treatments had worked, no one would use them because the treatments were impractical and they lasted too long. In treatment 19, the sulfuric acid carbonized the seed surface, thus prohibiting germination. Djavanshir and Fechner (1976) found that black carbon had to be removed by hand rubbing before seed would germinate. Carbon removal would be impractical when dealing with large seedlots.

The best treatments' germination ranged from 55% to 22% (Table 5). No germination results were reported for treatment 17. An accurate count could not be made because so much seed germinated in stratification. I was unable to determine when the seed had germinated during the second cold period and the seedlings had deteriorated into a gelatinous mess.

The heavy seed in each size class responded similarly to treatments resulting in a small variance around the mean. The majority of seed germinated in the first 2 weeks - signifying vigorous seed. Rapid germination is desirable in the nursery due to the many unforeseen environmental factors that can hinder seed germination.

Although viability of the five subsamples averaged 91%, the seed only germinated around 50% (Table 2). About 40% of the seed did not germinate, even though they stained red in the tetrazolium test. The tetrazolium test only indicated whether the seed was alive, not whether it was vigorous enough to germinate. The test did not predict germination, but was used as a benchmark of seed quality. From past experience, nursery managers have been developing a target number of seedlings from the tetrazolium results.

Since the best treatments in this study were also the best in other experiments even when the seed had low germination, the process should work on medium and light weight seed classes. They may give a more variable response to treatments, due to the presence of more ungerminable seed in the sample. Freshly harvested and cleaned seed may not require as long a warm/cold stratification period as stored seed. With the periodicity of seed crops, Rocky Mountain juniper seed will have to be stored, so these treatments will apply to most of the seed being planted.

Future recommendations

The first step is to try the best five treatments in a nursery. Will treatments act similarly in the nursery as they did in the laboratory where the seed were given the optimum conditions for germination? The germinator was set at a constant 59°F with only small temperature fluctuations. Nursery beds' temperatures fluctuate greatly between day and night. The ambient temperature may not be at the optimum when seed are ready to germinate, therefore inhibiting germination.

The second step is to try the treatments on other seed sources. Treatments worked well on this seed source, but what about the more westerly sources where introgression with *Juniperus virginiana* has

probably not occurred? Warm-moist stratification has not proved effective on enhancing germination of any juniper species where winter precipitation is dominant and summers are almost completely dry (Young and others 1981). Young and others (1981) have found that oxygenated, refrigerated water baths worked best for Utah juniper (*Juniperus osteosperma* Torr.) and western juniper (*Juniperus occidentalis* Hook.). This treatment may also increase germination for Rocky Mountain juniper and will be considered in the next stage of this study.

Statistical analysis of the data was not included in the nursery proceedings, but will be added in future reports.

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