CONTROLLED POLLINATION IN EASTERN REDCEDAR AND ROCKY MOUNTAIN JUNIPER[1]

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ABSTRACT.--Pollination with forced and fresh eastern redcedar pollen was compared to wind pollination (and unpollinated controls). This is the first attempt at controlled pollination in juniper. Seeds were extracted, and cutting tests and germination tests were conducted to evaluate the success of pollinations. Artificial crosses were also made with eastern redcedar pollen on a single Rocky Mountain juniper female tree. First-year fruits were collected and evaluated. Preliminary results indicate that wind pollination is less reliable than control-pollination in obtaining sound seed set of eastern redcedar. This may explain the high proportion of empty seeds found and the low reproduction obtained in many natural stands.

INTRODUCTION

The low proportion of filled seeds in developed fruits of *Juniperus* L. has been observed frequently. This low set of sound seeds may be the result of poor pollination due to a lack of synchronization between pollen release from male trees and ovular receptivity on female trees in the vicinity. If this were true, artificial pollinations could enhance sound seed set, provided that pollen viability could be maintained. However, the only previous report of artificial pollination in juniper is that of Djavanshir (1974) in which pollen was applied to non-isolated female strobili of *Juniperus polycarpos* C. Koch in Iran.

Rocky Mountain juniper (*J. scopulorum* Sarg.) has long been considered a western form of eastern redcedar (*J. virginiana* L.). In fact, early western explorers and botanists (James 1823; Torrey 1828) did not recognize the subtle differences that were later used to separate these two species. Recently field studies of several North American junipers have revealed individuals possessing combinations of morphological characteristics belonging to different species. Intergrades have been noted between eastern redcedar and Ashe juniper (*J. ashei* Buchholz) (Hall 1952a,1952b, 1955), between eastern redcedar and Rocky Mountain

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juniper (Fassett 1944; Van Haverbeke 1968), and between Rocky Mountain juniper and creeping juniper (*J. horizontalis* Moench) (Van Haverbeke 1968). These intergrades have been presumed to be the result of interspecies hybridization. However, no controlled crossing attempts intended to verify such presumptions have been reported.

The objectives. of this study were, therefore, (1) to compare the fruit and seed set, seed germination, and initial seedling development resulting from controlled pollination with stored or fresh eastern redcedar pollen; and (2) to attempt artificial crosses between Rocky Mountain juniper and eastern redcedar.

METHODS

Beginning in October 1973 and continuing through February 1974, branches were collected at 10-day intervals from two eastern redcedar male trees (Tree A and Tree B) on the Colorado State University campus. The branches were forced in water at room temperature (25 to 27°C), and the pollen obtained was stored in cotton-stoppered vials at 0 to 4°C. Fresh pollen was extracted from the male trees on March 4, 1974. [3]

On February 27, 1974, before receptivity, strobili were isolated on three eastern redcedar female trees (JV-1, JV-2, JV-3) on the Colorado State University campus. First pollinations with fresh and stored pollen were made March 7 and 8 on trees JV-1 and JV-3 and on March 11 on JV-2. Receptive female strobili, terminal on short lateral shoots, were reddish-yellow to brownish in color. Repollinations were performed on March 11, 15, and 21. All bags received three pollinations; some received four, depending on the amount of pollen available. Unpollinated and wind-pollinated control bags were maintained on all three female trees Pollen treatments were assigned randomly to the bags.

Branches on a single Rocky Mountain juniper female tree were isolated on March 5, 1974, and pollinations with a single eastern redcedar male (Tree A) were made on April 25, when Rocky Mountain juniper male trees were actively shedding pollen, and again on May 2. Unpollinated and wind-pollinated controls were maintained.

Isolation bags were removed from the tagged branches of the eastern redcedar females on April 25 and from the Rocky Mountain juniper female on May 16, when wind-pollinated branches were also marked. On October 23 and 25, 1974, the eastern redcedar branches were collected and placed into labeled bags; the same was done for the Rocky Mountain juniper bags on December 20, 1974.

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3 Natural pollen shed began on the two male trees on March 6 and continued until March 15. Peak pollen shed was March 11.

Between October 1974 and January 1975, all of the developed fruits were measured to the nearest 0.1 mm, and the seeds of 90 percent of the fruits were cut to determine whether they were filled or empty. Seeds from 10 percent of the fruits from each female x male combination were washed, their tips cut (Djavanshir and Fechner 1975b) [4], and the seed stratified between moist paper toweling in petri dishes at 5°C.

On May 6, 1975, the stratified seeds were divided into two replicates from each of the pollination bags and transferred to an incubator for germination tests under an 18°C, 14-hour day/8 °C, 10-hour night regime. Germination of seeds was recorded at approximately 3-day intervals until June 12, 1975. Seedling length was also measured, and when the cotyledons had shed their seed coats, the seedlings were transplanted into plastic pots containing vermiculite; they were watered and kept at the same temperature-light regime as the seeds. Beginning on June 17, Hoagland's nutrient solution was used for watering at 3-day intervals until the study was terminated July 8, when the seedlings were measured and their dry weights obtained.

RESULTS AND DISCUSSION

Fruit Set

No fruit was set on branches of either eastern redcedar or Rocky Mountain juniper in the absence of pollination, [5] but undeveloped female strobili were still identifiable on unpollinated control branches when the branches were collected.

Average fruit set per unit dry weight of bagged branchlet varied among the pollen sources and among the three eastern redcedar female trees. Higher fruit set was obtained on each female tree with fresh pollen than with stored pollen from the same male tree. Fresh pollen from Tree B produced a higher fruit set than fresh pollen from Tree A or wind-borne pollen. Pollen from Tree A produced a lower fruit set than wind-borne pollen on two of the trees (table 1). Variation in fruit set among the female trees may simply reflect difference in the number of strobili produced in the study year.

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4 Djavanshir, K. and Gilbert H. Fechner. 1975b. Epicotyl and hypocotyl germination of eastern redcedar and Rocky Mountain juniper. Unpublished manuscript.

5 An exception was a single fruit containing a single empty seed that may have resulted from unknown contamination (see table 1).

Table 1.--Fruit set from controlled pollination on eastern redcedar and Rocky Mountain juniper

Female parent [1]	Date, type pollination by male parent [2]	Bags	Fruit set: Total	Fruit set: Per gram branch weight
		Number	Number	
INTRASPECIFIC CROSSES				
JV-1	A Feb. 10	1	2	0.26
	A Fresh	2	162	5.83
	B Dec. 31	3	48	2.50
	B Feb. 10	3	94	1.82
	B Fresh	2	300	6.22
	Wind	5	117	1.70
	Unpollinated	3	1	0.04
JV-2	A Jan. 31	1	11	1.01
	A Fresh	3	55	1.52
	Wind	5	152	1.66
	Unpollinated	2	0	0.00
JV-3	A Jan. 31	2	26	0.56
	A Fresh	4	140	1.22
	B Jan. 10	3	149	1.71
	B Fresh	3	192	2.60
	Wind	5	201	1.48
	Unpollinated	2	0	0.00
INTERSPECIFIC CROSSES				
JS-1	A Fresh	17	122	0.36
	Wind	5	218	2.48
	Unpollinated	4	0	0.00

1/ JV = *J. virginiana*; JS = *J. scopulorum*.

2/ "A" and "B" are *J. virginiana* male trees

Fruit set was obtained in 13 of 17 bags on Rocky Mountain juniper cross-pollinated with eastern redcedar, but fruit set per unit branch weight was only about 1/7 that obtained by wind pollination. Fruit set per cross-pollinated bag was extremely variable, averaging 7.6 fruits per bag t 5.46 (2S.E.); in contrast, wind pollination resulted in an average set of 43.6 fruits per bag. The most likely source of wind-borne pollen is several Rocky Mountain juniper male trees near the JS-1 female.

The fruits of both study species are irregularly ellipsoidal in shape, usually elongated along the cone axis. Fruit length varied from 4.0 to 8.1 mm (average 5.8 mm) for the four trees, and fruit width varied from 2.8 to 6.5 mm (average 4.5 mm). The Rocky Mountain juniper fruits were significantly larger than those of the grouped eastern redcedar trees, primarily due to exceptionally small fruits on one of the latter, according to Duncan's multiple range test (95 percent level of probability).

Seed Set

The set of sound seed on eastern redcedar varied among the female trees studied, JV-1 averaging 0.90 sound seeds per fruit, significantly higher than the other two trees (table 2). Seed set differed significantly among the pollen sources within the JV-2 and JV-3 female trees, primarily due to one pollen treatment, Tree A Jan, 31, which was high on one tree and low on the other tree. Of particular interest, however, is the fact that stored pollen was usually as effective, or more so, in bringing about sound seed set as fresh pollen from the same male parent or wind-borne pollen were. This supports the suggestion that tube length of eastern redcedar pollen *in vitro* after storing is a measure of its ability to affect *in vivo* fertilization (Djavanshir and Fechner 1975a). The considerable bag-to-bag variation observed suggests that other factors were more important in affecting sound seed set than simply the pollen sources used. No doubt the precise timing of receptivity of the female strobili varied from bag-to-bag, and even though several pollen applications were made, ideal pollination conditions may not always have existed.

Seed set did not differ significantly between the cross-pollinated and the wind-pollinated branches of the Rocky Mountain juniper female, averaging 0.90 sound seeds per fruit. Eastern redcedar pollen was as effective in producing sound seed in fruits that were set as was wind-borne pollen. Thus, incompatibility barriers between these two species are not absolute.

The set of empty seeds was high. In 28 percent of the fruits set on both species studied, only empty seeds were found. In an additional 14 percent, one or two empty seeds were found with at least one filled seed. The presence of pollen is apparently essential to the development of fruit; furthermore, the presence of pollen in the fruit or in the ovule also assures seed development. [6] It therefore seems clear that empty seeds in

6 In a single fruit on one eastern redcedar tree, no seeds were found.

Table 2.-- Seed set from controlled pollinations in eastern redcedar and Rocky Mountain juniper

Female parent[1]	Date, type pollination by male parent[2]	Fruits	Empty seeds	Filled seed set: Total	Filled seed set: Average per fruit
		Number			
INTRASPECIFIC CROSSES					
JV-1	A Feb. 10	2	0	2	1.00
	A Fresh	145	71	119	0.84
	B Dec. 31	43	19	49	1.14[3]
	B Feb. 10	84	40	66	0.79
	B Fresh	267	118	279	1.04
	Wind	105	50	73	0.70
	Unpollinated	1	1	0	0.00
	Mean				0.90
JV-2	A Jan. 31	9	8	2	0.22
	A Fresh	49	35	33	0.67
	Wind	137	91	73	0.53
	Mean				0.55
JV-3	A Jan. 31	23	5	25	1.09
	A Fresh	128	80	91	0.71
	B Jan. 10	135	88	99	0.73
	B Fresh	172	96	141	0.82
	Wind	179	90	111	0.62
	Mean				0.74
INTERSPECIFIC CROSSES					
JS-1	A Fresh	112	50	98	0.88
	Wind	196	78	180	0.92
	Mean				0.90

1/ JV = J. virginiana; JS = J. scopulorum.

2/ "A" and "B" are J. virginiana male trees.

3/ Differed significantly from wind pollination of same female tree.

the study species cannot be attributed to the lack of synchronization between pollen release and ovular receptivity. However, the high proportion of empty seeds suggests either that fertilization is not essential to seed development, or that some post-fertilization incompatibility reaction brings about a tissue breakdown within the ovule. There was no noticeable difference in the size of filled or empty seeds, so the presence of pollen must trigger some mechanism which allows ovular development to proceed. As long as 50 years ago, the Russian physiologist Doroshenko (1928) claimed that the function of pollen in ovules is physical and that development could take place without fertilization. Our results in juniper do not discount Doroshenko's claim.

The number of seeds per fruit varied from 1 to 3 in both eastern redcedar and Rocky Mountain juniper. A single seed developed in 69.0 percent of the eastern redcedar fruits and in 66.1 percent of the Rocky Mountain juniper fruits. Three seeds per fruit occurred in very low frequency, 1.9 and 1.3 percent, respectively, for the two species. There was no significant difference in the number of seeds per fruit among the female trees nor among the pollen sources.

According to Mathews (1939), three types of female strobili are found in eastern redcedar:

1. Only one ovule, borne in the axil of one member of a sporophyll pair (2/3 of the fruits are of this type).

2. Two ovules, side by side in the axil of one member of a sporophyll pair; the other sporophyll is sterile (1/6 are of this type).

3. Two ovules, each separately on a fertile member of a sporophyll pair (1/6 of this type).

Mathews further found that if three seeds develop, a combination of the latter two types is usually the explanation. More than four seeds are produced rarely, and only when a second pair of sporophylls participates. Thus, one and two seeds per fruit are expected to be in the approximate proportions that our data show.

Seed Germination

The real germination percent (germination percent of filled seeds) was generally high among all eastern redcedar female x male parental combinations producing adequate numbers of seeds to test (table 3). On all three female trees, however, real germination of wind-pollinated seed was somewhat lower than control-pollinated seed from the same mother trees. Possibly, the presence of foreign pollen (e.g., that of another juniper species) could bring about seed filling without a viable embryo.

Table 3.--Germination of eastern redcedar and Rocky Mountain 1/, 2/ juniper seeds from controlled and wind pollinations

Female parent 3/	Date, type pollination by male parent 4/	Total	Seeds germinated	Real germination 5/	Plantable seedlings	
		Number		Percent	Number	Percent
INTRASPECIFIC CROSSES						
JV-1	A Fresh	21	16	94.2	12	75.0
	B Dec. 31	8	7	100.0	5	71.4
	B Feb. 10	13	9	90.0	1	11.1
	B Fresh	37	32	88.9	17	53.1
	Wind	12	6	60.0	0	0.0
JV-2	A Fresh	2	2	100.0	0	0.0
	Wind	6	2	40.0	1	50.0
JV-3	A Jan. 31	4	4	100.0	2	50.0
	A Fresh	10	10	100.0	9	90.0
	B Jan. 10	10	10	100.0	9	90.0
	B Fresh	12	12	100.0	10	83.3
	Wind	10	9	90.0	6	66.7
INTERSPECIFIC CROSSES						
JS-1	A Fresh	15	0	0.0	0	0.0
	Wind	25	0	0.0	0	0.0

1/ From two petri-dish replications for each parental combination.

2/ Following 150 days stratification at 5°C (98 days for Rocky Mountain juniper); seed tips cut.

3/ JV = J. virginiana; JS = J. scopulorum.

4/ "A" and "B" are J. virginiana male trees

5/ Real germination = germination percent of filled seeds.

Although real germination was surprisingly uniform, the number of plantable seedlings obtained varied among the eastern redcedar mother trees and among the pollen parents. Whereas, the JV-1 female was superior to the JV-3 in fruit set per unit branch weight and in the set of sound seeds per fruit, the reverse was true for the percentage of plantable seedlings developing from germinated seeds. The percentage of plantable seedlings was usually higher for fresh pollen than for stored pollen, but this difference was not statistically significant. The factors influencing early seedling vigor are not clear.

No germination of the one-year seeds from the Rocky Mountain juniper female was obtained from either the wind- or cross-pollinated treatments. Following the completion of the germination tests, the central portion of the filled seeds, considered to be an embryo cavity, was removed, killed in FAA, and prepared for microscopic examination to determine the stage of development. A total of 27 microscope slides were thus prepared. The material examined was entirely cellular female gametophyte tissue, with no evidence of the presence of archegonia. Seeds from both the cross- and wind-pollinations contained this material; no difference could be detected.

Interpretation of the one-year seed development in Rocky Mountain juniper is difficult. Unfortunately, the reproductive cycle of this species has not been worked out. However, the sequence of its developmental stages is evidently different from that observed in other juniper species and other gymnosperm genera. For example, in the one-year-maturing eastern redcedar, the female gametophyte develops during the spring, a few weeks after pollination, the archegonia differentiating and fertilization occurring by late June or July (Ottley 1909; Mathews 1939). This is not a great deal different from other one-year-maturing gymnosperms such as spruce (Picea A. Dietr.), whose female gametophyte is at the free-nuclear stage at the time of pollination (Fechner 1964).

In the three-year-maturing common juniper (J. communis L.), the female gametophyte develops during the second season, the megaspore mother cell not differentiating until April of the year following pollination (Ottley 1909). And in the two-year-maturing pine (Pinus L.), the megaspore mother cell is differentiated at the time of pollination, and the ovules overwinter in the early free-nuclear gametophyte (Ferguson 1904; McWilliam 1958).

None of the above examples describes the two-year-maturing Rocky Mountain juniper seeds observed in this study. We found that the cellular female gametophyte stage was reached by late autumn of the first year, although it had only replaced about one-fourth of the nucellus tissue. It is not known if interspecies crosses occurred, since no developing embryos were found. However, the similar first-year development of the cross-pollinated and wind-pollinated seeds strongly suggests that hybridization is possible between Rocky Mountain juniper and eastern redcedar.

Seedling Development

Seedling survival to 27 to 46 days following transplanting was variable among both eastern redcedar female trees (JV-1, JV-3). Nor could a pattern be recognized relative to seedling vigor; some of the seedlings resulting from stored pollen were as vigorous as those from fresh pollen.

Pollen source did not significantly affect the dry weight of the surviving seedlings. Average seedling weight at the end of the study was 0.0034 grams, ranging from 0.0018 to 0.0055 grams. The ratio of top length divided by root length was variable within and between female x male parental combinations (table 4), ranging from 4.20 for the smallest tree (0.0018 grams dry weight, 52 mm total length) to 0.60 for the largest tree (0.0055 grams dry weight, 134 mm total length). The average ratio was 1.00, and the average length was 94 mm.

Table 4.--Development of eastern redcedar seedlings from controlled and wind-pollinations

Female parent[1]	Date, type pollination by male parent[2]	Surviving seedlings July 8[3]		
		Number	Percent[4]	Average top-root ratio
JV-1	A Fresh	4	33.3	1.41
	B Dec. 31	3	60.0	.79
	B. Feb. 10	1	100.0	.69
	B Fresh	9	52.9	1.10
JV-3	A Jan. 31	2	100.0	2.13
	A Fresh	5	55.6	1.51
	B Jan. 10	8	88.9	.95
	B Fresh	8	80.0	.80
	Wind	3	50.0	1.93

1/ JV=J. virginiana. 2/ "A" and "B" are J. virginiana male trees. 3/ Seedlings apparently living but without root systems not included. 4/ Percent of seedlings transplanted, May 23-June 12.

CONCLUSIONS

This study shows that the presence of pollen is necessary for the development of fruit in both eastern redcedar and Rocky Mountain juniper, and the presence of pollen in the fruit or in the ovule assures seed development. The occurrence of empty seeds cannot be attributed to lack of synchronization between pollen release and ovular receptivity. Furthermore, pollen of eastern redcedar extracted as much as 2 months prior to normal pollen shedding and then stored is effective in producing sound seed set and vigorous seedlings to at least 40 days.

The results of this study also suggest that on the basis of fruit set, sound seed set, and first-year female gametophyte development,

hybridization is possible between eastern redcedar and Rocky Mountain juniper. Further study is necessary to determine the degree of compatibility between them.

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