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Early Pollen Forcing in a White x Black Spruce Hybrid and its Parental Species

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Early Pollen Forcing in a White x Black Spruce Hybrid and its Parental Species [1]

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ABSTRACT — Vioble pollen was successfully forced from winter-collected cuttings of white ond black spruce, as well as of a hybrid of them. The natural time difference in flowering was overcome, thus providing a tool for interbreeding these species. During forcing, and naturally on the trees, pollen always shed from white spruce first, then the hybrid and finally from black spruce. Using departures from long-range weather averages, a heat-requirement difference was postulated for each species to account for the time difference in their flowering.

White and black spruce are morphologically distinct species that occur together throughout most of their transcontinental range, a wide band extending from the eastern parts of Canada and northeastern United States westward through the Lake States region and central Canada, thence northwest to Alaska. In northeastern Minnesota white and black spruce are valuable for pulp. Thus, any genetic improvement in these species would likely be of major interest to the forest industry of the state.

One of the methods currently used to improve white and black spruce is selection. Another possible method involves hybridization of the native species. Since white spruce normally sheds pollen seven to ten days before black spruce, however, the possibility of making reciprocal crosses with fresh pollen is remote. This time difference in normal pollen release is probably responsible for the natural isolation of these species. In fact, it was generally believed by many workers (Wright, 1955) that white and black spruce do not hybridize either naturally or artificially. But one white x black spruce hybrid tree (Rosendahl spruce) was discovered in 1955 near Cromwell, Minnesota (Little and Pauley, 1958). This tree is flanked by a specimen of white spruce and one of black spruce, offering an excellent opportunity to investigate the cytology and phenology of one representative of each of these three species.

The purposes of this study were to compare the cytology and phenology of normal and forced microsporogenesis of white, the hybrid and black spruce, and to determine the feasibility of obtaining viable pollen by early forcing in order to facilitate hybridization between these species.

Materials and Methods

During the abundant flowering year of 1962, male-bud bearing branches were collected between January 19 and May 14 from ten white spruce trees planted on the St. Paul Campus, and between March 20 and May 21 from white, the hybrid and black spruce trees growing near Cromwell. These branches were forced in the greenhouse at St. Paul. For each collection, the stages of microsporogenesis present were determined cytologically (by an acetocarmine squash method) at the time of collection, as well as at frequent intervals during forcing.

Results and Discussion

During normal and forced development, microsporophylls and strobili significantly increased in length with the initiation of meiosis. Although initiation of normal meiosis in the field was accompanied by a noticeable swelling of male buds no such correlation was observed in the greenhouse.

Cytological examinations of microsporocytes of white, the hybrid and black spruce disclosed the presence of 12 bivalents prior to anaphase I for all three species. The average percentages of chromosomal aberrations (bridges and lagging chromosomes) occurring during anaphase I and anaphase II were 3.5, 8.1 and 2.4, respectively, for white, the hybrid and black spruce. These percentages were not significantly different.

On the basis of the cytological evidence, there does not appear to be any barrier to hybridization between white and black spruce which can be associated with a lack of chromosomal compatibility.

The days of forcing required to obtain pollen from male strobili from each collection are illustrated in Fig. 1 for one white spruce tree (#10) at St. Paul. A pollen-release curve has been drawn through the points on the graph to show the decrease in the time of forcing required for subsequently later collections in the season. During the winter months, apparently, there was a slow but constant physiological development within the microspore mother cells laid down the previous summer. This was also the conclusion reached by Johnson (1945) after working with cuttings of Norway spruce in southern Ontario, Canada. But, in addition, at St. Paul, after the arrival of above freezing temperatures in spring, the rate in the decrease of forcing time for subsequent collections was accelerated in white spruce until normal pollen release occurred in early May. This fact indicates that physiological processes increase significantly with the ad-

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vent of warm weather, although normal meiosis did not begin on the white spruce tree at St. Paul until April 21, after five consecutively warm days with an average temperature of 49° F.

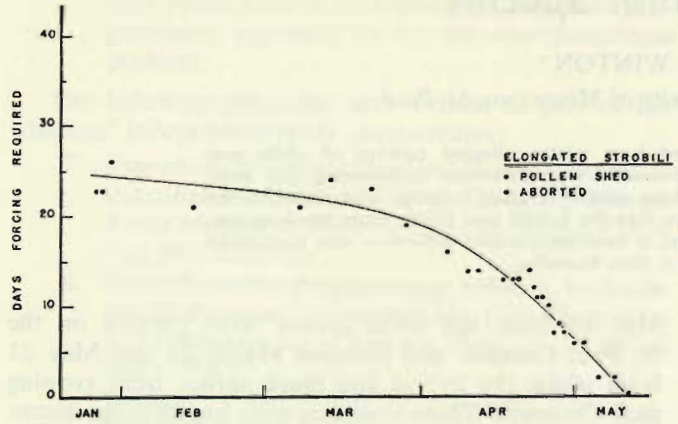

FIGURE 1. Pollen-release curve for white spruce tree #10 at St. Paul during the 1962 forcing season. Normal meiosis occurred between April 20-26, and normal pollen release May 11.

Pollen-release curves are also shown (Fig. 2) for white, the hybrid and black spruce near Cromwell. During forcing, pollen was shed from male strobili of the hybrid within one day after pollen release from white spruce cuttings of the same collection, but two to seven days before black spruce. In contrast, pollen was normally shed from the hybrid in the field six days after white spruce but only three days before black spruce. The relative time of forcing for the hybrid was intermediate between white and black spruce during normal as well as forced development. The normal tendency of the hybrid to shed pollen closer to the time of black spruce, however, was in some way modified during forcing to cause pollen to be shed closer to the time of white spruce.

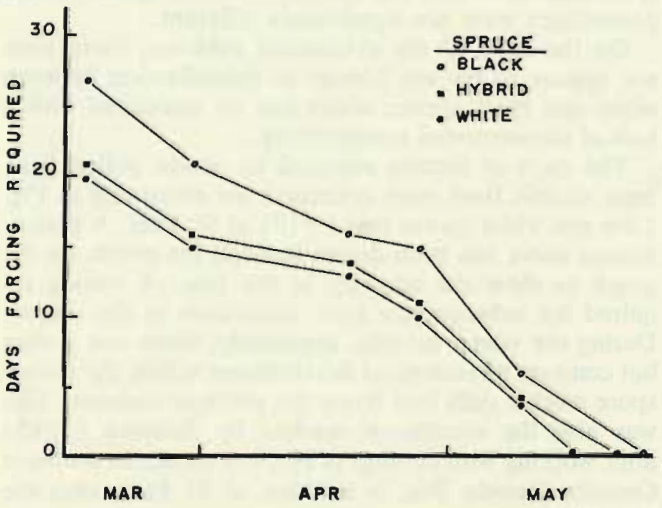

FIGURE 2. Pollen release curves for white, the hybrid and black spruce trees near Cromwell in 1962. Normal meiosis occurred on the trees in the field between April 23-May 3 for white spruce, April 27-May 4 for the hybrid, and May 1-9 for black spruce. Normal pollen release occurred from the same trees, respectively, May 19, 25 and 28.

The early-forced pollen from most collections was tested *in vitro* immediately after shedding. The remaining pollen in each sample was stored in cotton-plugged vials over $CaCl_2$ at 3° C, and was again tested in July, 1962, as well as a year later in June 1963. In each case, a small amount of pollen was placed in 0.5 ml of a five per cent dextrose solution. After 50-60 hours incubation at 70°-80° F, one drop of the mixture was observed on a slide at 100X and the germination of about 150 pollen grains was determined.

The germination results for white spruce tree #10 at St. Paul are shown in Fig. 3 for the three testing dates. These data show that for branches collected January 29, pollen shed after 26 days forcing had 57 per cent germination (solid line). After about five months storage, however, the germination of the same pollen sample had

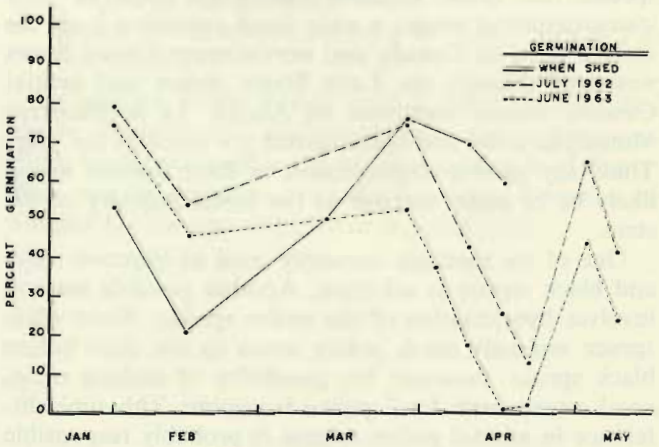

FIGURE 3. Per cent germination of pollen shed from male strobili of the white spruce tree #10 at St. Paul. For pollen shed from separate collections, the per cent germination is shown when shed, and again on July 2, 1962, as well as a year later on June 4, 1963.

risen to 79 per cent (dashed line). These results are interpreted as indicating that immature pollen was shed during forcing and then continued to develop during storage by means of an afterripening process. The good storage capacity of this pollen was illustrated by its 74 per cent germination after more than one year (dotted line). On the other hand, from branches collected from the same tree April 16, pollen shed after 13 days forcing had good germination of 60 per cent when shed, but only 26 per cent by June, and one per cent a year later. The low germinative capacity after storage of this pollen was probably related to the unusually high greenhouse temperatures (in excess of 100° F) recorded during the first two weeks in April. After the greenhouse was whitewashed April 18, the germinability of stored pollen increased rapidly with each collection.

The earliest that branches were collected from the trees near Cromwell in 1962 was March 20. Pollen forced from this collection gave initial germination of 63 per cent for white spruce after 20 days forcing, 53 per cent for the hybrid after 21 days forcing, and 78 per cent for black spruce after 27 days forcing. Germination after storage remained between 50-80 per cent for white

spruce pollen shed from collections made during the warm period of early April. The germinative capacity of stored pollen from the same period for the hybrid and black spruce, however, was respectively 10-30 and zero per cent.

In vivo tests were not specifically designed to determine the viability of early forced pollen. Early-forced pollen obtained during this study, however, was used in a preliminary study conducted in May 1962, to investigate the possibility of obtaining artificial hybrids between white, the hybrid and black spruce. Female conelets were not found on black spruce. One putative F_1 hybrid seedling was recovered from the white x black spruce crosses made at St. Paul, as well as three white x hybrid and five hybrid x black putative backcross seedlings from crosses made on the trees near Cromwell.

On the basis of this study, the use of early forced pollen provides a tool for artificial hybridization in spruce improvement programs. Under greenhouse conditions approximating those found out of doors during the early part of the growing season, viable pollen of white and black spruce, as well as their hybrid, may be forced from male strobili collected during winter. Thus, for spruce with different times of flowering, sources of pollen are now available for reciprocal crosses at the time they are needed in the spring. This method of obtaining pollen also eliminates the need to store for three or four years normally shed pollen from one good pollen year until another occurs.

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