3. The smallest pair of chromosomes having near-median centromere showed peculiar structure at proximal region. The proximal region (about 30% of the total length of the chromosome) of the chromosome showed light staining or negative heteropycnosis at late prophase. This segment is totally discolored at metaphase and can not be distinguished from the centromere. This chromosome pair may be used as a good cytological marker in the study of chromosomal evolution in the species of family Taxodiaceae.

Key words: Sequoiadendron giganteum, Chromosome.

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Literatur Cited

Buchholz, J. T.: The morphology and embryogeny of Sequoia gigantea. American Journal of Botany 26: 93–101 (1939a). — Buchholz, J. T.: The generic Segregation of the sequoias. American Journal of Botany 26: 535–538 (1939b) — Harlow, W. M. and E. S. Harrar: Textbook of dendrology. McGraw-Hill Book Co., New York, Fifth Edition 173–180 (1969). — Hirayoshi, I. and Y. Nakamura: Chromosome number of Sequoia sempervirens. Bot. Zool. 2: 73–75 (1943). — Jensen, H. and A. Levan: Colchicine-induced tetraploidy in Sequoia gigantea. Hereditas 27: 220–224 (1941). — Levan, A., K. Fredga, and A. A. Sadnberg: Nomenclature for centromeric position on chromosomes. Hereditas 52: 201–220 (1964). — Saylor, L. C.: A karyotypic analysis of selected species of Pinus. Silvae Genetica 10: 77–84 (1961). — Saylor, L. C. and H. A. Simons: Karyology of Sequoia sempervirens: Karyotype and accessory chromosomes. Cytologia 35: 294–303 (1970). — Stebbins, G. L., Jr.: The chromosomes and relationships of Metasequoia and Sequoia. Science 108: 95–98 (1948). — Tsuchiya, T.: An improved acetocarmine squash method, with special reference to the modified Rattenbury's method of making a preparation permanent. Barley Genetics Newsletter 1: 71–72 (1971).

Pollen Germination and Pollen Tube Growth of Juniperus from Autumn and Winter Collections

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Introduction

The occurrence of a low proportion of filled seeds in developed fruits of Juniperus has been observed frequently. This low set of sound seeds may be the result of poor pollination due to a lack of synchronization between the dates of pollen release from male trees and those of ovular receptivity on female trees in the vicinity. If this were true, artificial pollinations from forced and stored pollen could enhance sound seed set, provided that pollen viability could be maintained. However, no information is available on the forcing or storage of Juniperus pollen, collected during the dormant period, or on the most suitable media for testing pollen germination and pollen-tube development of Juniperus.

The germination of pollen and pollen-tube development of conifers have been studied on different artificial media. Several authors (Dengler and Scamoni, 1939; Fechner, 1958) have found that aqueous solutions of less than 20 percent sucrose stimulate the germination of both fresh and stored pollen of Pinus, Picea, and Abies, compared to that on plain-water media. Fechner and Funsch (1966) found that for pollen stored eleven years the optimum concentration for germination of blue spruce pollen was 15 percent and that it was 5 percent for ponderosa pine pollen. This constituted a change, during storage, in the optimum concentration from 10 percent sucrose for unstored pollen of both species (Fechner, 1958).

Reichle (1960) showed that 0.01 percent, or even 0.001 percent, boric acid in a medium of 0.75 percent agar plus 7.5 percent sucrose stimulated the pollen-tube growth of Pinus, Picea, and Abies, but at a concentration of 0.1 percent boric acid, pollen germination and pollen-tube growth of most species that he studied were inhibited. Pinus contorta Dougl. was not stimulated at any of the three boric acid levels tested by Reichle. Vasil (1964) explained that the stimulatory effect of boron in pollen germination may be due to: a) increased absorption, translocation, and metabolism of sugars because of the formation of sugar-borate complexes, b) increased oxygen uptake, and c) the role of boron in the synthesis of pectic materials for the walls of the actively-growing pollen tubes.

Other substances are known to affect pollen germination and pollen-tube growth. For example, Brewbaker and Kwack (1964) found that calcium increased pollen germination and pollen-tube growth of angiosperms. The authors also pointed out that the effect of calcium is directly a function of the balance between potassium, magnesium, and hydrogen ions. Fins (1972) found that silver solutions in very low concentrations stimulated pollen-tube growth of Engelmann spruce (Picea engelmannii Parry); however, she also found that silver nitrate was highly toxic to pollen, limiting both germination and pollen-tube growth at a concentration of 0.1 ppm.

Benner (1972), studying the pollen-tube growth of alsike clover used four different nutrient solutions: those proposed by Kendall and Taylor (1965), Kendall (1967), Brew-

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[2]) For a single collection, December 10, a few branches were forced in a 1.0 percent boric nitrate solution, with no noticeable difference in pollen shedding or subsequent pollen germination or pollen-tube growth, compared to those forced in water.

BAKER and KWACK (1963), and a modification of the latter. BENNER suggested that the formula of KENDALL and TAYLOR, which consisted of 25 percent sucrose (w/v), with 100 ppm $CaCO_3$, and 50 ppm H_3BO_3, was the most suitable for the pollen that he tested.

Materials and Methods

Collection and handling — Two male trees (A and B) of *Juniperus virginiana* L. on the campus of Colorado State University, Fort Collins, were selected for study. Branches 30 to 40 cm. long bearing male strobili were collected approximately every ten days from October 3, 1973 until March 20, 1974. On each collection date, a portion of the branches from each tree were used in each of the following ways:

1. Fresh pollen was extracted by crushing male strobili. Some of this pollen was cultured immediately on artificial media; the remainder was placed in refrigerated storage (4 to 6° C) in cotton-stoppered glass vials until March 15, 1974, when samples of the stored pollen were cultured. No special humidity control was administered during storage.
2. Branches bearing male strobili were placed in water at room temperature (18 to 23° C) and normal daylight conditions, their tops covered with plastic bags for isolation and for observation of strobilus dehiscence and pollen shedding. Pollen was collected each morning from glass vials attached to openings near the base of the plastic bags. A portion of this forced pollen was cultured immediately on artificial media; the remainder was placed in cotton-stoppered glass vials in the refrigerator (4 to 6° C) until March 15, 1974, when stored pollen was cultured.[2])
3. Branches bearing male strobili were placed directly into plastic bags and stored in the refrigerator for later study. Pollen from these strobili was extracted and cultured along with other stored pollen on March 15, 1974.

Culturing — Between October 3 and December 31, 1973, the ten different culture media listed below were compared. In later cultures, only the more suitable media were used to measure pollen viability and pollen-tube growth. Since *Juniperus* pollen is unwinged and sinks in aqueous media, thereby causing oxygen deficiency for germination, our culture media all contained agar usually 0.5 to 1.0 percent, in distilled water. The following media were used:

I — 1.0 percent sucrose plus 1.0 percent agar (w/v) in distilled water.
II — Same as I plus 10 ppm thiamine.
III — Same as I plus 100 ppm glutamic acid.
IV — Same as I plus 10 ppm thiamine plus 100 ppm
V — Same as I plus 10 ppm thiamine plus 100 ppm glutamic acid.
VI — Same as I plus 10 ppm thiamine plus 100 ppm glutamic acid plus 100 ppm asparagine.
VII — Same as I plus 10.0 percent foliage extract.
VIII — Same as I plus 100 ppm boric acid, 300 ppm calcium nitrate, 200 ppm magnesium sulfate, 100 ppm potassium nitrate (modification of BREWBAKER and KWACK, 1964).
IX — Same as I plus 1.0 percent terpene.
X — 10.0 percent sucrose plus 0.5 percent agar.

Following sterilization, the culture media were poured hot into plastic petridish culture chambers (35 mm × 10 mm), and the covers were immediately replaced to minimize contamination. Two to five culture chambers were prepared in this manner for each medium used. After solidification of the medium, pollen was dusted gently on the surface, and the culture chambers were incubated in the dark at 20 to 23° C.[3])

Estimating germination and pollen-tube length — Germination counts were made at the end of the test to obtain the maximum germination for each culture. Within each culture chamber, 100 pollen grains were counted, some in each of five locations in the culture chamber. True randomization was not possible due to occasional clumping of pollen grains and the consequent difficulty in determining their condition. Counting was done at approximately 100-power magnification, and an eyepiece grid micrometer was used to minimize personal bias. A pollen grain was considered as germinated, when the pollen-tube reached a length of 60 microns, or about 2 to 2½ times the diameter of the pollen grain.

Pollen-tube length was estimated by measuring a sample of the germinated pollen grains each day, with the aid of an eyepiece micrometer, as long as pollen-tube growth continued. In each culture chamber, the 10 longest pollen-tubes in each of seven to ten different locations in the culture chamber were measured at 100-power magnification, for a total of 70 to 100 pollen tubes in each chamber.

Results and Discussion

Strobilus dehiscence and pollen shedding — Branches which were collected during October dried within four to six weeks without shedding any pollen. However, strobili collected from Tree A on November 10 started to dehisce after 21 days in water at room temperature, and pollen shedding began six days later, continuing for 11 days. The time of predehiscence and dehiscence for this tree was consistently longer than for Tree B throughout the study. During dehiscence, the strobili of both trees approximately doubled in length, from an average of 3.1 mm to 6.0 mm. Pollen shedding always occurred during the night. The time necessary for pollen shedding to begin decreased from 27 days in the November 10 collection to just overnight in the late February and early March collections *(Fig. 1)*.

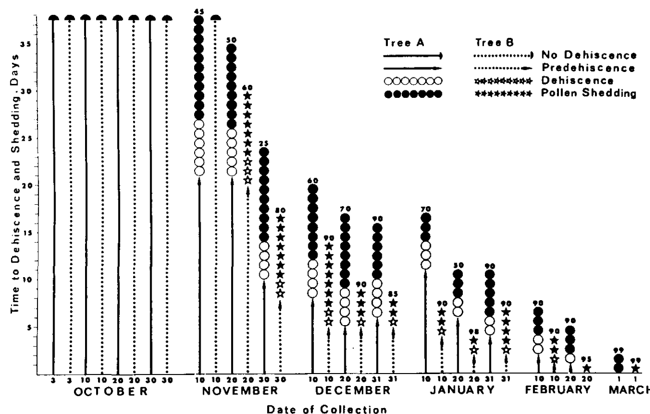

Fig. 1. — Predehiscence, dehiscence, and pollen shedding periods for *Juniperus virginiana* strobili forced in water at room temperature. Circles and stars each equal 24-hour days.

[3]) A few petri pishes were placed in an incubator illuminated for 14 hours of each 24 hours with a fluroescent lamp, 115 volts, 5700 lumens. Pollen germination and pollen-tube growth were not noticeably different from that obtained when germinated in the dark.

Germination mechanics — The exine of pollen grains capable of germinating was shed before germination occurred *(Fig. 2a)*, as also observed by MÜLLER-STOLE (1948). On an agar medium, this shedding occurred in about one hour, but in water, the exine was shed when within two to ten minutes. As the exine became detached, it forced the pollen grain away 20 to 30 microns, and the shape of the pollen grain immediately changed. Before the exine was shed, the pollen grains were spherical, averaging 22 to 26 microns in diameter, but immediately after exine shedding, they became elliptical *(Fig. 2a)*, with an average length of 31 microns. This is similar to the splitting of the exine in Douglas-fir illustrated by HO and SZIKLAI (1972) in their Figure 7.

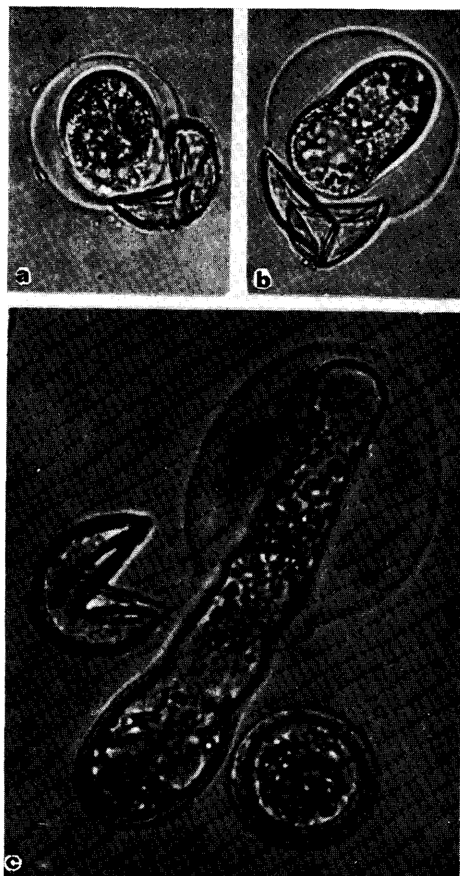

Fig. 2. — Germination of *Juniperus virginiana* pollen: a. Immediately after exine shedding; b. Initial tube elongation; c. Shed exine, elongated tube and ungerminated pollen grain (1000×).

Germination percent — The percent pollen germination varied among the dates of pollen collection, whether the pollen was extracted immediately or forced, and among the culture media used. For a given collection date, there was no consistent difference in pollen germination between the two trees studied.

Germination of forced pollen increased from an average of about 25 percent in the November collections to about 90 percent in the March collections. Whenever pollen was obtained by forcing, some germination occurred. Germination of the freshly-extracted pollen was very poor, and no germination of this pollen occurred prior to the December 10 collection *(Tab. 1)*. This contrast in germination suggests that pollen maturation occurred during the forcing period in the laboratory.

Table 1. — Comparison of extracted and forced pollen of *Juniperus virginiana* L. on Medium X.

Collection Date	Tree A Extracted[1])	Tree A Forced	Tree B Extracted[1])	Tree B Forced
	Germination Percent			
Nov. 10	0	25	0	[2])
Nov. 20	0	50	0	30
Nov. 30	0	25	0	25
Dec. 10	4	35	5	30
Dec. 20	10	45	18	90
Dec. 31	1	35	5	90
Jan. 10	7	55	6	75
Jan. 20	8	42	15	55
Jan. 31	4	87	4	87
Feb. 10	5	76	5	74
Feb. 20	[3])	33	[3])	79
Mar. 1	[3])	73	[3])	87
Mar. 10	[3])	92	[3])	90

[1]) Extracted on collection date.
[2]) No shed pollen available for culturing.
[3]) Pollen matured; no separate extractions made.

No germination was obtained on media II, IV, VI, VII and IX for either freshly-extracted or forced pollen. Of the remaining culture media used in this study, Medium X (10.0 percent sucrose plus 0.5 percent agar) produced the highest pollen germination. Because of this, it was the only medium used to study germination and pollen-tube growth from collections made in January and thereafter. *Tab. 2* shows that freshly-extracted pollen from strobili collected in autumn did not germinate on Media III and V, although forced pollen from the same collections did. This suggests that glutamic acid (present in Media III and V, as well as in VI) decreased the germination of extracted (immature) pollen. When thiamine was present alone in the medium, the germination of forced pollen was prevented, and thiamine depressed the germination percent when present with glutamic acid. Medium I produced higher germination of freshly-extracted pollen than Medium VIII, but Medium VIII produced higher germination of forced pollen than Medium I. Hence, the ability of pollen to germinate must be related to chemical changes in the pollen during forcing.

Tabe 2. — Germination of *Juniperus virginiana* L. pollen on different culture media.[1])

Medium	Freshly Extracted[2])	Forced
I	Yes	Yes
III	No	Yes
V	No	Yes
VIII	Yes	Yes
X	Yes	Yes

[1]) From collections made November 10 to December 31, 1973.
[2]) Extracted on collection date.

Cultures made on March 15, 1974, of stored pollen showed that storage did not affect pollen germination. Extracted pollen which germinated poorly before storage did not improve during storage, and forced pollen which germinated well before storage was not damaged.

Pollen-tube growth — Noticeable pollen-tube growth began three to five days following culturing *(Fig. 2b)*, considerably later than has been reported for pine and spruce, which produces a pollen tube within 24 hours on media similar to those used in this study (FECHNER, 1958). Pollen-tube growth proceeded slowly, reaching its maximum length on most culture media tested in 16 to 17 days, provided that mold did not occur in the culture chambers.

However, our sampling indicated that the growth was not a continuous process but that growth stoppage of one-half day to two days occurred intermittently during the culturing period. Usually the pollen-tube was unbranched *(Fig. 2c)*, but sometimes pollen from Tree A, especially that which was extracted on the date of collection, developed branched or large tubes or tubes with very large ends.

Pollen-tube growth differed on the several culture media used *(Fig. 3)* and whether the pollen was freshly-extracted or forced. The maximum pollen-tube length obtained in this study was 484 microns, more than 20 times the diameter of the pollen grain and more than twice the distance required for fertilization in an ovule. This length was attained with forced pollen collected from Tree A (February 20) and Tree B (January 31), after 14 days and 17 days of culture, respectively. Maximum pollen-tube length achieved from freshly-extracted pollen was 308 microns for Tree A (January 10 collection) and 242 microns for Tree B December 20 collection). Pollen tube length attained with forced pollen was always longer than attained with extracted pollen of the same collection dates.

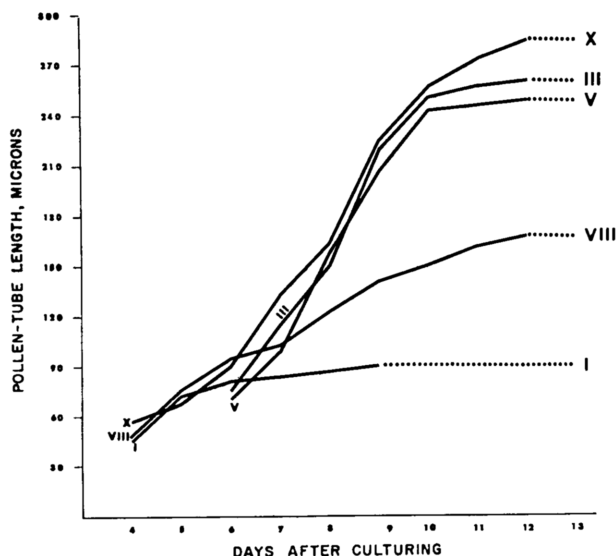

Fig. 3. — Comparison of pollen tube growth of *Juniperus virginiana* on different culture media (See Methods and Materials for formulas). Collection date November 30, 1973; Tree B.

Problems — The occurrence of mold in the culture chambers made comparison of pollen germination and pollen-tube growth from different collections and between Trees A and B very difficult. This fungal contamination sometimes occurred in the medium before pollen germination or sometimes after pollen germination. The presence of fungi in the culture chambers apparently interferred with pollen germination and pollen-tube growth. The fungi developed more readily, however, in those culture chambers in which the pollen was not well-distributed on the medium or in which the total amount of pollen in the chambers was small. Whether or not the presence of pollen is antagonistic to fungal growth as well as the converse, is not clear, however.

Freshly-extracted pollen produced much more fungal growth in the petri dishes than did forced pollen. No doubt crushing the strobili to extract the pollen spread the fungal spores. Furthermore, Medium VII (foliage extract) produced more fungal growth than any of the other media studied.

Another problem involved evaporation from the culture media and the consequent increasing of the sucrose concentration. This problem was most pronounced in those culture chambers in which pollen-tube growth was studied, because the chambers were necessarily opened often for examination under the microscope. As a result, the volume of some media decreased to ½ to ⅓ of their original volume. This was true, for example, for the pollen collected from Tree B January 31; hence, its sucrose concentration must have increased to 20 or 30 percent. In petri dishes which were kept closed until after the 14- to 16-day study period, and consequently kept more moist than those opened daily for pollen-tube measurement, pollen-tube growth was not satisfactory. Perhaps this means that a higher sucrose concentration is necessary for late pollen-tube growth than for pollen germination and early pollen-tube growth, for which 10 percent sucrose was found to be superior to 15 or 20 percent.

Summary

Branches of *J. virginiana* L. collected periodically from October to March were forced in water and pollen shedding began with November 10 collections. The periods of predehiscence, dehiscence and pollen shedding shortened gradually from November through March, and pollen germination percent increased during that period. Germination percent and pollen-tube growth of forced pollen were much higher than those of pollen extracted from the strobili at the time of collection. Storage of up to 3 months did not affect germination percent or pollen tube growth of either forced or extracted pollen. Germination of viable pollen occurred within 3 to 5 days after culturing, and pollen-tube growth proceeded slowly to its maximum in 16 or 17 days. The best medium for germination and pollen-tube growth was 10 percent (w/v) sucrose plus 0.5 percent agar. The increase in concentration of sucrose in the culture chambers, caused by evaporation of the medium, produced greater pollen-tube growth than constant sucrose concentration did. This study suggests that pollen forced from autumn or winter collections could fertilize female strobili of *Juniperus* in spring.

Key words: Juniperus virginiana L., Pollen-germination.

Literature Cited

Benner, L. R.: Growth of alsike clover pollen *in vitro*. Master's thesis. Colorado State University, Fort Collins. 33 pp. (1972). — Brewbaker, J. L. and B. H. Kwack: The essential role of calcium ion in pollen germination and pollen-tube growth. Amer. J. Bot. 50: 859—865 (1963). — Brewbaker, J. L. and B. H. Kwack: The calcium ion and substances in influencing pollen growth. *In* Pollen physiology and fertilization. H. F. Linskens, Edit.: 143—151. North Holland Publishing Company, Amsterdam (1964). — Dengler, A. and A. Scamoni: Über die Keimungsbedingungen von Waldbaumpollen. Zeitschrift für Forst- u. Jagdwesen 71 (1): 1—40 (1939) — Fechner, G. H.: Effect of storage conditions on the viability of Rocky Mountain tree pollens. Proceedings, Soc. Amer. Foresters, Syracuse, New York (1958). — Fechner, G. H. and R. W. Funsch: Germination of blue spruce and ponderosa pine pollen after eleven years of storage at 0° to 4° C. Silvae Genetica 15 (5/6): 164—166 (1966). — Fins, L.: Effect of cloudseeding materials on Engelmann spruce pollen germination. Master's thesis. Colorado State University. Fort Collins. 68 pp. (1972). — Harabetova, E. and J. Tupy: The growth effect of some sugars and their metabolism in pollen tubes. *In* Pollen physiology and fertilization. H. F. Linskens, Edit.: 95—101. North Holland Publishing Company, Amsterdam (1964). — Ho, R. H. and O. Sziklai: Germination of Douglas-fir pollen. Silvae Genetica 21 (1/2): 48—51 (1972). — Kendall, W. A.: Growth of red clover pollen II. Elongation *in vitro*. Crop Science 7: 342—344 (1967). — Kendall, W. A. and N. L. Taylor: Growth of red clover pollen. Crop Science 5: 241—243 (1965). — Müller-Stoll, W. R.: Zytomorphologische Studien am Pollen von *Taxus Baccata* L. und anderen Koniferen. Planta 35: 601—641 (1948). — Reichle, R. E.: A study of germination and growth of coniferous pollen *in vitro*. Master's thesis. Colorado State University. Fort Collins. 53 pp. (1960). — Vasil, I. K.: Effect of boron on pollen germination and pollen-tube growth. *In* Pollen physiology and fertilization, H. F. Linskens, Edit.: 107—119. North Holland Publishing Company, Amsterdam (1964).