Variable Seed Dormancy in Rocky Mountain Juniper[1]

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Abstract.-- Rocky Mountain juniper is difficult to grow in the nursery due to variable seed dormancy that spreads germination over time. In two experiments, six seed sources, five seed treatments, and 15 stratification treatments were tested. Although there were some seed source and stratification treatment differences, none of the treatments effectively enhanced germination amount or timing enough to be useful in nursery culture.

INTRODUCTION

Rocky Mountain Juniper (Juniperus scopulorum Sarg.) (RMJ) is widely planted for windbreaks and wildlife habitat in the Great Plains. The popularity and importance of this species resides in its cold hardiness, tolerance to drought, and relative freedom from insect and disease problems. In a windbreak, junipers provide a dense barrier throughout the year, resulting in excellent wind protection and snow control.

Despite its popularity, RMJ is difficult to grow in the nursery. Variable seed dormancy, and consequent low and variable germination are the underlying causes of these problems. The degree of seed dormancy varies by seed crop, seed source, seed age, and probably among and within individual trees (Van Haverbeke and Comer 1985, Young et al. 1988). Berries persist on the tree for 2-3 years, so a single collection could contain berries of varying age, including immature berries (Johnson and Alexander 1974). While seed dormancy is an ecologically important device to optimize the distribution of the species in time and space, it is an obstacle in nursery culture where prompt, uniform, and complete germination is required in order to grow high quality planting stock.

Juniper seed has both seed coat and chemical dormancy (Gerbracht 1937, Pack 1921). The seeds have a thick, semi-permeable seed coat that must be conditioned to imbibe water. Efforts to increase the permeability of the seed coat of Juniper seeds have included depulping (Afanasiev and Cress 1942); soaking seeds in sodium-lye (Webster and Ratliffe 1942), alcohol or boiling water (Chadwick 1946), concentrated sulfuric acid (Barton 1951), citric acid (Cotruto 1963, Van Haverbeke and Comer 1985), and hydrogen peroxide (Trappe 1961, Riffle and Springfield 1968); and freezing seeds in ice (Jelley 1937).

Although the usual method to overcome embryo dormancy of juniper seeds is classical cool/moist stratification for conifer seeds, stratification should not be restricted to cool temperatures. Van Haverbeke and Comer (1985) found that germination of eastern redcedar (Juniperus virginiana L.) seeds is enhanced by a combination of warm/moist stratification (75° F. for 6 weeks) followed by cool/moist stratification (41° F for 10 weeks).

The recommended stratification treatment for RMJ is warm/moist (68° F night / 86° F day) for 45-90 days, followed by cool/moist (41° F) for 30-120 days to induce germination (Johnson and Alexander 1974). This procedure is generally followed in Great Plains tree nurseries. A typical procedure is stratification for 60-240 days, sowing in mid- to late-summer, mulching the seedbeds, and keeping them moist through the fall. Germination occurs the following spring when temperatures reach 50° F (Benson 1976). Individual nurseries differ in the type of stratification used, sowing date, and type of mulch used.

Having said all this, I would like to quickly point out that the actual success of applying all of these techniques to germinate RMJ seed has been minimal, typically 20-50% germination. Consequently, seed germination of RMJ is identified as being one of the highest priority problems in Great Plains nurseries. In response to a request for research assistance, the

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Rocky Mountain Station contracted research on "Development of pre-germination treatments to best achieve predictable and uniform germination of Juniperus scopulorum in the shortest time" with SWCA, Inc. in Flagstaff, AZ. This paper reports the results of that research.

MATERIALS AND METHODS

The study consisted of two experiments conducted in 1987-88 and 1988-89, which will be presented separately.

Experiment 1

The experiment tested three seed sources, five seed scarification treatments, and six stratification treatments. Mature berries were collected in late October, 1987 from 10-20 trees in Coconino County, AZ, elevation 6840'; Iron County, UT, elevation 6400'; and in Cassia County, ID, elevation 6100'. The locations represented a general north-south transect, well west of the range of Junipers virginiana to avoid any intermixing. Within 30 days of collection, seeds were depulped, floated, and soaked in isopropyl alcohol to remove residues. The seeds were temporarily stored at 50° F and 10% RH until early December.

The seed scarification treatments consisted of: (1) none, (2) seeds dropped in boiling water, then removed immediately from the heat and allowed to stand 24 hrs; (3) seeds soaked in concentrated sulfuric acid for 0.5 hr; (4) seeds soaked in 1% citric acid for 96 hours; and (5) seeds soaked in 30% hydrogen peroxide for 0.5 hr. Following chemical treatments, seeds were stratified in 4-mil zip-lock bags in a 1:1 peat/vermiculte medium that was fully dampened and treated with a fungicide to prevent molding. Stratification treatments (fig. 1) were combinations of warm/moist (86° F 8 hrs, 68° F 16 hrs) and/or cool/moist (38-41° F). Treatments began on December 8, 1987, and germination tests were completed on July 6, 1988.

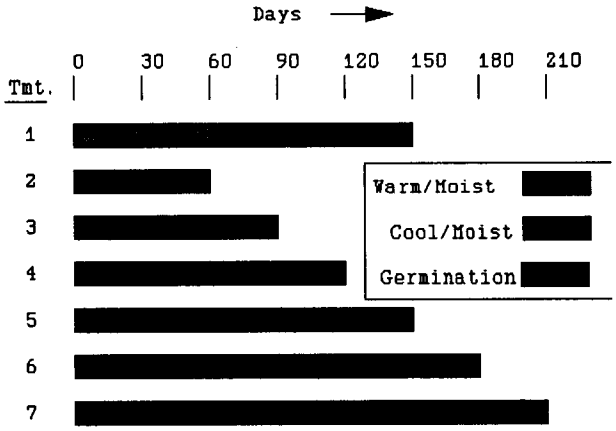

Figure 1. Stratification treatments and schedule for Experiment 1.

When stratification treatments ended the seeds were germinated in flats of 1:1:1 peat/vermiculite/perlite in a greenhouse. Each treatment combination was represented by 100 seeds; there was no replication of treatments. The flats were watered 3-4 times a week. Germination was checked daily for 30 days. Greenhouse temperatures during germination tests differed because of the varying duration of the stratification treatments:

	Jan	Feb
Avg.	54	56
Max.	67	70
Min.	38	45
Regime	2	3

Greenhouse temperature averaged 54-60° F during germination tests for treatments 1-5, and 64-69° F for treatments 6-7.

Experiment 2

The experiment tested four seed sources and eight stratification treatments. All seed was collected in fall, 1988. Seed from three sources was obtained from Great Plains tree nurseries, and the fourth was locally-collected by the contractor. They were: (1) Saguache County, CO, elevation 8500' (obtained from the CO State Nursery); (2) seed zone 600, Meade County, SD (obtained from Big Sioux nursery); (3) Cheyenne River, SD (obtained from USFS Bessey nursery; and (4) Coconino County, AZ, elevation 7000' (locally collected).

Other than depulping and lye-soaking the seed to remove residues, there were no seed scarification treatments in Experiment 2. The stratification treatments (fig. 2) consisted of combinations of warm/moist and cool/moist at the temperatures listed for Experiment 1, and freezing treatments at 5-10° F. Stratification treatments began on December 23, 1988 and germination tests were completed on July 24, 1989.

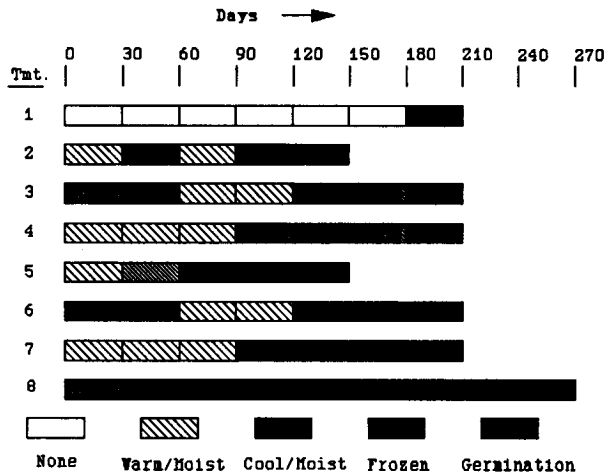

Figure 2. Stratification treatments and schedule for Experiment 2.

Seeds were germinated in the same medium and location as in Experiment 1. Each treatment combination was represented by five flats of 100 seeds. Germination temperatures were 71.5° F for stratification treatments 1-7 and 66.6° F for treatment 8.

RESULTS

Seed germination in Experiment 1 was very low; most treatment combinations had zero germination during the 30-day germination test. The only treatment that resulted in any seed germination was the combination of no seed scarification and warm/moist 60 days + cool/moist 60 days (stratification regime 1). Seed germination was 10%, 1%, and 3% for the Arizona, Utah, and Idaho sources, respectively.

In Experiment 2, samples of the seed were sent to the USFS National Tree Seed Laboratory for viability testing (tetrazolium method) before the stratification treatments were begun. Average percent viability was 70, 57, 82, and 71 for seed obtained from the Colorado State Nursery, Big Sioux Nursery, Bessey Nursery, and locally collected near Flagstaff, Arizona, respectively. Analysis of Variance of germination data and application of Tukey's Studentized Range Test revealed significant differences by seed source and stratification treatments. Treatment effects were the same using percentage germination data based on all seeds or viable seeds. For consistency with Experiment 1, germination data presented are based on all seeds tested.

Effect of seed source

Seed collected in Arizona had significantly lower germination rates than did seeds provided by the Bessey Nursery and Colorado State Nursery (Fig. 3). Germination of Arizona seeds was lower, but not significantly lower, than Big Sioux Nursery seeds. Big Sioux seeds, while lower in germination, were not significantly different from Bessey and Colorado State Nurseries.

Effects of stratification treatment

Stratification treatments 2-8 were not significantly different from the control (treatment 1) (Fig. 4). The lowest germination rate was from treatment 5. The highest germination rate was recorded for treatment 3; this stratification treatment was significantly different from all other stratification treatments.

Seed Source	% Germination	Significance
Bessey Nursery	2.8	
Colo. St. Nursery	2.6	
Big Sioux Nursery	1.8	
Arizona	0.7	

Figure 3. Percent germination by seed source for Experiment 2.

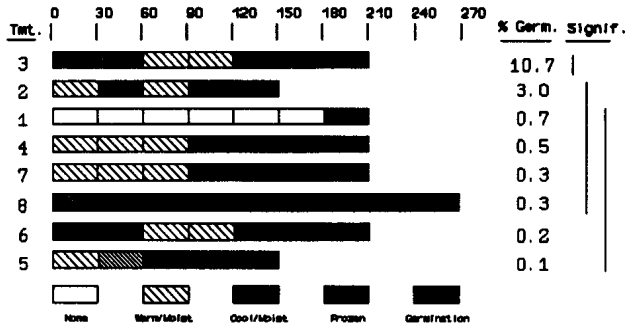

Figure 4. Percent germination by stratification treatment for Experiment 2.

Overall, the highest germination rates were recorded for Bessey Nursery seed (2.88), and stratification treatment 3 (10.78).

DISCUSSION

Seed germination was unexpectedly low in both experiments. In Experiment 1, the highest germination (10%) was attained for Arizona seed in stratification treatment 1 (warm/moist 60 days followed by cool/moist 60 days). In Experiment 2, the highest germination (18.6%) was attained for Bessey Nursery seed in stratification treatment 3 (cool/moist 60 days, followed by warm/moist 60 days, followed by cool/moist 60 days). Thus, the pattern was not consistent. Experiment 2 contained three stratification treatments with freezing treatments, which were intended to simulate a cycle of warm and cold seasons, but the resulting germination of seeds subjected to treatments including freezing ranked lowest in the experiment.

The low germination rates in both experiments suggests that some confounding factor was involved. Possible sources of the problem are: (1) collection of immature seed, (2) stratification of seed in plastic bags may have restricted air exchange, and (3) higher germination temperatures may have retarded germination. Collection of immature seed can probably be ruled out since seed obtained from three nurseries in Experiment 2 also had low germination. Restricted aeration from stratification in plastic bags can also be ruled out because 4-mil plastic is sufficiently thin to allow gas exchange (Bonner,1971). However, restricted aeration due to the impermeable seedcoat may be an important factor limiting the effectiveness of stratification, as discussed later. The most likely confounding factor in the experiments is high temperature inhibition of seed germination. Juniper seeds germinate best at 50-55° F, and germination is retarded above 65-70° F (Chadwick 1946, Meines 1965, Wycoff 1961). However, none of these authors specified whether these were average temperatures, or if the inhibition occurs when daily highs exceed 70° F. Average germination temperatures in Experiment 1 were generally favorable through May, however

high temperatures exceeded 70° F as early as February.

In summarizing the results of 10 years of research to enhance seed germination of Utah juniper (Junipers osteosperma (Torr.) Little) and western juniper (J. occidentalis subsp. occidentalis Hook.), Young et al. (1988) reported 10 to 37% germination achievable through various stratification treatments. Variations in substrate, moisture content of substrates, or temperature were generally not effective in enhancing germination. An alternative method of stratification that enhanced germination was prolonged immersion in refrigerated (41° F) water baths where oxygen was kept near saturation in the water by actively bubbling compressed air into the bath. This treatment for 12-14 weeks brought germination up to 43 to 58%. Germination was not enhanced by prolonged soaking in non-aerated refrigerated water. Germination was further enhanced to 64 to 84% by addition of 0.284 m mol L^{-1} of Gibberellic acid to the oxygenated cool water baths. These results suggest that the key factors required to accomplish enhanced germination of juniper seed include: (1) time, because of the restricted permeability of the seed coat and slowness of the processes involved; (2) oxygenation; and (3) a growth promoter.

There are two concepts of seed dormancy that pertain to junipers. A brief discussion of these will assist in understanding the complexity of the problems. The hormone interaction model (Kahn 1975) asserts that dormancy or germination results from the balance among gibberellic acid (GA), cytokinins (CK), and an inhibitor (presumably abscisic acid, ABA). The model assumes that GA is a primary hormone that induces germinative enzymatic processes, and CK and ABA are secondary competitive factors. Cytokinins oppose (i.e. neutralize) the inhibitory effects of ABA. The hormonal balance changes during the after-ripening process, and eventually leads to a condition that allows germination. This may include: (1) the disappearance of ABA, or (2) synthesis of GA and/or CK. The model allows that seeds can be either dormant or capable of germination under several alternative hormonal conditions, for example:

#	GA vs.	CK vs.	ABA
1	+	+	+
2	+	+	-
3	+	-	+
4	+	-	-
5	-	-	-
6	-	-	+
7	-	+	-
8	-	+	+

This model helps explain many of the anomalous hormonal situations in seeds, e.g. (1) dormancy without the presence of inhibitors (#5,#7), (2) germination in the presence of high levels of inhibitors (#1), and (3) dormancy in spite of high levels of GA or CK (#3,#7,#8). The response to the GA treatments by Young et al. (1988) could have been due to the existence of situations #7 or #8, resulting in germination as in situation #2 or #1, respectively. Their results also suggest that considerable time may be required for the changes to occur.

A second concept of seed dormancy pertaining to junipers is shifts in oxidative pathways (Roberts 1973). The change from dormant to germinating seeds is often accompanied by an increased functioning of the pentose-phosphate pathway of glucose use, an important pathway of respiratory metabolism in seeds. The model asserts that dormancy is caused by the enzyme catalase which causes the destruction of metabolically-derived hydrogen peroxide needed for the oxidation of $NADPH^{+}$. Dormancy release, resulting from the oxidative inactivation of catalase, would allow the respiratory pathway to function at an accelerated level. The results of Young et al. (1988) could also be explained by this model. The seed coat may restrict the entry of oxygen into the seed; but with high oxygen concentrations, sufficient oxygenation may occur over time to inactivate catalase. Once germination begins, the gibberellic acid is needed for germinative enzymatic processes, e.g. synthesis of hydrolytic enzymes which mobilize stored substrates.

Evidence to date does not support this model, but further investigation is clearly needed. Several investigators (e.g. Hendricks and Taylorson 1975) have reported that compounds such as nitrites and thiourea that inhibit catalase activity are effective in releasing seed dormancy in certain species. Bonner et al. (personal communication) have tested thiourea at 1% and 2% concentrations, with and without added cytokinin, on eastern redcedar seeds, and found that the treatments reduced germination. The concentrations applied were quite high, however, and likely caused germination inhibition from the thiourea. Hendricks and Taylorson (1975) found thiourea concentrations greater than 20 mM were inhibitory, and cautioned against using high concentrations for short periods of time.

A study on changes within the seeds of RMJ during the processes of after-ripening and germination (Afanasiev and Cress 1942) also introduces some uncertainty about the catalase-dormancy model. During stratification at low temperatures they found: (1) a slight increase in peroxidase content, (2) appearance of oxidase, and (3) an increase in catalase activity. During germination, the activity of oxidizing enzymes increased markedly. The increase in catalase activity associated with the completion of after-ripening and germination is the opposite of that asserted by the catalase-dormancy model, but the increase in activity of oxidizing enzymes is consistent.

CONCLUSIONS

Although there were some seed source and stratification treatment effects in Experiment 2, none of the treatments tested in this study effectively enhanced germination amount or timing to be useful in nursery culture.

Virtually every treatment known to seed technology has been tried with seeds of various species of junipers. The majority of experimentation, including the present study, has focused on seed scarification treatments and stratification treatments, with minimal success. What is truly needed is basic research to understand the physiological and biochemical mechanisms responsible for the dormancy. Then we will have the basis to develop treatments to achieve prompt and uniform germination.

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