Fertilization in Forced Quaking Aspen and Cottonwood

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(Received for publication February 28, 1967)

Introduction

Triploid quaking aspen (Populus tremuloides MICHX.) appears to have many more desirable characteristics as a pulpwood source than does the normal diploid form of this species (VAN BUIJTENEN et al., 1958). In addition, it is reasonable to expect that triploid forms of other species of Populus, including cottonwood (P. deltoides MARSH.), would also exhibit faster growth and increased fiber length. The large-scale production of triploid aspen and cottonwood, however, first requires a number of male tetraploid parentc for use in diploid X tetraploid crosses.

EINSPAHR (1965) recovered 50 putative tetraploid quaking aspen seedlings in two treatments with colchicine. Starting at 6, 12, 18, 24, and 30 hours after pollination, whole catkins were immersed in a 0.3 percent aaueous solution of colchicine for six hours. The purpose was to obtain tetraploids, presumably by the doubling of the two sets of chromosomes in each of the relatively few diploid cells of the newly-formed embryos. The recovery of some putative triploids, in addition to the expected tetraploids, led to the speculation that premature applications of colchicine possibly caused some prefertilization doubling of the chromosomea in the gametes.

NAGARAJ (1952) presented a detailed camera-lucida study of the floral development of cottonwood in the Chicago, Illinois area. He also included one drawing of megagametophyte development in quaking aspen which showed the eight-nucleate stage (having two unfused polar nuclei). The following observations made by NAGARAJ for both species provided important guide lines for the present study. (1) The two polar nuclei fuse before the entrance of the pollen tube into the megagametophytc, (2) both the polar body and the egg cell are fertilized by male gametes from a single pollen tube, and (3) the polar body divides and starts to form the endosperm before the zygote divides and embryogeny begins.

ERLANSON and HERMANN (1927) earlier published one camera lucida drawing of a two-celled embryo and one of very young ovules but gave no information about the rate of development. Their main contribution was the first correct report in the literature of the haploid and diploid chromosome numbers of quaking aspen cf 19 and 38, respectively.

LESTER (1963) studied the time and sequence of natural floral differentiation in quaking aspen in the New Haven, Connecticut area. He reported that floral initiation occurred in late May or early June, and pistillate flowers were receptive and anthesis occurred in early April of the following year. CAMPO (1963) reported that after fertilization, the endosperm began to develop immediately in one bisexuai P. deltoides tree in northern Italy. However, the zygote remained dormant ten days before the first transverse division occurred.

In our work, the primary objective is to produce tetraploids. Hence, colchicine should be applied during the one-celled zygote stage, which occurs from the time of fertilization until the first mitotic division. A cytological study was thus initiated to determine the rate of development after pollination for two species of Populus.

Material and Methods

During April and May of 1965, three collections of five to seven branches each were made from one female quaking aspen tree growing in east-central Wisconsin. The branches were placcd in a greenhouse with their cut bases immersed in ice water. The female flowers were receptive fourteen days after the first collection and progressively earlier for the second and third collections.

Catkins of the first collection were pollinated March 16 with pollen forced in the greenhouse. At periods of 2, 4, 6, 8, and 24 hours after pollination, whole catkins were placed in FAE, FPA, Craf III, and BOUIN's fixative solutions (SASS. 1961). Individual capsules from the center of the catkin were then dehydrated in an ethanol-tertiary butanol series (RANDOLPH, 1935) and embedded first in Parawax and finally in Tissuemat. Longitudinal, as well as a few cross and oblique sections were cut, all at 10 microns. Mounted sections were stained in safranin then fast green in a xylene-ethanol schedule, and made permanent by mounting with balsam in xylene.

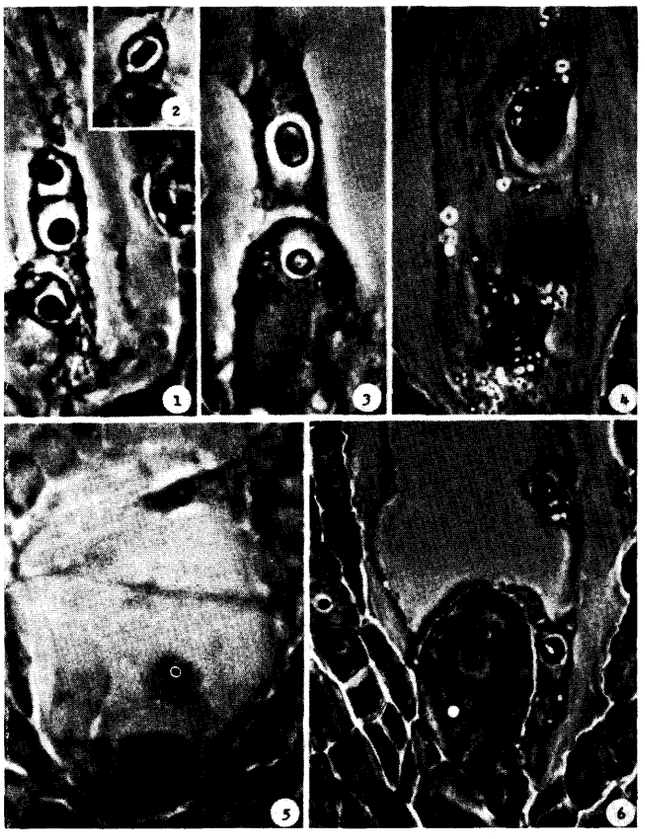
Catkins from the second collection were pollinated April 1 and collected daily in Craf III fixative for the first eight days, and thereafter in FAE fixative every 3 or 4 days until 18 days after pollinatian. Branches of the first two collections were left in the greenhouse. However, branches of the third collection were moved into a growth chamber after pollination on April 14. Catkins were placed in FAE fixative at half-hour intervals between five and eight hours after pollination, and thereafter 1 to 5 times daily until seed was shed on the twelfth day. Permanent slide preparations were made from 2 or 3 capsules per treatment. Photomicrographs were taken with Kodak 35 mm Panatomic-X film in a phase-contrast Zeiss Photomicroscope.

Branches, bearing flower buds, were also collected from one female cottonwood tree in the same vicinity and forced in the greenhouse. After pollination on April 22, these branches were placed in the growth chamber. Material was collected in FAE fixative 6, 8, and 12 hours after pollination, then daily on days 1 through 12, and finally when seed was shed on the eighteenth day after pollination. Sections were prepared in the same manner described above.

Results

At time of pollination, both species had either two polar nuclei (Fig. 1) or one polar body (Fig. 3) in each mature megagametophyte. Figure 2 shows what appears to be the fusion of two polar nuclei seen in cross section. Receptivity was signaled by a faint pinkish blüsh in quaking aspen, and a yellowish translucent color in cottonwood, caused by the emerging stigmas.

*) The author is Research Fellow, The Institute of Paper Chemistry, Appleton, Wisconsin. He wishes to ucknowledge the financial support of this work by the Louis W. and Maud Hill Family Foundation of Bt. Paul, Minnesota and the nine pulp and paper companies who are members of the Lake States Aspen Genetics and Tree Improvement Group. Appreciation is also expressed to DEAN EINSPAHR for his help with the manuscript and with the collections, to MILES BENSON and DELMAR SCHWALBACH for their assistance in the greenhouse, and to Mrs. SUSAN LEBERGEN and Mrs. DOROTHY OLSON for their assistance in the cytology laboratory.



Figs. 1—6. — Populus tremuloides (ca. 1000×). — *Fig. 1:* Two polar nuclei above the egg cell. — *Fig. 2:* Fusion of polar nuclei (cross section). — *Fig. 3:* Polar body above, egg cell in center. — *Fig. 4:* Fertilization. Egg cell out of focus in center. — *Fig. 5:* First division of polar body. Egg cell in micropylar cavity. — *Fig. 6:* First transverse mitotic division of zygote. Note development of the endosperm.

In the quaking aspen flowers of the third collection, which were placed in the growth chamber after pollination, pollen germinated on the stigma at 6½ hours, and double fertilization *(Fig. 4)* occurred between 30 and 48 hours after pollination. The zygote migrated into the micropylar cavity during the next 24 hours, followed shortly by the division of the polar body *(Fig. 5)* and the early development of the endosperm. The first transverse mitotic division of the zygote occurred between 54 and 72 hours after pollination *(Fig. 6)*. Embryogeny then continued fairly rapidly, forming the mature embryo before the seed was shed on the twelfth day after pollination.

In contrast, aspen left in the greenhouse after pollination showed considerable variation in the rate of development. Some fertilization did occur as early as 8 to 24 hours in the first collection, but not until 48 to 72 hours in the second. Differences in the rate of development may have reflected differences in greenhouse temperatures. The times of fertilization were, respectively, about one day earlier and one day later than the 30 to 48 hours observed in the third collection.

The cottonwood material, unfortunately, was cut mostly in oblique sections, making a detailed analysis difficult. Fertilization probably occurred between 24 and 72 hours, and the endosperm was well developed by the fifth day. First division of the zygote apparently occurred on either the sixth or seventh day, because the young embryo was observed on the eighth day after pollination.

The placental epidermis hair-cells on the funiculus divided longitudinally in aspen as early as 5½ hours after pollination, but only after the fusion of the two polar nuclei. Hair cells were approximately one half the length of the ovules during fertilization, and almost as long as the ovules at the first division of the zygote. The onset of embryogeny was accompanied by the rapid elongation of both the ovules and the hair cells. Growth of the hair cells of cottonwood was difficult to follow, but may have been slightly faster than in aspen.

Discussion

During this study, quaking aspen exhibited much variation in development depending upon its post-pollination treatment. Fertilization can apparently occur in the greenhouse either one day faster or slower than in the growth chamber according to the current weather conditions. In the controlled environment of the growth chamber, fertilization occurred between 30 and 48 hours after pollination. Einspahr (1965) recovered putative tetraploids from all colchicine treatments given from 6 to 30 hours after pollination. However, the greatest number was from the six-hour treatment. One explanation is that warm greenhouse conditions at the time merely hastened fertilization. Another is that the colchicine applied at the earlier times remained active in the megagametophyte until after fertilization, and caused doubling during the first mitotic division. The small percent of putative triploidy can possibly be explained by an unreduced colchicine division of the generative cell in the pollen tube. Smith (1943) found that this division took place from 10—15 hours after the *in vitro* germination of quaking aspen pollen. If such an unreduced generative cell fertilizes the egg, one might then ask if the polar nucleus is fertilized, and by what, or if it develops by apomixis.

Summary

Flowers on branches from quaking aspen and cottonwood were forced in the greenhouse, pollinated, and either left in the greenhouse or placed in a growth chamber. In the growth chamber, fertilization occurred after 30 to 48 hours in quaking aspen, and the zygote divided between 54 and 72 hours after pollination. Fertilization probably occurred in cottonwood before the third day, and the zygote divided either on the sixth or seventh day after pollination. Fertilization occurred in quaking aspen left in the greenhouse, over a period of 8—72 hours after pollination. Colchicine applied before fertilization probably produces triploids by prefertilization doubling of chromosomes in the male gametes. The relative length of hair cells to ovule length, as observed in the greenhouse, may be a reliable indicator of critical stages of development, particularly in quaking aspen.

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