Seed Set and Germination of Eldarica Pine Influenced by Cone Hierarchy¹

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Harrington, John T.; Mexal, John G.; Fisher, James T. 1989. Seed Set and Germination of Eldarica Pine Influenced by Cone Hierarchy. In: Landis, T.D., technical coordinator. Proceedings, Intermountain Forest Nursery Association; 1989 August 14-18; Bismarck, ND. General Technical Report RM-184. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station: 65-69. Available at: http://www.fcnanet.org/proceedings/1989/harrington.pdf

Abstract.-- Tree and cone hierarchy effects on eldarica pine seed quantity and quality are examined. Hierarchy, or location of cone whorl on a branch, and tree significantly influenced total number of seed/cone, number of viable seed/cone and percent viable seed. Whorl 2 cones, the distal whorl, had 54% more seed/cone and a 65% greater percent viable seed. Percent germination, total germination, G_{50} and number of cones/whorl were not effected by either tree or hierarchy. Relationships between total number of seed/cone and number of seed germinating/cone and percent germination are provided. Potential causes for hierarchal effects are discussed.

INTRODUCTION

Eldarica pine (*Pinus eldarica* Medw. (= P. *brutia* subsp. <u>eldarica</u>) is a member of the *Pinus brutia* group of mediterranean pines (Spencer 1985). The only naturally regenerating population of eldarica pine occurs from 200-600 meters elevation in the semi-arid steppe region in the Russian republic of Georgia (Zimina 1978, Mirov 1967 as cited by Spencer 1985). This species, however, has been introduced to numerous countries in Europe, the middle east and Asia, as well as the United States and Australia (Fisher 1985). Eldarica pine was first introduced to the United States in 1961 in southern California (Spencer 1985).

Eldarica pine is used for Christmas and ornamental trees, and in windbreaks throughout its potential range (fig. 1). Eldarica pine can tolerate alkaline soils (Fisher 1985), high levels of salinity (Manuchia 1986) and demonstrates growth rates comparable to *Pinus radiata* and Pinus *caribaea* when well watered (Fisher et al. 1986). Eldarica pine's polycyclic growth habit and deep root system allow it to fully utilize the long growing seasons and deep soil moisture reserves found in regions of the southwestern United States. Furthermore, tests on the

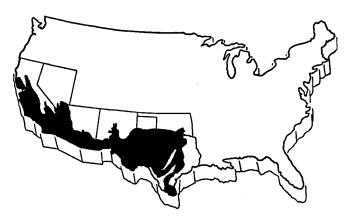


Figure 1.--Potential distribution of eldarica pine in the United States (Fisher 1985).

wood properties of eldarica pine indicate it has potential for manufactured wood, paper pulp and fuelwood production (Fisher 1985).

Russia, Iran, Afghanistan and Pakistan are the leading growers of eldarica pine worldwide. Because of international relations with these countries and the lack of a worldwide seed certification program for eldarica pine, obtaining seed can be time consuming, difficult and expensive. While seed companies in the United States sell eldarica pine seed, seed costs and quality vary dramatically. Costs incurred by local (Las Cruces, NM) nurserymen in 1989 ranged from \$110 to \$310 per kilogram (\$50 to 140/lb) and seed germination varied from 30 to 60% following float sorting of the seed.

¹ Paper presented at the Intermountain Forest Nursery Association Annual Meeting (Bismarck, ND, August 14-17, 1989). New Mexico Agric. Exp. Sta. Scientific Paper no. 338

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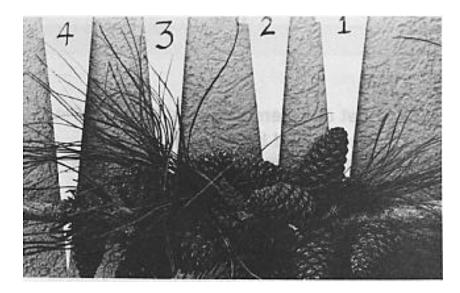


Figure 2.—Photograph of 4 cone whorls produced on an 17 yearold eldarica pine branch growing in Las Cruces, New Mexico.

Eldarica pine has a relatively unique cone production habit, similar to *Pinus clausa* and *Pinus caribaea* (USDA 1974). Individuals can set multiple flushes or whorls of female cones during one growing season (fig. 2); however, this response is site specific (USDA 1974). This trait affords eldarica pine growers in this region the ability to produce large quantities of seed in a short time (relative to single whorl species). The purpose(s) of this study was to determine if cone heirarchy influenced seed quantity and quality in trees demonstrating this multi-whorl habit, and to examine the tree-to-tree variation in this response.

METHODS AND MATERIALS

Plant Material

Three, 10-year old plantation-grown eldarica pine trees at the Fabian Garcia Science Center in Las Cruces, NM were randomly selected and used in this study. The two most recently matured cone whorls were harvested on two branches of each tree on November 2, 1988. The two whorls of cones per branch were labeled. Whorl I was the first whorl of cones produced on the branch; therefore, the whorl closest to the bole of the tree. Whorl 2 was the second whorl of cones produced on the branch during the growing season, and was the whorl closest to the periphery of the crown. No information was taken on the difference in time between cone whorl production.

Eldarica pine has serotinous cones, so seed was extracted by hand using a grafting knife to peel back the cone scales and expose the seed. Total number of seed and total seed weight were recorded following extraction. Total number of seed includes all developed seed and large second year aborted ovules (i.e. pops). According to Bramlett et al. (1977), developed seed can fall into three catagories: filled, partially filled and empty

seed (pops). Total seed number does not include first-year aborted ovules (seedless wings) that were observed but not counted. These terms are described in detail by Bran-Jett et al. (1977) and are addressed in the discussion section of this report.

Germination

Eldarica pine has no reported stratification requirement so none were performed. Following extraction, seeds were soaked for 10 hours in distilled water (25°C). The soaking served two purposes, it separated filled (sinkers) from unfilled (floaters or pops) seed, and allowed the seed to soak up enough water to initiate the germination process. The floaters were air-dried for 48 hours, and their weight and numbers were recorded. These seed were subsequently examined for filling and relatively few (ca 0.7%) were filled.

Filled seed was hand sown in flats of steam sterilized vermiculite, and covered with clear plastic to maintain a moist environment. Flats were placed on lab benches where temperatures ranged from 21 to 25°C. Germination was monitored for the next 30 days. A seed was considered germinated when the hypocotyl broke the vermiculite surface. Percent germination, total germination and G_{50} , or date at which 50% of the seed germinated, were determined from this information.

Statistical Considerations

The experimental design was a randomized complete block design with blocking by tree and branches serving as repetitions per block. Table 1

Table 1.--Analysis of variance partitioning of degrees of freedom for the study design.

SOURCE	df
Tree (Block)	2
Whorl	1
Interaction	2
Exper Error	7
Total	11

illustrates the partitioning of the degrees of freedom of the design. Analysis of variance was performed to test the effects of source (tree) and whorl position on cone number per whorl, total number of seed per cone, total number of viable seed per cone, percent viable seed, mean weight of viable seed, total germination, percent germination and G₅₀. Analysis was performed using the PROC GLM of SAS-Version 5 (SAS Institute Inc. 1989).

Regression analysis was performed using number of sound seed per cone as an independent variable on the dependent variables, number of seed germinating per cone and percent germination. Analysis was performed using the PROC REG of SAS Version 5 (SAS Institute Inc., 1989).

RESULTS

Tree significantly impacted both seed quality and quantity (table 2). Among individual trees, tree 2 had both the greatest number of seed per cone, number of viable seed per cone as well as the greatest percent viable s eed. Tree 2 also had the highest percentage germination. While not statistically significant (alpha = 0.05), source appeared to influence mean weight of viable seed and G_{50} (PR > F = 0.116). Trees 1 and 3 had comparable numbers of viable seed per cone but total number of seed per cone and percent viable seed differed.

Cone heirarchy or whorl position also impacted seed quantity and quality attributes. Whorl 2 cones, those closest to the periphery of the crown and latest to develop, had significantly greater total seed, number of viable seed and percent viable seed (table 3). Mean weight of viable seed, percent germination and G_{50} were unaffected (P < 0.05) by cone position in this study. As was the case with source, whorl position did not influence the number of cones per whorl.

The relationship between the number of germinating seed and total number of seed extracted per cone (fig. 3) is a positive, linear relationship where an increase in the total number of seed per cone yields an increase number of seed germinating. Tree 1 data appear to follow a less steep, positive relationship than do data points from trees 2 and 3. When tree I data is dropped from the data set, the model improves with the line becoming steeper and the confidence interval narrowing.

Table 2.-- Mean cone and seed attributes and observed significance levels (PR>F) for the three plantation-grown eldarica pine. Seed Wt. (mg) = mean weight of sound seed; '-' = tree 1 material failed to reach 50% germination

Parameter	1	2	3	PR>F
Cones/whorl (no.)	3.8	3.3	2.8	0.488
Total Seed/Cone (no.)	54	103	62	0.004
Sound seed/cone (no.)	28.2	11.1	27.5	0.003
Sound Seed (%)	41.7	68.8	38.2	0.050
Seed Wt (mg)	70.7	56.9	61.7	0.116
Germination (%)	24.4	79.8	64.0	0.004
G ₅₀ (d)	-	12.9	21.3	0.262

Table 3.--Mean cone and seed attributes and observed significance levels (PR > F) for whorl 1 and whorl 2 of eldarica pine. Seed Wt. (mg) = mean weight of sound seed

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Parameter	1	2	PR>R
Cones/whorl (no.)	3.2	3.3	0.804
Total Seed/cone (no.)	57	88	800.0
Sound Seed/cone (no.)	26.6	58.0	0.004
Sound Seed (%)	38.9	64.1	0.019
Seed Wt. (mg)	64.6	61.6	0.546
Germination (%)	51.1	60.8	0.333
G ₅₀ (d)	19.5	14.7	0.503

The relationship between germination percent and total number of extracted seed per cone is also positive and linear (fig. 4). However, this relationship is weaker as indicated by the lower correlation coefficient and the wider confidence limits.

DISCUSSION

The relatively large percentages of unfilled seed found in this study may be caused by three agents. First, several insects including seedbugs (*Tetyra bipunctata, Leptoglossus corculus*) attack southern pine cones (Bramlett et al. 1977). Second, attack by several species of fungi can cause unfilled or partially filled seed (Bramlett et al. 1977). A third potential cause for unfilled seed is selfing, or more precisely, homozygous recessive embryonic lethal genes. Most members of the Pinus genus have varying numbers of these genes (Bramlett pers. comm.³). When this condition occurs, it can result in either a first-year aborted ovule, (i.e. a seedless wing), or a second-year aborted ovule, (Le. an unfilled seed). A seedless wing is an unfilled seed that never enlarges to full seed size, while an unfilled seed is a full-sized, empty seed

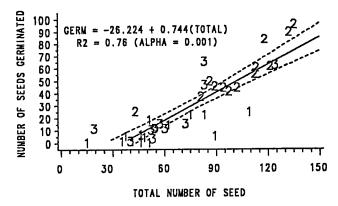


Figure 3.-- Relationship between total number of seed per cone and number of seed germinating per cone. Data points 1, 2 and 3 are those obtained from trees 1, 2 and 3 respectively. Solid line is calculated regression equation and dashed lines represent a 95% confidence limit on the mean.

coat. The material used in this study, while not examined microscopically, had no evidence of insect or fungal attack. This could indicate selfing as the likely cause of the empty seed and the greater percentage of empty seed in whorl 1 found in this study. Possibly, whorl 1 cone receptivity coincides with the time when its own pollen is shed, resulting in greater percentages of selfing in these cones.

The reduced total amount of seed (empty and filled combined) in whorl 1 may be attributable to selfing. Homozygous embryonic lethal genes may result in first-year aborted ovules. Seedless wings were observed in this study, but no data were recorded. If the timing of receptivity for the whorl 1 cones coincided with that of pollen dispersion for the same tree, this may have resulted in first-year aborted ovules, which would have decreased the total seed numbers in this whorl.

A second explanation for the decreased total number of seed in whorl 1 cones may be the result of an overall decrease in the amount of pollination of these cones. While pollen production and cone development timing were not examined in this study, whorl 1 cones possibly were receptive before the majority of pollen production in the stand. This would result in the reduced levels of seed production in these cones.

Cone hierarchy did not statistically impact G_{50} in this study. This may be because of the overall poor germination percentage of tree 1. Only one whorl of cones of this tree exceeded 50% germination during the evaluation period. Overall, whorl 2 seed of tree 1 germinated faster than whorl 1 seed. The approximate 5 days difference between the G50 , in the complete data set, of whorl I and whorl 2 cones amounts to a 25% decrease in G_{50} of whorl 2 cones.

The number of cones per whorl varied from three to five cones, and was not under any strong hierarchy or

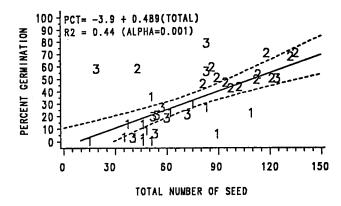


Figure 4.-- Relationship between total number of seed per cone and percent germination. Data points 1, 2 and 3 are those obtained from trees 1, 2 and 3 respectively. Solid line is calculated regression equation and dashed lines represent a 95% confidence limit on the mean.

tree influence. Most eldarica pine grown in the plantation averaged between three and five cones per whorl. However, eldarica pine can set as many as 20 cones per whorl in southern New Mexico and southeastern Arizona (Harrington and Mexal pers. obs.). It appears number of cones per whorl is primarily environmentally controlled.

As would be expected, tree impacted some of the attributes examined in this study. While eldarica pine is believed to have originated from a relatively small, 550 ha, naturally regenerating stand in Soviet Georgia (Spencer 1985), its genetic base is variable enough to justify the implementation of some screening regime when selecting potential seed trees.

Number of seed germinating per cone appears to be related to total number of seed per cone. However, this relationship is strongly influenced by tree. As can be seen in figure 3, tree 1 data appear to follow a less steep line than do data from trees 2 and 3. A positive relationship between these variables would be expected because more seeds per cone provides more opportunities for seed to germinate. The relationship may afford a grower a criterion suitable for selecting potential seed trees.

The relationship between percent germination and total number of seeds per cone is linear and positive, but is not as defined as the relationship between number of seeds germinating and total number of seeds per cone (r2 = 0.44 vs. 0.76 respectively). While total number of seeds per cone accounted for only 44% of the variation in percent germination, this relationship was unexpected. A possible explanation for this relationship may be that more fertilized ovules result in a stronger carbohydrate sink such that seed vigor improves concomitantly with overall seed set. Shifriss (pers. comm. 4) found pepper fruit size was positively correlated with number of seed set. Possibly as seed set increases, fruit size increases, and seed vigor, as measured by germination, improves. Further work is needed to identify easy-to-measure attributes that reliably predict seed quality and quantity

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³ Bramlett, D.L. 1989. Personal correspondence. USDA For. Ser. Southeastern Forest Exp. Station, Dry Branch GA 31020.

⁴ Shifriss, C. 1989. New Mexico State University Seminar 9/15/89. The Volcani Center, Bet Dagan, Israel.

to be used when selecting potential seed trees. Attributes such as cone length or width may be potential candidates because they are measured easily in the field and may have predictive value.

IMPLICATIONS

Improving seed yields in eldarica pine cone crops may be dependent on supplemental pollination of whorl 1 cones and/or basal pruning seed trees. The majority of staminate cones are produced on the lower one-third of the crown. Removing this pollen source would decrease the likelihood of first and second-year aborted ovules resulting from genetic constraints. If a sufficiently large seed crop is produced, it may be feasible to preferentially collect whorl 2 cones to reduce costs of seed extraction by concentrating efforts and energy on cones with higher proportions of sound seed. Finally, further work is needed to determine the causes of empty seed in eldarica pine, to develop criteria for screening potential seed trees and to understand the influence of cone hierarchy on these criteria.

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