SELECTION AND IDENTIFICATION OF MONOPLOID POPULUS TREMULOIDES MICHX.

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Homozygosis may be achieved in one generation through the selection of monoploid plants and the subsequent doubling of the chromosomes either as a spontaneous occurrence (Chase 1952) or induced by a chemical treatment such as colchicine. This method of producing pure line has been employed in the breeding of maize, Zea mays L. (Chase, 1952), the common potato, Solanum tuberosum L. (Hougas, et al.1968), tobacco, Nicotiana tabacum L. (Stokes 1963), and is now being attempted in forest tree breeding.

The method usually employed for easy identification of monoploids developing from unfertilized eggs is classification of the seed or seedlings for a recessive trait of the female parent that had been crossed with a male homozygous for the dominant allele. Hougas, et al. (1958) crossed the common potato with related species which possessed dominant genes. The feasibility of using the interspecific cross of trembling aspen, Populus tremuloides Michx., and the European white poplar, P. alba L., for the identification of monoploid trembling aspen occurred to the senior author upon first seeing the hybrid at the Institute of Paper Chemistry, Appleton, Wisconsin. This hybrid, described by Heimburger (1936), can be distinguished from trembling aspen in 20-day-old seedlings, the third to fourth leaf stage, on the basis of epidermal hairs on the upper leaf blade, leaf margin, and petiole. The difference is more obvious in older seedlings.

Three methods designed to increase the efficiency in producing trembling aspen monoploids are being or have been tried at Syracuse. They are: (1) the labeling of P. alba pollen with Phosphorus-32 to act as a dominant marker to distinguish hybrid from monoploid seed developing without fertilization by the presence or absence of radioactivity, (2) chemical treatment of the staminate parent to prevent normal gametogenesis so that a single diploid sperm or none forms in the pollen tube, and (3) treatment of pistillate flowers which had not been pollinated or which were pollinated with dead pollen with naphthaleneacetic acid (NAA), a synthetic plant hormone used to prevent premature fruit drop (Gardner, 1951). Since nonpollinated aspen catkins usually fall before capsule dehiscence, this treatment should increase the chance of obtaining fully matured catkins, hence monoploid seed.

The ornamental cultivar of the white poplar, P. alba 'Bolleana', has been used as the male parent in all experiments. Various trees of trembling aspen occurring near Syracuse, New York, were used as the female parent.

P^{32} AS A MARKER FOR SPERM DNA

Radioactive phosphorus, as sodium radiophosphate, was added to 200 ml, of water in vases to give solutions with total radioactivity of 5, 10, 15, 20, and 30 mc. A sixth vase lacking P^{32} served for a control. Short floral bud-bearing branches of P. alba were put in the vases prior to meiosis for forcing flower development. The anthers failed to dehisce in the first attempt so new branches were cut and water was added to each vase to restore the volume to 200 ml. The radioactivity of the solutions had reduced to 2.4, 5.4, 11.1, 9.6, and 16.3 me., respectively. This attempt

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was successful. The seed resulting from the pollinations was adhered to felt paper at one-half-inch spacing with dried rubber cement. The sheets were then placed over Kodak Medical Bluebrand X-ray film in holders and stored for about two weeks at 4 °C and low humidity. The films were developed and scored for the presence or absence of an image caused by B-ray emissions from P^{32} at each position corresponding to that of a seed. Seeds causing no image or a questionable image were planted as well as samples of seed causing an image and seed from the control cross. The results are summarized in table 1. Approximately one-sixth of the seed from the P^{32} treatments failed to cause an image or it was so faint it was questionable. Seed from the two lowest concentrations of P^{32}, 2.4 and 5.4 mc per ml., included 446 of the 572 seeds in this group. All but 44 of the total number of six- to eight-week-old seedlings, however, proved to be hybrid. All seedlings of a questionable phenotype were classified as putative monoploids. It should be noted that four of the 44 putative monoploids were from seed causing a film image. Upon the senior author 's return to Syracuse following a year of sabbatical leave, the two-year-old trees, including the samples of the control and of seedlings from seed causing images, were classified. All but one were hybrid. The trembling aspen-like tree was from the lowest P^{32} treatment group. Its height at two years was about eight inches which compared with hybrid siblings of more than three feet. Cytological determination of chromosome number using the stem apex was attempted, but was not successful. The next spring, three lateral buds developed, and within two to three weeks, one became dominant. It dwarfed the rest of the tree in height and had leaves two to three times the size of those on the other two branches. Again stem apices were sampled for chromosome counts, but very few metaphase figures were obtained and no definitive counts were possible. One very clear polar view of a metaphase or early anaphase was obtained from leaf tissue of the dominant branch. The chromosome number was definitely not 38, the diploid number for trembling aspen and white poplar, but it also appeared to be greater than 19.

The low frequency of putative monoploids among seedlings from seed lacking or showing a questionable X-ray film image led us to question the use of P^{32} as a specific marker of the DNA of the sperm effecting fertilization. It certainly shows that the amount of label was too little to always cause an image, even in treatments of higher P^{32} concentration. Seed causing an image, therefore, must have incorporated additional P^{32}.

The experimental results given in table 2 show that P^{32} was resorbed from nonfunctioning pollen tubes and translocated to the developing embryo. Two successive pollinations of each trembling aspen female were made, the first using a non-P^{32}-labeled pollen and the second, two hours later with P32-labeled pollen of another species. Ten mc of P^{32} per 200 ml. of water was used in the vases to label the second pollen parent. Procedures described for the previous experiment were followed to identify labeled seed. The results of these crosses are shown in the first two columns of table 2. The results from two different control crosses, both lacking P^{32}, are shown in the two columns to the right. For the control shown in the far right column, equal quantities of the two pollens by weight were mixed and used.

Two groups of seedlings demonstrate that sufficient P^{32} is translocated to the embryo to cause a film image in seed resulting from fertilization by nonlabeled pollen (fig. 2) . In the first column 17% of the seedlings developing from seed that caused an image were hybrid, yet the P. alba pollen lacked P^{32}. In the second column, the definitive group is the trembling aspen-like seedlings, representing 92.9% of the seedlings from seed causing an X-ray film image. These results clearly show that P^{32} cannot serve as a dominant marker of the sperm DNA for distinguishing at the seed stage the hybrid diploid embryos and the monoploid embryos developing from unfertilized eggs.

Table 1. -- Seed and seedlings from P. tremuloides x P. alba 'Bolleana' pollen labeled with P^{32}.

	Control	Number of seeds or seedlings for different levels of P^{32} label of pollen					Totals
P^{32} in vases, mc/200 ml.	0	2.4	5.4	11.1	9.6	16.3	--
Radioactivity per seed in cpm	0	0.54	0.73	0.89	0.86	2.43	--
Total number of seeds	546	1174	574	749	413	510	3420
Seeds with X-ray film image:							
Total number	0	835	467	726	341	479	2848
Number planted	--	54	92	63	110	83	402
Percent germination	--	53.6	68.4	71.3	67.2	65.1	65.8
Putative monoploids, 6-8 weeks	--	0	0	0	4	0	4
Number 2-yr.-old trees	--	12	18	10	19	7	66
Putative monoploids	--	--	--	--	--	--	0
Seeds without X-ray film image:							
Total number	546	339	107	23	72	31	572
Number planted	220	326	101	23	70	23	543
Percent germination	74.9	75.9	62.0	73.9	68.4	30.2	74.9
Total seedlings, 6-8 weeks	--	251	35	4	24	3	317
Putative monoploids	--	28	7	1	3	1	40
Total no. 2-yr.-old trees	32	26	6	1	2	1	36
Putative monoploids	--	1	0	0	0	0	1

Table 2. -- Summary of results demonstrating the translocation of P^{32} from non-functioning pollen tubes to embryos.

Initial pollination	alba	trem.	trem.	trem. + alba (1:1)
Pollination 2 hours later	trem.-P^{32}	alba-P^{32}	alba	none
Total number of seeds	1086	3669		
X-ray film image	883	1276		
Planted	784	1216		
Seedlings, 8 weeks	376	630		
Tremuloides-like	70.2%	92.9%		
Hybrid	17.0%	2.1%		
Other	12.8%	5.1%		
No film image	203	2393		
Planted	40	182		
Seedlings, 8 weeks	31	89	1440	1099
Tremuloides-like	87.1%	94.4%	90.2%	91.4%
Hybrid	12.9%	5.6%	9.8%	8.6%

A gas flow counter was used to determine average radioactivity of sample seed lots and samples of P. alba pollen. The mean activity per seed was 1.36 cpm for seed resulting from pollinations with labeled P. tremuloides pollen and 0.27 cpm for the P. alba labeled pollen. This compares with a mean activity of 0.12 cpm per pollen grain of P. alba, showing that the seed contained more P^{32} than that contributed by the pollen.

CHEMICAL TREATMENT OF STAMINATE FLOWERS

Chemical treatment of P. alba 'Bolleana' floral buds with colchicine has been used to prevent the normal mitotic divisions during microgametogenesis. Colchicine prevents spindle formation but not chromosome replication so that the chromosome number is doubled during a C-mitosis (Eigsti and Dustin, 1955).

Two methods of treatment have been tried: (1) uptake via the cut stem of twigs and translocation to the developing flowers, and (2) dipping the catkin for 10 seconds daily in an aqueous solution. Concentrations of colchicine of 0.2, 0.4, and 1.0% were used for both methods when the majority of the microgametophytes were in the two-nucleate stage. Treatments lacking the chemical served as a control. The pollen was used for crosses with trembling aspen females.

In the initial experiment in 1967 employing only the translocation of colchicine via cut stems method, two putative monoploids were obtained, one from the 0.2% and the other from the 1.0% colchicine treatment group. These seedlings, in addition to having a low frequency of epidermal hairs, were slow growing with leaves about one-third to one-half the size of hybrid siblings and a finer dentation. They distinctly differ from each other in leaf morphology, however, which may reflect a heterozygous condition of the female parent as the seedlings in all treatment and control groups exhibited an obvious dimorphism for several leaf traits. All progeny seedlings, including the putative monoploids, were classified for each of the contrasting traits and found to fall into three groups. Thirty-five seedlings are characterized by large leaves (mean length 8.4 cm. and mean width 6.8 cm.), a low blade length to width ratio (mean of 1.24'0.12), shorter stomata (mean of 26.3-1-1.3

microns), coarse dentation, and a higher frequency of epidermal hairs. They appear to be intermediate in leaf type to the two parents. Seventeen seedlings have smaller leaves (mean length 6.9 cm. and mean width 3.9 cm.), a high blade length-to-width ratio (mean of 1.78 ± 0.19), longer stomata (mean of 30.3 ± 2.0 microns), fine dentation, and a lower frequency of epidermal hairs. Nineteen seedlings, including the two putative monoploids, exhibit a mixture of certain of these traits. The mean stomata size for each of the two monoploids (26.6 ± 2.2 and 24.7 ± 2.9 microns) is within the range observed for seedlings in the first group (23.3 to 28.2 microns), which is in contrast to Von Kopecky's findings (1960) for European white poplar seedlings which he considered to be monoploids.

The segregation for leaf characters approximating that of a test cross suggests that one of the parent trees used is heterozygous for one or more genes controlling leaf characteristics, some of which are linked. Since the P. alba 'Bolleana' male parent has been used for a number of years in crosses with P. tremuloides and this dimorphism had not been observed, the female parent most likely has contributed the contrasting alleles to the progeny. This could explain the differences in leaf traits exhibited by the two putative monoploids.

The analyses of this year's experiments are still in progress so that only preliminary results can be reported. The initial classification of three-week-old seedlings included ten tremuloides-like seedlings among 119 from the colchicine dip treatment group and none among 23 seedlings from the stem translocation treatment group.

NAPHTHALENEACETIC ACID TREATMENT OF PISTILLATE FLOWERS

Attempts to obtain monoploids from seed produced by nonpollinated catkins or ones pollinated with dead pollen, each with or without NAA/treatment, have not been successful, but the method appears promising. A year ago, two seeds were obtained from nonpollinated, NAA-treated catkins and germinated. Unfortunately, they were killed by damping off in the cotyledon stage. This year two seeds were obtained by a student who undertook this as a special project in the introductory forest tree breeding course. One seed was from a cross using only dead pollen, and the other, dead pollen followed by NAA treatment. Neither seed germinated, but this was most likely due to dessication during germination. These studies have shown, however, that premature catkin drop is decreased both by the use of dead pollen and by spraying the developing catkins with NAA. All but one of the seven groups of catkins that were neither pollinated nor sprayed with NAA fell prior to capsule dehiscence. This compares with approximately half when dead pollen only, NAA only, or the dead pollen followed by NAA was used. The general appearance of the NAA-treated catkins throughout development was better than that of the non-NAA treatment groups. A control in which live pollen was followed by NAA treatment was not made, so that we cannot recommend NAA treatment following pollination as a general procedure for aspen, but it would appear to be promising and will be tested next year.

Confirmation of the haploid condition of putative monoploids depends upon chromosome counts or measurement of DNA content. Repeated attempts with limited amounts of material have failed. Several reasonably good polar metaphase figures have been found for the putative monoploid first described, and the chromosome number appeared to be about 19. It certainly was not 38, the diploid number for this species. Efforts to confirm the chromosome number of the three putative monoploids will be continued.

3/ Sodium salt of B-naphthoxyacetic acid as the commercial product, Fruitone, Amchem Products, Inc., Fremont, California.

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