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PROCEEDINGS OF THE TWELFTH LAKE STATES FOREST TREE IMPROVEMENT CONFERENCE

AUGUST 1975

NORTH CENTRAL FOREST EXPERIMENT STATION
FOREST SERVICE U.S. DEPARTMENT OF AGRICULTURE

PROCEEDINGS OF THE TWELFTH LAKES STATES FOREST TREE IMPROVEMENT COMFERENCE

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FOREST TREE IMPROVEMENT PROGRAM FOR THE NATIONAL FORESTS IN THE LAKE STATES

R. G. Miller and J. D. Murphy[1/]

ABSTRACT.--The Eastern Region of the USDA Forest Service has been conducting a Forest Tree Improvement Program on the national forests in the Lake States since the early 1960's. This paper presents a general review of the program, including objectives, organization, species priorities, and basic steps and procedures. Also discusses accomplishments and future plans.

A tree improvement program for the more than 5.6 million acres of commercial forest land on the eight national forests in the Lake States was begun in the early 1960's. Objectives for the program are: (1) to develop an aggressive, practical tree improvement program; (2) to produce seed for seedling production and/or direct seeding that will yield fast-growing, high-quality, pest-resistant forest trees; and (3) to develop and demonstrate cultural methods for producing timber and other products by applying sound genetic principles.

The program is carried on by a Regional Geneticist, a Zone Geneticist; in addition each forest has one or more technicians. The Regional Geneticist develops and coordinates the tree improvement, nursery, and seed procurement programs. The Zone Geneticist implements and coordinates the tree improvement program which includes training and supervising a full-time seed orchard manager, training technicians for each national forest, and keeping all personnel informed of progress and status of the tree improvement program. The technicians perform the initial screening of superior tree selections, do the seed and scion collection work, and assist in collecting data and maintaining records.

SPECIES

The first step in the program was to establish species priorities and program intensities. Factors considered included acres of commercial forest land in the various timber types, genetic variation within the species, plantability of the species, current research programs, estimated

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1/ Regional Geneticist, USDA Forest Service, Eastern Region, Milwaukee, Wisconsin 53203, and Zone Genticist, Nicolet National Forest, Rhinelander, Wisconsin 54501.

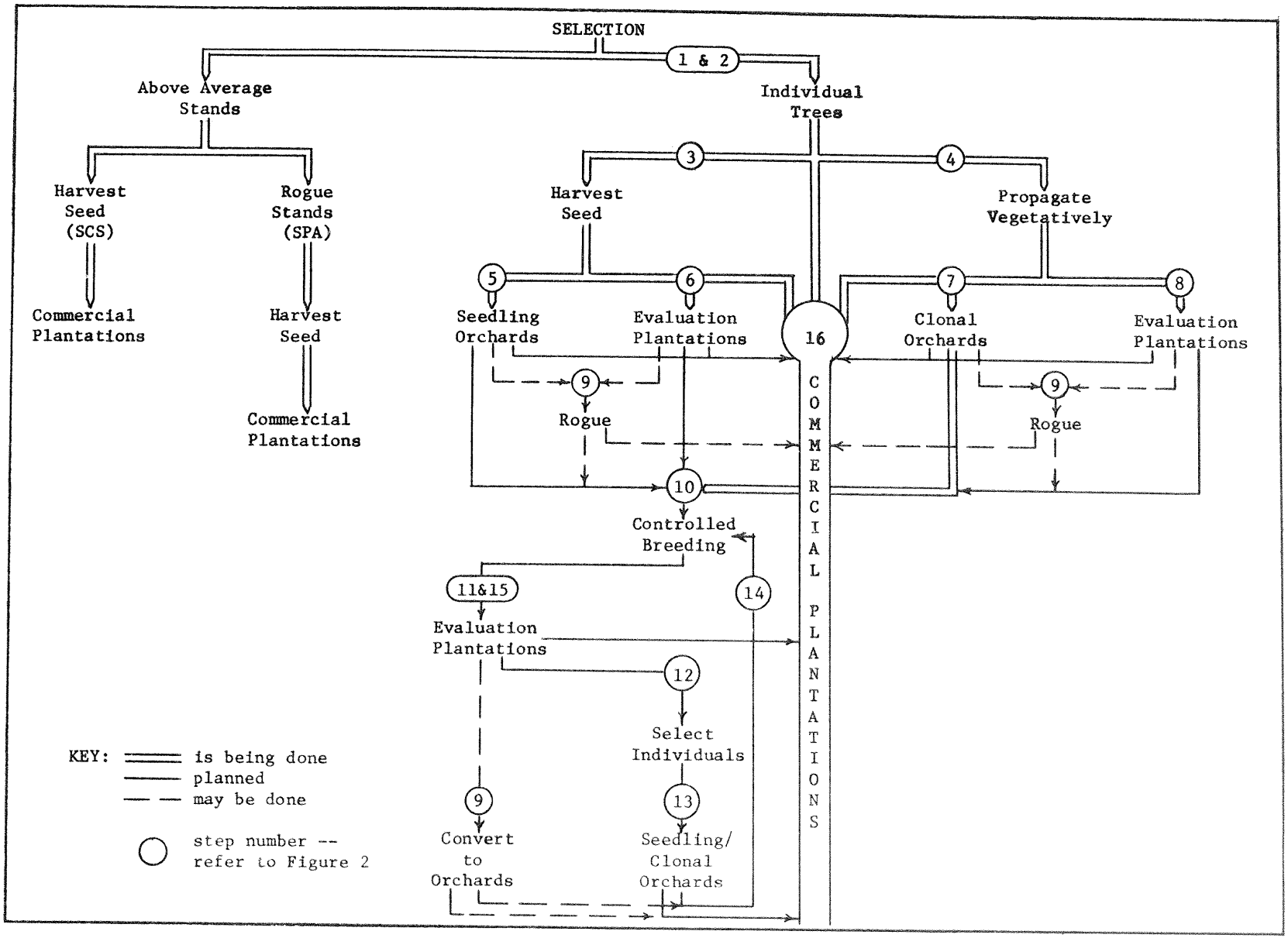

Figure 1.--Basic steps in the Region 9 forest tree improvement program.

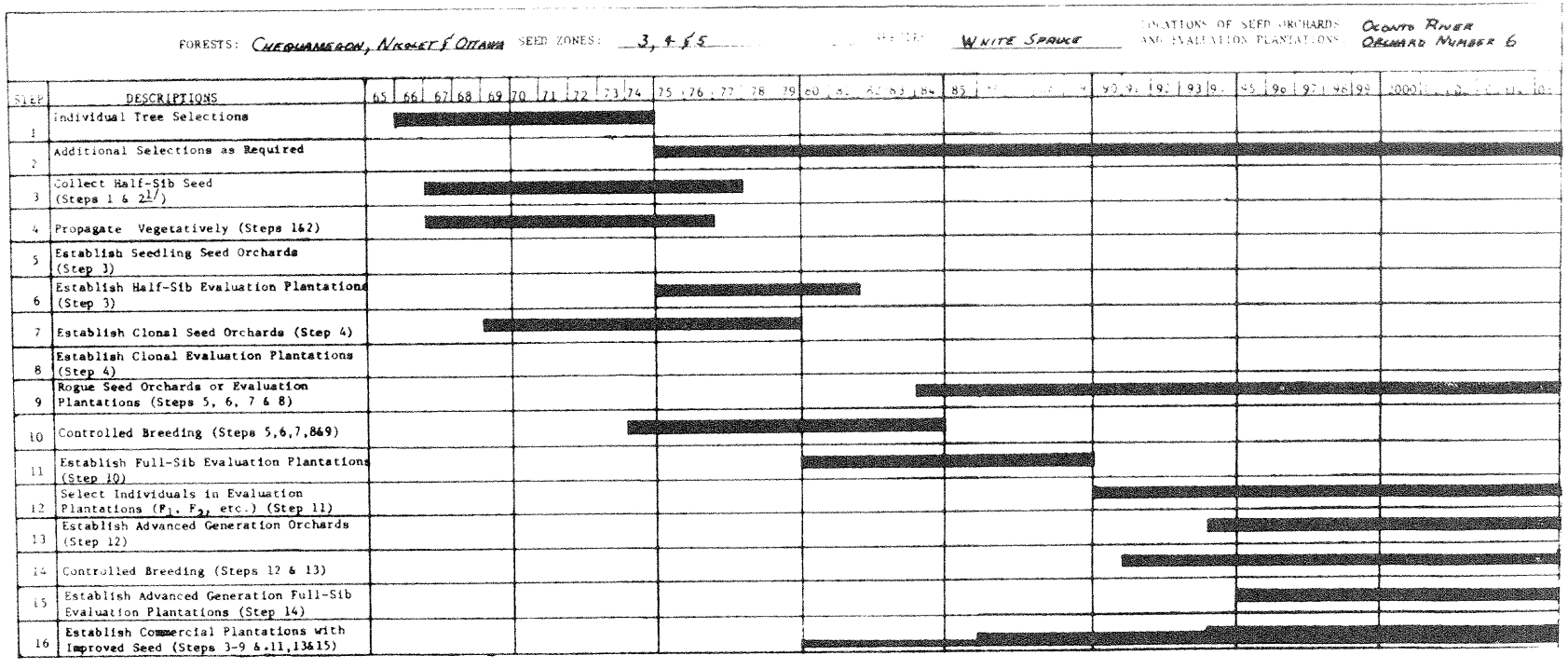

Figure 2.--Region 9 forest tree improvement time schedule for white spruce on the Chequamegon, Nicolet and Ottawa National Forests. The schedule indicates the time span for those steps applicable to the intensity of the program. (1/ The numbers in parenthesis refer to previous steps that this action is dependent on.)

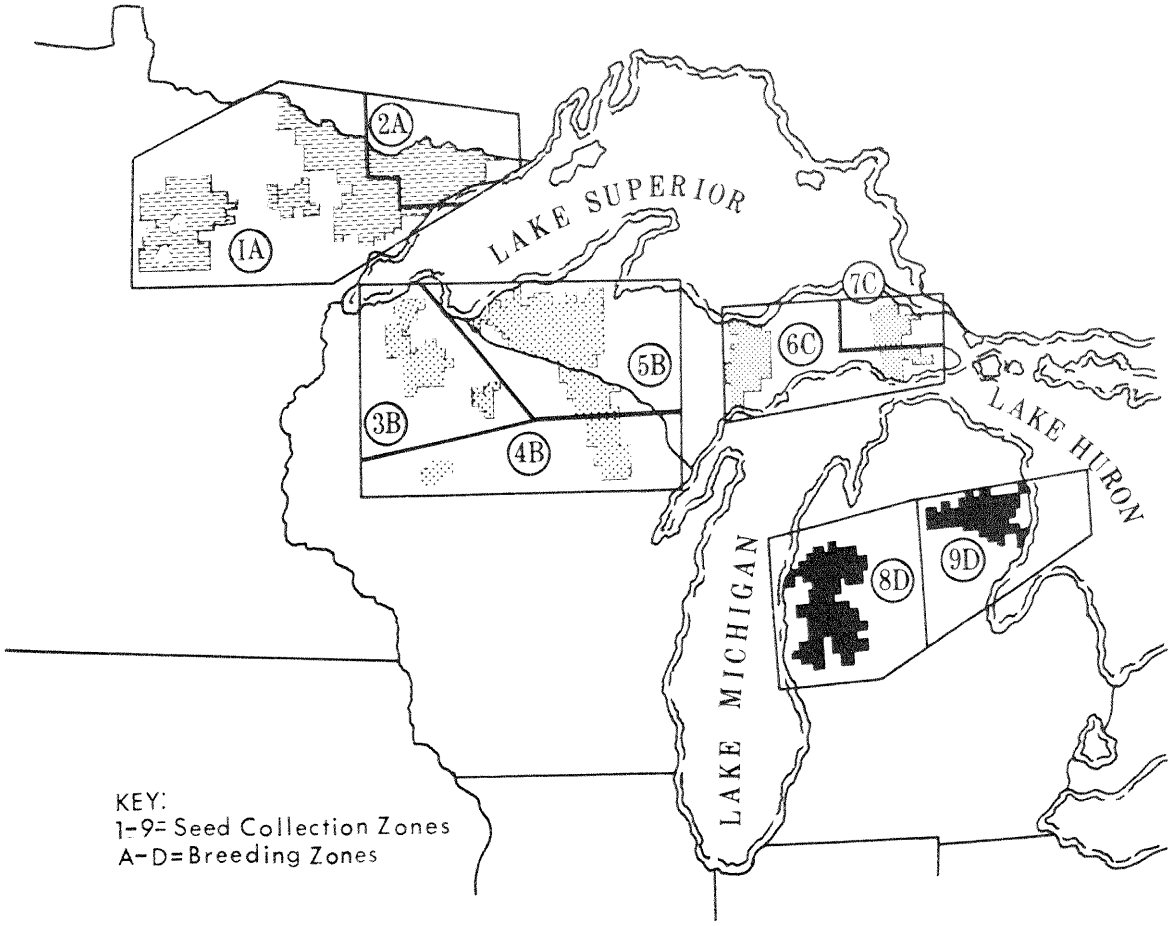

Figure 3.--Seed collection zones and breeding zones.

economic gains, and, to some extent, development programs carried on by others (table 1).

Table 1.--Species priority and types of programs

Species (in order of priority)	Priority group	Program: SCS and SPA	Selection	Seed orchard	Breeding	Evaluation plantation
White Pine	A		X	X	X	X
White Spruce	A	X	X	X	X	X
Aspen	A					X
Jack Pine	A	X	X	X	X	X
Black Spruce	A	X	X	X	X	X
Red Pine	A	X	X	X	X	X
Yellow Birch	A		X	X	X	X
Paper Birch	B		X			X
Black Cherry	B		X			X
Sugar Maple	B		X			X
Red Oak	C		X			X
Basswood	C		X			X
Red Maple	C		X			X
Larch	C		X			X

The program has been divided into 16 basic steps (fig. 1). The program for each species varies considerably in scope and degree. Time schedules are used to show the anticipated work for each species and to coordinate the work that involves several species over a period of years. The schedule for white spruce (fig. 2) is one of the most involved.

SEED COLLECTION ZONES AND BREEDING ZONES

The second major action in the program was to delineate seed collection zones for the eight national forests (fig. 3). The zones are based on the average January temperature and the total number of degree days above 50°F during the growing season (Rudolf 1956). To simplify the establishment of the seed orchards, the nine seed collection zones have been combined into four breeding zones (fig. 3). These breeding zones are based on the desired adaptability of the improved materials and may have to be modified as data become available from evaluation plantations.

SEED COLLECTION

Seed collection stands (SCS) have been located for black spruce and red pine within each seed collection zone. Timber sales within these selected stands are coordinated with good seed crops, and cones are

collected from the downed tops. White spruce and red pine seed production areas (SPA) were established in each seed zone where the species occurred naturally. Cones have been collected in the white spruce SPA's during good crop years by shooting or cutting the tops out of selected trees and picking the cones off the tops on the ground. The trees usually produce four to six new tops and another bumper cone crop within 6 years. Nienstaedt 2/ has included white spruce seed collected from one of the SPA's in his research and found that the SPA seedlings were 10 percent taller than the control seedlot at age 4. Small amounts of seed have been harvested in the red pine SPA's, mainly by climbing the trees. Collecting red pine cones in SPA's has been more difficult and expensive than similar work in white spruce SPA's. The time is rapidly approaching when all seed used on national forest land will originate from SCS, SPA, or individual tree collections.

SELECTION

Individual trees exhibiting fast growth, good form, and pest resistance are located on each national forest by trained technicians. Comparison trees are used to guide selection. Although there are inherent weaknesses in this process, comparison trees do provide a guide for the initial phases of the selection work (Ledig 1974). As workers become more familiar with the selection process, they depend less on comparison trees and more on how the candidate compares with previous selections. Several outstanding trees have been included in the program based on their own merit. More than 2,000 selections representing 11 species have been made.

VEGETATIVE PROPAGATION

All grafts are made at the J. W. Toumey Nursery, Watersmeet, Michigan, or at the Oconto River Seed Orchard. The scion collection work during January and February is closely coordinated with the grafting operation, enabling the grafting of all scions within 7 days of collection. The completed grafts are removed from their pots and lined out at the nursery or seed orchard after the danger of frost is past. The grafts are cared for from 1 to 3 years, depending on the species, and then moved to their specific seed orchard. Between 1969 and 1973, grafting success averaged 87 percent for white pine, 74 percent for white spruce, 81 percent for black spruce, and 83 percent for yellow birch. During the past 2 years, secondary scions have been collected from established grafts growing in the seed orchard. There has been no problem with graft incompatibility.

SEED ORCHARD

In 1967, we consolidated all seed orchards for the Lake States national forests in one area called the Oconto River Seed Orchard, about 30 miles

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2/ Personal communication. USDA Forest Service, North Central Forest Experiment Station, Institute of Forest Genetics, Rhinelander, Wisconsin 54501.

east of Antigo, Wisconsin on the Nicolet National Forest. This made it possible to employ and train a crew, as well as purchase the necessary equipment and supplies to intensively manage the seed orchard. The area includes 500 acres of cultivated farmland, 180 acres of woodland, a pond, and a trout stream.

During the summer of 1968, a survey crew established a grid system and constructed a topographic map using a 1-foot contour interval. Soil pits were dug adjacent to several grid stakes, and soil types were plotted on the topographic map. The soil in the cultivated fields is mostly Antigo silt loam, deep and shallow phases. This is an excellent agricultural soil and no fertilization was recommended for tree species at the time. A deer exclosure fence was erected around the entire section in the summer of 1969. The administrative site, including warehouse, office, headhouse, and greenhouse, was developed between 1970 and 1974. The warehouse also serves as an inoculation chamber for the blister rust-resistant eastern white pine program.

Pollen contamination among seed orchards of a particular species is reduced by locating the individual orchards far apart and by planting orchards of other species between them. The wooded areas, composed mainly of aspen, maple, and balsam fir, also serve to reduce pollen contamination.

The first seed orchards were established on cultivated fields during the spring of 1969. A computer program developed by Stairs et al. at the University of Wisconsin was used to generate a completely random planting design that maximizes out-crossing. The grafts are planted, staggered 30 feet apart in rows 15 feet apart--an actual spacing of about 22 feet by 22 feet. The initial orchards were kept clean-cultivated for about 3 years, and the grass was mowed around several extra grafts that were planted at a 10 feet by 10 feet spacing. Because the cone production on clones (white pine and white spruce) growing in both mowed and cultivated seed orchards seemed to be about the same, cultivation was reduced and the orchards were seeded to grass. Now the grafts are planted in the sod, the area around each graft is treated with an herbicide, and a mulch of sawdust or other suitable material is placed around the grafts. This method has increased accessibility within the orchards, reduced maintenance costs, and maintained excellent survival and growth.

Cone production in the white and black spruce and white pine orchards has been increasing each year. In 1974 it ranged from 18 to 31 percent of the total number of grafts in two white pine orchards, from 12 to 55 percent in white spruce and from 2 to 41 percent in black spruce. White pine and black spruce produced no male strobili. Most of the seed collected in the orchards is being reserved for establishing evaluation plantations.

The eastern white pine blister rust-resistant development program is also centered at the Oconto River Seed Orchard (Miller 1972).

EVALUATION PLANTATIONS

The evaluation plantations, like the seed orchards, are being consolidated as much as possible to facilitate the work and ensure proper care. Five major evaluation sites have been selected--one in Minnesota, Upper Michigan, and Lower Michigan, and two in Wisconsin.

Efforts have been concentrated on producing and evaluating full-sib material, but half-sib evaluation plantations are planned for white spruce, black spruce, and jack pine. Whenever possible, plant materials will be evaluated in each of the five major evaluation plantation sites. One objective is to identify material that will perform well in several of the sites. Seedlots that produced these selected materials would be sown in the nursery each year as a small part of the normal production and used within appropriate zones where stock shortages exist.

CONTROLLED POLLINATION

After reviewing several mating designs, it was decided to use a multiple four-parent disconnected half diallel (Squillace 1973). This design provides great flexibility in making crosses within and among the seed orchards for specific zones, with the production of a minimum number of half-sib families. In order to broaden the genetic base of the breeding program, the program was developed in cooperation with the North Central Forest Experiment Station, Institute of Forest Genetics, and will include their selections. This design was used in the white spruce breeding program, which began in the spring of 1975.

Evaluation plantations will be established as the full-sib seed becomes available from a sufficient number of crosses. As soon as the trees begin to flower, selections will be made in these plantation and used to upgrade existing orchards and/or establish new ones, and will also be included in an advanced generation breeding program. The main objective will be to create new generations as rapidly as possible with the available material and data. The older plantations will continue to be evaluated and new selections added to, or previous selections eliminated from, the breeding programs and seed orcahrds. This will result in a continuous genetic gain in the seed orchards.

RECORDS

An ADP system has been developed to help maintain the identity and status of all plant material used in the tree improvement program. The system also summarizes and analyzes the data collected in seed orchards, evaluation plantations, and breeding programs.

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WHITE PINE POLLEN SPECIES AND YEAR DO NOT AFFECT CONELET DROP OR CONE SIZE IN *PINUS STROBUS*

H. B. Kriebel[1]

ABSTRACT--Statistical analysis of 6 years' breeding experiments on *Pinus strobus* L. showed that conelet drop was controlled by female parent but not by white pine pollen species or year of pollination. Crossing with a species never yielding viable seed did not increase conelet drop. The degree of loss was the same after self- and open-pollination as it was after controlled crossing. The length and weight of mature cones also depended only on female parent. A possible relation is suggested in pines between the presence or absence of a pollen effect on cone retention and the type of crossability barrier.

Premature cone drop in pines is a widespread and serious problem in many species. It is especially frequent in the first part of the conelet stage, i.e., early in the first year of cone development.

Abscission of conelets during the first few weeks after pollination may result from severe competition with vigorously growing vegetative shoots for photosynthates and mineral nutrients (Sweet and Bollman 1970). It also appears to be associated with a threshhold proportion of aborted ovules per cone (Burdon and Low 1973b).

In regions where pollen supply is limited, wind pollination may be insufficient for cone retention. If most of the ovules are not pollinated, they collapse in a few days and the conelet drops off (Sarvas 1962).

Cone losses due to wind, animals, birds, and insects may occur at any time during cone development but are most common at later stages, expecially during the second year.

The degree of maternal control over conelet drop varies among individuals. Clonal tests of *Pinus sylvestris* L. have shown that trees vary widely in the amount of pollen required to retain conelets (Brown 1970). Clonal variation in conelet drop in *Pinus radiata* D. Don, where pollen supply is not a problem,

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may be due to clonal differences in spring growth patterns because of the critical competition with vegetative growth (Sweet and Bollman 1970).

The pollen parent had no effect on conelet drop in intraspecific crosses of Pinus resinosa Ait. (Fowler 1965b). In Hagman's crosses between species and between subgenera of pines, there was no effect in crosses of Pinus peuce Griseb. and Pinus cembra L. with other white pines, regardless of crossability. A pollen parent effect was apparent, however, in crosses of these pines with hard pines. In P. sylvestris, the male effect varied from nil to high in species crosses within the hard pines and was pronounced in crosses with white pines.[2]

Conelet drop is a serious problem in P. strobus. In Ohio, heavy losses occur during the first 4 weeks after pollination. Records suggest that there were differences in conelet drop among trees used in breeding experiments in Ohio. This led to analysis of crossing records to estimate male and female parental effects as an aid in the selection of breeding materials. The availability of 6 years' data permitted analysis of the possible effect of year of pollination as an indication of nongenetic effects.

METHODS

Crossing Technique

Crosses were made in young stands and plantations of P. strobus in north central Ohio. They included selfs, P. strobus crosses, and crosses with other white pines. Standard pollination procedures were used with adequate precautions for protection from contamination. For each cross combination, records were kept of the pollen parent, number of strobili pollinated, number of conelets bagged for insect protection, and number of cones collected at maturity. Yield of filled and empty seed was recorded. Lengths and weights of cones from 4 years' collections were measured after air-drying and seed extraction.

Analysis

Analysis of conelet drop in interspecific crosses followed the procedure in the generalized least squares program for partial regression developed by Barr and Goodnight (1971). An analysis of variance was run on cone yield data per tree x tree combination for the 6 years 1962, 1963, 1964, 1965, 1966, and 1969 in relation to the number of strobili pollinated. Estimates of the effects of year, male species, and female tree were obtained. Because of the large number of levels involved, the individual male parent effect was excluded from the analysis. This effect, if any existed, would be smaller than the effect of pollen species. If pollen species were nonsignificant, the individual male parent could

[2] Personal communication with M. Hagman.

therefore be assumed to have no significant effect on conelet drop.

Comparisons of the effects of selfing vs. outcrossing and of controlled vs. open pollination on cone yield per flower were made by "t" tests of trees on which both types of cross were made.

Estimates of the effects of year, pollen species, individual pollen parent, and female tree on cone length and cone weight were also obtained from a least squares analysis of variance. The analysis was based on the mean of each tree x tree combination for all crosses made from 1962 through 1965.

The effects of selfing vs. outcrossing and of controlled vs. open-pollination on cone length and weight were compared by "t" tests.

RESULTS

Mean cone yield, cone length, and cone weight are summarized in table 1 by species cross. Analysis by tree combination showed:

1. Conelet drop was strongly controlled by the individual female parent (p of a larger F = 0.0001).

2. Pollen parent had no significant effect on conelet drop even when it was another species of white pine, either crossable or noncrossable in terms of sound seed yield.

3. Neither selfing nor open pollination affected conelet drop in comparison with controlled intraspecific cross-pollination.

4. Year of pollination had no effect on conelet drop when adjusted for male and female parent.

5. There was no effect of pollen species, pollen parent, year, selfing, or open pollination on length or weight of dry mature cones.

DISCUSSION

Biological Relations

The absence of a pollen parent effect on conelet drop in *P. strobus* is typical of at least two other white pines, as previously noted. A negligible pollen parent effect on cone length has also been observed in other pines (Fowler 1965a, Burdon and Low 1973a).

The accumulated evidence from these experiments and others covers a diversity of species and suggests that there is a common factor in

the pollen of white pines favoring cone retention, cone scale development, and seed coat formation.

Table 1.--Cone yield, cone length and weight, and sound seed yield of *P. strobus*

Cross combination	Female parents	Strobili pollinated	Mean cone yield 1/	Mean dry cone length 1/	Mean dry cone weight 1/	Mean sound seeds per cone
	No.	No.	Percent	cm	gm	No.
INTERSPECIFIC CROSSES						
P. strobus x						
griffithii	15	1,067	21	10.6	7.9	9.4
monticola	14	3,619	21	9.0	5.4	4.4
peuce	17	2,572	19	8.4	5.4	1.7
peuce x *strobus*	5	334	19	10.4	6.8	.5
parviflora	9	360	18	9.5	5.9	6.8
flexilis	10	1,056	23	9.4	5.8	.0
albicaulis	2	343	05	10.0	5.8	.0
cembra	10	879	15	12.0	7.3	.0
koraiensis	4	274	11	2/	2/	.0
INTRASPECIFIC CROSSES						
P. strobus x						
strobus outcross	41	10,464	19	9.4	6.3	19.3
strobus open	9	438	20	9.6	6.8	19.6
strobus self	17	911	19	9.4	5.9	11.5

1/ Differences nonsignificant at p = 0.05 (see Methods).
2/ No measurements taken.

Diffusible pollen-wall enzymes appear to play a role in pollen-tube nutrition and growth (Stanley and Linskens 1965, Knox and Heslop-Harrison 1970) and might be involved in cone nutrition. Genetic factors in pollen controlling the production of sugars and critical to ovule development may function in the nucellar tissue (Hagman 1975) but in at least some species, e.g., *Pinus thunbergiana* Franco, pollen viability is not a requirement for conelet retention (Katsuta 1971).

White pines seem to have embryo inviability as an isolation mechanism (Hagman and Mikkola 1963, Kriebel 1972). It is possible that

a common pollen factor in these pines initially induces cone scale development and later stimulates pollen tube growth and fertilization. Subsequent control is through genome interaction, leading either to full seed formation or embryo abortion, depending on the species combination.

In contrast, incompatibility with arrested pollen tube growth is the typical reproductive barrier in P. sylvestris and other hard pines investigated (McWilliam 1959, Vidaković and Jurković-Bevilacqua 1970). Hagman found that P. sylvestris, unlike the soft pines, has a higher rate of conelet drop in inter- or intra-sectional crosses than in crosses within the species. Clearly, some pollen stimulus to cone retention occurs in P. sylvestris, because all the cones in incompatible species crosses do not abort, but the effect on the strobilus is insufficient for full pollen tube growth and fertilization. All hard pines may not have male-induced conelet drop, but it is evident that they do not share a pollen factor favoring cone retention in interspecific crosses.

The finding that conelet drop was no higher in P. strobus after selfing than after outcrossing agrees with results of other selfing experiments on both soft and hard pines. Pollen-tube incompatibility has never been observed in either subgenus of pines in response to self-pollination (Hagman 1964, 1975). The reaction to selfing occurs after fertilization. Therefore, the results of selfing P. strobus tend to support the suggested relation between post-fertilization ovule abortion and polen-induced cone retention in inviable crosses.

Practical Implications

From the standpoint of the breeder or silviculturist, the dominant female influence on conelet drop in P. strobus implies that individual tree selection for cone retention is of critical importance, especially for seed orchards. Past performance is a good indication of future cone yield. This criterion must, of course, be integrated into the overall selection index and not made at the expense of desirable tree characteristics.

ACKNOWLEDGEMENTS

Mr. J. H. Roberds, USDA Forest Service, Genetics Department, North Carolina State University, Raleigh, North Carolina, provided invaluable

assistance in application of the generalized least squares analysis program to the data. Professor M. Hagman of the Forest Research Institute, Maisala, Finland, kindly provided records of conelet drop analysis from his extensive pine breeding experiments.

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JACK PINE SEEDLING SEED ORCHARD ESTABLISHMENT AND PROJECTED SEED YIELDS

Richard M. Jeffers[1]

ABSTRACT.--Jack pine test plantings of open pollinated progenies from proven seed sources can be converted to seedling seed orchards. A combined selection index based on individual as well as family performance can be used to select the best individuals to retain in the orchards. Use of the index will result in retention of more families than under alternate schemes and permit more rigorous selection within families. A broad genetic base is maintained, the dangers of inbreeding are reduced, and the greatest genetic gain is assured. This scheme was applied to a set of data from a 90-seed-source test, a complement of seed sources was selected, and flowering and cone data from these seed sources were used to predict early seed yields in jack pine seedling seed orchards. After initial thinning, 1 hectare of orchard established according to the suggested scheme may yield 266.8 M full seed annually. This is probably a conservative estimate.

When grown under short rotation on good sandy soils, jack pine grows rapidly and produces a greater volume of pulpwood than red pine (Wilde *et al.* 1965). Although small amounts of jack pine are being planted in the Lake States, it is expected to play an increasing role in Lake States planting programs as the demand for paper and paper products increases (King 1973).

Jack pine grown under standard nursery and field conditions produces male and female strobili at 3 to 6 years of age. However, by forcing growth with long photoperiod, fertilization, and wide spacing in the greenhouse and nursery, flowering can be advanced to as early as 17 months after sowing seed (Rudolph 1966, Jeffers and Nienstaedt 1972). Thus, the time between sexual generations in jack pine can be reduced to less than 3 years (Rudolph 1966). With rapid generation turnover, repeated cycles of selection, progeny testing, and breeding can be used to genetically improve jack pine in a short time (King 1973).

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Establishing seedling seed orchards appear to be the best method for mass producing genetically improved jack pine because this species flowers at an early age and presents serious graft incompatibility problems.[2]

Methods for establishing jack pine seedling seed orchards have been suggested by King (1973), Yeatman (1974a) and Klein (1974). This paper includes a brief outline of their schemes. Results obtained from a nursery and field test of jack pine seed sources are used to describe an alternate scheme and to estimate projected early seed yield from the seed orchards.

JACK PINE SEEDLING SEED ORCHARD ESTABLISHMENT

King's Method

King's (1973) scheme begins with selecting--on the basis of seed source tests--the better seed collection areas for the planting region. Between 300 and 400 trees are chosen--no more than 15 individuals per stand--and open-pollinated seed collected and sown in a replicated nursery test. After 2 years' nursery growth, the 200 fastest growing families are selected and used to establish 2 or 3 test plantings on different sites in the planting area. The progeny test plantings are converted to seed orchards when the trees begin to bear regular pollen and cone crops--about 7 to 8 years after field planting. The plantings are measured at this time and thinned by removing all trees from below-average seedlots and some of the poorer trees from above-average seedlots. The plantings are remeasured at age 12 to 14 years and thinned to leave 1 out of every 4 trees of the best 25 seedlots.

Yeatman's Method

Yeatman (1974a) suggests improving the natural populations for seed collection by converting seed production areas into seedling seed orchards. When original stands are cut, replant them with local plus-tree progenies. Yeatman recommends systematically sampling 100 to 200 plus-trees on a unit area basis to avoid selecting close relatives. Selected trees are grafted and included in breeding archives. Open-pollinated progenies are used in first generation orchards followed by controlled-pollinated families (full-sib) from selected parents. Parents are selected for their breeding value determined initially from clonal tests (stem and branch form) and subsequently from progeny tests (survival and growth). Small half-sib family plots are initially established at 1.0 to 1.5 m spacings. A two-stage thinning operation is used: first thinning at 6 to 8 years to about half the original stocking, and second thinning at 11 to 14 years. Ultimately, 30 to 50 percent of the families are selected and family plots are thinned to a single tree per plot.

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[2] Unpublished data on file at the Institute of Forest Genetics, North Central Forest Experiment Station, Rhinelander, Wisconsin.

Klein's Method

Klein (1974) reports a design composed of 20 half-sib families, that have little or no co-ancestry from each of 11 geographic areas. The orchard consists of 24 blocks; each block contains 11 plots (area collections); each plot contains one tree from each of the 20 half-sib families from each area collection. Selection thinnings ultimately reduce the number of trees to one per plot. After thinning, each block contains 11 trees, each from a different area; thus, mating occurs predominantly among sources.

My Method

The scheme I suggest follows King's (1973) closely, deviating only in the selection processes used to rogue the orchards. All families are field tested and selections are based on a combination of seed source, family and individual tree performance. Some outstanding individuals within below average families may be selected but more families are included in the orchards and the dangers of inbreeding are reduced.

The scheme will be applied to a set of data from a 90-seed-source test, a complement of seed sources will be selected, and flowering and cone data from these seed sources used to predict early seed yields in jack pine seedling seed orchards.

THE EXAMPLE SEEDLING SEED ORCHARD

Seed Source and Parent Tree Selection

The 20 most promising seed sources were chosen in our northern Wisconsin planting of the rangewide seed source study initiated by the Canadian Forestry Service, Petawawa Forest Experiment Station at Chalk River, Ontario, in 1962 (Yeatman 1974b). (Table 1). Total heights of the trees from the 90 seed sources at age 11 was the sole selection criterion. The local source from Nokomis, Wisconsin, (2233) ranked 13 among the 20 selected sources. Trees from the Cloquet, Minnesota, source (2242)--the best of the 20 seed sources--were about 9 percent taller than those from Nokomis, while the poorest trees from the Constance Bay, Ontario, source (2208) were about 4 percent shorter than local trees.

For our purpose, the procedure used rather than the actual seed sources selected is the important point. In a seed source study of 90 to 100 sources tested at a single location in the planting area, we should select 10 to 25 of the most promising sources for retesting at additional test locations in the planting region. Inclusion of material from 10 or more sources will help to assure a broad genetic base for advanced generation breeding. And increased adaptive variability may result from crosses between individuals with different adaptive characteristics (Zobel 1971).

Table 1.--Projected seed yield from a young jack pine seedling seed orchard[1]

Source No. IFG	Source No. PFES	State or Province	Local Name	Lat. °N	Long. °W	Female strobili[2] tree	Female strobili[2] ha	Cones[3] ha	Seed[4] ha
2242	3279	MN	Cloquet	46.7	92.6	10.8	1,728	864	21,600
2230	3267	WI	Nekoosa	44.3	89.7	2.8	448	224	5,600
2236	3273	MI	Marl Lake	44.5	84.7	4.7	752	376	9,400
2231	3268	WI	Waupaca	44.3	89.0	8.6	1,376	688	17,200
2241	3278	MN	Brainerd	46.3	94.2	5.1	816	408	10,200
2211	3247	QUE	Harry Lake	46.4	76.2	5.9	944	472	11,800
2238	3275	MI	Gladstone	46.0	86.5	9.7	1,552	776	19,400
2246	3283	MAN	Hadashville	49.5	95.8	7.5	1,200	600	15,000
2209	3244	QUE	Fort Coulonge	45.8	76.7	9.1	1,456	728	18,200
2243	3280	MN	Cass Lake	47.3	94.6	5.8	928	464	11,600
2235	3272	MI	Fife Lake	44.6	85.4	4.9	784	392	9,800
2229	3266	WI	Wisconsin Dells	43.8	89.8	2.0	320	160	4,000
2233[5]	3270	WI	Nokomis	45.6	89.8	8.1	1,296	648	16,200
2210	3246	ONT	Petawawa Plains	45.8	77.4	10.3	1,648	824	20,600
2207	3242	ONT	Douglas	45.5	76.9	8.6	1,376	688	17,200
2232	3269	WI	Mosinee	44.8	89.7	9.0	1,440	720	18,000
2225	3262	ONT	Gowganda Lake	47.6	80.7	4.5	720	360	91,000
2192	3227	QUE	St. Louis de France	46.4	72.6	9.3	1,488	744	18,600
2228	3265	WI	Lone Rock	43.6	90.2	2.8	448	224	5,600
2208	3243	ONT	Constance Bay	45.5	76.1	3.9	624	312	7,800
				Mean or Total		6.7	21,344	10,672	266,800

1 Seed yields per hectare of orchard; 3,200 trees/ha
2 Data from a 5-year nursery test; 160 trees/source
3 50 percent female strobili to mature cones
4 Twenty-five full seed/cone
5 Local source

The example in Table 1 includes only 12 seed sources from the Lake States: more Lake States seed sources would have been desirable. The other eight seed sources are from Manitoba, Ontario, and Quebec. Of these, the Manitoba source probably is very similar to sources in northern Minnesota. Early results in our Wisconsin test suggest that the other seven seed sources are adapted to environmental conditions similar to our test site. The choice of such geographically widely separated seed sources for inclusion in a regional breeding program may be questionable. Advantages of such broad sampling have not been proven biologically and collection from widely separate origins may not be economically feasible. Until more research data is available, one may choose to restrict selection of seed sources to the planting region.

Phenotypic selection of 10 trees in each of the 20 selected stands will provide 200 half-sib families for establishing the progeny test seedling seed orchard. Selected trees should be at least 500 feet apart in a stand to minimize common ancestry. Consequently, it is best to select from relatively "large" stands on good sites, rather than from "small" stands on poor sites. The trees selected should be dominant and with good stem form, a wide branch angle, little evidence of serious second flushing characteristics (Rudolph 1964), and no insect or disease damage. It will not be worthwhile to spend much time and money on the selection of the parent trees using systems such as the "comparison tree method." Simple phenotypic selection of the individual parent trees after a quick survey of the stand is all that is required.

Establishment of Progeny Test-Seedling Seed Orchard

For the greatest return from a jack pine breeding program, genetically improved stock should be planted on the best jack pine sites. Therefore, the test plantings should be established on better sandy loam soils with a climate similar to the planting region (Wilde et al. 1965).

A randomized, complete block design with two to four tree family plots is recommended. Rows should be spaced wide enough (2.5 m) to permit use of equipment in the planting for the first few years after establishment. Trees within rows should be planted at close spacings (0.5 m) to permit early culling of the poorest trees. Thus, at an initial spacing of 0.5 by 2.5 m, 1 hectare of planting will contain 8,000 trees (20 sources, 10 families per source, and 40 seedlings per family).

Six to 8 years after establishment the trees in the progeny test should be evaluated for height and diameter growth, form, and pest incidence. A combined selection index based upon individual as well as family performance is then used to select the best individuals (Falconer 1960).

Thinning schemes similar to those recommended by King (1973), Yeatman (1974a), and Klein (1974) that result in elimination of a large proportion

of families based on average family performance ignore differences in variation patterns among individuals within the families. Combined selection assures the greatest genetic gain and because a large number of families are retained after roguing, a broader genetic base will be maintained in first generation orchards. This may result in greater potential genetic gain in advanced generation orchards (Canavera 1975).

A second generation of orchards can then be established using controlled pollinated full-sib families produced from the best individuals from the first generation orchard. Selected parents from two or more orchards in the planting area should be included in the crossing scheme and new selections should be added to the program periodically to keep the genetic base as broad as possible.

PROJECTED SEED YIELD

The number of female strobili per tree (table 1) at the beginning of the fifth growing season in a nursery test was used to estimate annual seed production from a young jack pine seedling seed orchard established according to the scheme recommended in this paper.

If an average of 160 individuals from each of the 20 seed sources are retained in the seed orchard after the initial thinning, then 1 hectare of orchard may produce 266.8 M full seed annually, an equivalent of 213.4 M plantable seedlings. The basic assumptions used in this example are: (1) the trees in the orchard will produce the same number of females as in the nursery test, (2) 50 percent of the female strobili will mature into cones, (3) there will be 25 full seed per cone, and (4) 80 percent of the full seed will yield plantable seedlings.

The 50 percent female abortion rate is based on actual observation over a number of years. Twenty-five full seed per cone is an average based on a preliminary study of the seed yield from individual cones (Jeffers 1972). Newer studies now in progress suggest that 25 seeds per cone may be a conservative estimate. We set the percent plantable seedlings at 80 percent of full seed because the USDA Forest Service (1974) reports germination capacities from 69 to 87 percent and because seed from seed orchards should be of high quality in a physiological sense as a result of insect control and optimum growing conditions. However, the goal should be germination capacities exceeding 90 percent because plant production should be under optimum conditions in the nursery or greenhouse and culling should not exceed 10 percent.

Thinning is likely to remove some of the provenances and perhaps some families within the remaining provenances from the production seed orchard. Depending on the provenance and family variation in strobili production, this could affect the potential for seed yield after roguing.

The seed sources are arranged in order of decreasing heights in table 1 (352 cm to 310 cm); it is clear that the correlation between heights and seed potential is not significant (r = 0.170). Therefore, the effect of roguing on the basis of heights would be random in terms of seed yield. Family variation within provenances in potential seed production is significant (Jeffers and Nienstaedt 1972), but there is nothing that would suggest that the roguing would reduce the seed production potential.

The projected seed yields are probably conservative. Because seed for the rangewide seed source test was sown directly in the nursery and the trees were grown at a 1 by 1 foot spacing, the number of females produced per tree probably is less than the number produced by orchard trees grown at wider spacings. By sowing progeny test seeds in the greenhouse and raising the seedlings under intensive greenhouse, nursery, and field techniques, earlier and heavier flowering will occur (Rudolph 1966), and thinning of seed orchards will promote crown development resulting in additional increased flower production among remaining trees.

The projected seed yield data indicate that a five-fold difference in seed yields may exist among trees from different seed sources. And variation in number of females per tree among trees within sources in our nursery test were even greater. This great variation in seed yield among different genotypes will continue to exist throughout the life of a seed orchard, but we have no assurance that the early good seed producers will continue to be the best. And we don't know if the best seed producers will have continued fast vegetative growth. Studies on other species suggest that this will not be the case. This lack of information necessitates continued research on flowering and potential seed yield. In addition, much valuable information may be obtained if seed orchard managers in the future will keep accurate records of growth, flowering, and seed production throughout the life of the orchards.

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

Gilbert H. Fechner[2]

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 polliation 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	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 ± 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 explantation. 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	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 developimg 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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GEOGRAPHIC VARIATION OF GROWTH AND WOOD PROPERTIES IN JAPANESE LARCH IN SOUTHWESTERN LOWER MICHIGAN[1]

Chen Hui Lee[2]

ABSTRACT--Growth and wood characteristics at age 10 from planting were assessed on the 22 seedlots of Japanese larch outplanted in the Kellogg Forest, Augusta, Michigan using a randomized complete block design. Results did not indicate any geographic trends for most traits studied but did suggest the operation of genetic drift and inbreeding. Fast growing seedlots continued to perform well in southwestern Lower Michigan. Recommends that seed for plantings in the Lake States area should be from the Mt. Nantai area in the northeast species range.

Japanese larch (*Larix leptolepis* (Sieb. et Zucc.) Gordon) is one of the most important economic species of Japan. It is found in the subalpine regions of Central Japan and attains sizes up to 30 meters in height and 1 meter in diameter (Japan For. Tech. Assoc. 1964). The wood is heavy, decay resistant, holds nails well, and pulps readily using the sulfate process. The species is extensively used for reforestation outside its natural range in northern Japan, Europe, the United States, and Canada because it grows fast and is valued for ornamental uses. It can be propagated readily from cuttings collected from young trees (Chandler 1967).

The natural range of Japanese larch is small (about 200 kilometers square, from 900 to 2,500 meters in elevation) and is composed of several genetically isolated small populations, the largest being several kilometers across and the smallest a hectare or so in size (Wright 1962). Considerable improvement of this species can be expected through a careful selection and breeding program because previous studies indicated that there were significant differences in growth performance. These include

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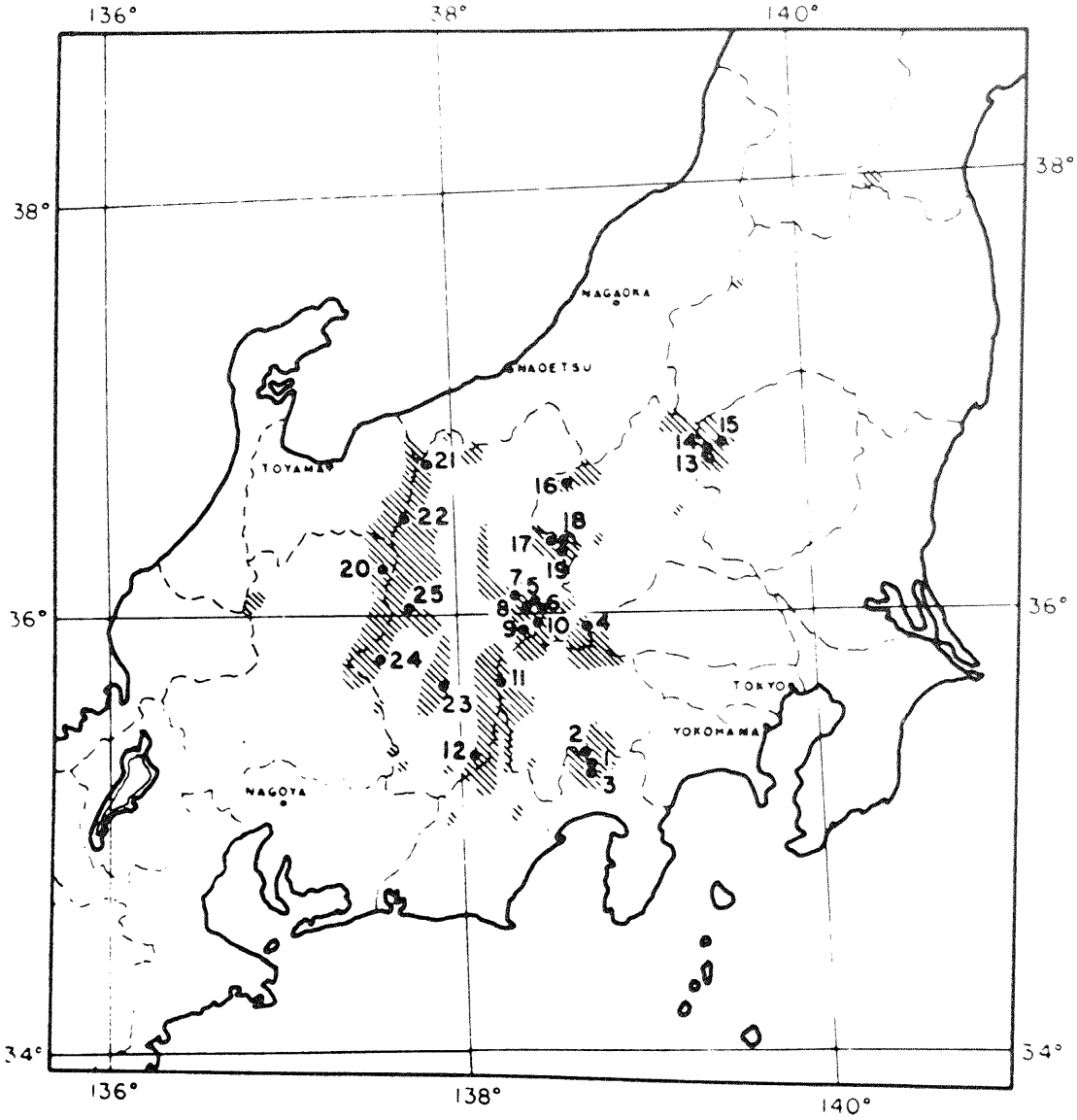

Figure 1.--Natural range (shaded) and location (numbered dots) of seedlots of *Larix leptolepis* after Farnsworth *et al.* (1972).

Alcohol-benzene extractives were removed because their presence tends to overestimate specific gravity (Taras and Saucier 1967). The specific gravity was determined following Smith's recommendation (1954).

Wood fibers were macerated in an equal mixture of glacial acetic acid and 30 percent hydrogen peroxide at 50-55°C for 72 hours. After several changes with distilled water, the macerated fibers (tracheids) were stained with 1 percent Bismarck Brown Y aqueous solution overnight and then mounted on slides. No dehydration or cover glass was used (Echols 1969), but fibers were measured directly to the nearest millimeter with 90 X magnification under the Bausch and Lomb No. 2700 projector.[3] They were then converted back to the actual tracheid length. Mean lengths of 20 tracheids were used as items in statistical analysis.

In the analysis of variance of growth and wood data, the degrees of freedom were 21, 9, and 189 for provenances, replication and error, respectively. However, there were 16 missing plots; the error term degrees of freedom were reduced accordingly. Rank correlation (d.f. = 20) was used to study the relations among growth, wood, and origin data of parent stands.

VARIATION IN GROWTH TRAITS

Height Growth

The between-seedlot differences in total height were significant at the 1 percent level (table 2). At age 10 after planting, seedlot 15 from Mt. Nantai continued to outgrow others.

Significant differences in juvenile height growth among 25 provenances were also reported by Langner (1961) at Schmalenbeck, North Germany, and by Hattemer (1968, 1969) at 13 localities throughout Germany; by Farnsworth et al. (1972) on the 18 test sites in north-central United States using 7 and 22 of the seedlots; By Lester (1964) who observed 6 seedlots in Wisconsin; by Stairs (1966) in New York (20 seed sources); and by Genys (1972) in Maryland (16 seedlots). All these studies were part of the international Japanese larch provenance testing program.

Despite it's narrow species range, variation in height growth was large. At age 10 from planting, seedlot means varied from 540 to 714 cm, a difference of 32 percent. This is roughly equivalent to 300 kg/m^3 or 20 lbs/ft^3 (62.4 lbs. x (1.32 - 1.00)) more wood production. The corresponding values obtained for eastern white pine (Pinus strobus L.)

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3 Mention of trade names does not constitute endorsement of the product by the USDA Forest Service.

Table 2.--Growth and wood properties of 22 *Larix leptolepis* provenances at age 12 from seed in southwestern Lower Michigan

Origin No., Mountain of origin	Height	Diameter at stump height	Height/ diameter ratio	Specific gravity	Tracheid length	Index wood production 1/
	(cm)	(cm)	(cm/cm)	(number)	(mm)	(number)
1 Fuji	663	11.7	57	0.400	2.08	1.05
2 Fuji	674	12.0	56	.424	2.11	1.19
4 Azusa	696	12.9	54	.392	2.17	1.31
5 Yatsuga	714	12.2	59	.388	2.12	1.18
6 Yatsuga	670	12.3	54	.374	2.06	1.09
7 Yatsuga	693	12.5	55	.405	2.07	1.27
8 Yatsuga	600	10.4	58	.404	2.08	.76
9 Yatsuga	673	12.1	56	.378	2.09	1.08
10 Yatsuga	683	12.6	54	.399	2.07	1.25
11 Akaishi	594	10.0	59	.412	2.13	.71
12 Akaishi	615	10.9	56	.393	2.13	.83
13 Nantai	636	11.4	56	.398	2.06	.95
14 Nantai	686	12.9	53	.386	2.03	1.27
15 Nantai	714	12.6	57	.405	2.13	1.32
16 Shirane	609	10.8	56	.403	1.99	.83
17 Asama	673	12.3	55	.390	2.00	1.14
18 Asama	655	11.1	59	.405	2.08	.95
19 Asama	540	9.5	57	.413	1.98	.58
23 Komaga	600	10.4	58	.441	2.05	.83
22 Hida	649	11.2	58	.411	2.06	.96
24 Hida	626	11.9	53	.379	2.03	.97
25 Hida	600	11.5	52	.366	2.04	.84
Mean	647	11.6	56	0.398	2.07	1.02
F	3.26**	2.49**	1.58*	1.79*	1.27	--

1/ Index $= \frac{\text{Height}}{\text{Mean Height}} \times \frac{(\text{Diameter})^2}{(\text{Mean Diameter})^2} \times \frac{\text{Specific Gravity}}{\text{Mean Specific Gravity}}$

and European black pine (*Pinus nigra* Arnold) from the same Kellogg Experiment Forest were 23 and 34 percent, respectively (Lee 1974, Lee and Wright 1975). These three species are test planted in the same general area with a high degree of precision as reflected in the small-sized (2.45 to 3.88 percent) coefficients of variability (the standard deviation to mean ratio).

The correlations between growth rate and the latitude, altitude, and mean annual precipitation at localities of parent stand were not significant.

Similar results have also been reported by Pauley et al. 1965; Lester 1964; Farnsworth et al. 1972; Langner 1961; Schönbach et al. 1966, and Genys 1972). Fast growing seedlots were from the northern as well as the southern species range and, likewise, from low as well as high altitudes. However, I observed a distinct geographic trend between the 1971 height growth, longitude, and the mean annual temperature at seed origin. The rank correlation coefficients were 0.497 and 0.514; both significant at the 5 percent level. Schönbach et al. (1966) also observed a clearcut correlation between early frost resistance and the time of growth cessation. This suggests that clinal variation patterns in height growth essentially are temperature related; trees from the species' eastern range and from localities where mean annual temperatures are high grew faster and suffered less from the early autumnal frost.

Professor Jonathan W. Wright of Michigan State University generously furnished the 1974 height data (13 years after planting). Therefore, it was possible to compute the following age-age correlations.

Age in years \ Age in years	2	5	6	10	13
2	1.00	0.69	0.63	0.46	0.51
5		1.00	0.97	0.75	0.83
6			1.00	0.82	0.88
10				1.00	0.91
13					1.00

The rank correlation coefficients were all statistically significant at the 1 percent level; however, they tended to decrease as the plantation grew older. In general, fast growing seedlots at two years of age were still growing well at age 13, but some changes in the growth pattern have occurred. For example, seedlot 15 from Mt. Nantai ranked fifth in 1963 at age 2. It was the top performer in 1964, when it was 60 percent taller than the slowest growing seedlot. In 1966 (age 5) it was 70 percent taller than the shortest seedlot. This figure dropped to 37 in 1967 and to 32 percent in 1971; by 1974 - 13 years from seed - seedlot 15 ranked sixth. The story is somewhat similar to that of the southern Appalachian seedlots in eastern white pine (Lee 1974). Therefore, continued observation on the growth pattern is necessary because changes in relative height may continue to occur in the future.

It is important to determine the genotype x environment interactions for each provenance. My study was based on a single plantation and provided no information. However, according to Wright (1962), the Germany-Michigan correlation in height data was low. Provenances that were fast-growing in Germany were not necessarily good in Michigan. Langner and

Stern (1965) attributed the absence of a growth relation to severe winters in Michigan. In the recent study by Farnsworth et al. (1972), there were 7 common seedlots represented in 18 plantations throughout the north-central United States. They reported a strong genotype x environment interaction in height performance. Seedlot 15 was tallest at most test sites but grew poorly in Nebraska and Ohio. The interaction was described as unintelligible. Hattemer (1969) was not able to explain genotype x environment interactions in height growth of Japanese larch. This means that the tallest seedlots in one plantation may not be the best in other plantations.

However, Genys (1972) found that Maryland growth data were more strongly correlated to data from Germany than to data from north-central United States. His study was based on 16 different seed sources.

Diameter Growth 1 Ft. Above Ground

There were significant (1 percent level) differences in diameter growth among the 22 seed sources (table 2). No geographic pattern was observed. The rank correlation coefficients between the diameter and origin data were weak.

The range in seedlot means varied from 9.5 to 12.9 cm, a difference of 36 percent. This range was far smaller than that observed at the two Maryland test plantations (Genys 1972). However, Genys' and my data are similar in one respect--both have established a strong positive height-diameter correlation ($r = 0.720$ in my study). The two growth traits may be inherited together.

The between-seedlot differences in the height-to-diameter ratios (cm/cm) were significant at the 5 percent level (table 2). A seedlot having a high h/d ratio is more desirable because it has less stem tamper.

The h/d ratios were correlated with the 1971 height and diameter. Both correlation coefficients were insignificant ($r = 0.170$ and -0.297, respectively).

DIFFERENCES IN WOOD QUALITY

Specific gravity and tracheid length are the most extensively studied wood properties, both are under moderate to strong genetic control (Smith 1967, Zobel 1961); thus, significant gains can be expected through selection breeding. The wood characteristics of Japanese larch are not well known and information based on rangewide material is not available.

Use of increment core sample extracted at the breast height or stump height (1 foot above ground) has been a common practice for the evaluation

of wood quality. However, whether the entire increment core (pith to bark) or portion of the core should be used is not known. I found that a single growth increment formed during the same growing season was adequate to show the geographic variation pattern.

The stump height (or breast height)-whole tree values relation was not determined. However, there are numerous studies for pines that indicate a strong correlation between the two values (Wahlgren and Fassnacht 1959).

Specific Gravity

The between-seedlot differences were significant at the 5 percent level (table 2). A single seedlot (Schmalenbeck No. 23) from Mt. Komaga had a higher specific gravity than those from the rest of the species range.

There was no clearcut geographic variation pattern in specific gravity; the correlations between specific gravity and origin data were weak (the rank correlation coefficients were from 0.044 to 0.326). Heavy wood may be characteristic of seedlots from northern as well as from southern localities, or from both high and low altitudes.

Nor was there a significant correlation ($r = 0.031$) between specific gravity and height growth measured in 1971, 10 years after planting. This is similar to loblolly pine (*Pinus taeda* L.), for which Matziris and Zobel (1973) attributed only 7.3 percent of the total variation in specific gravity to growth rate ($r = 0.271$ with 353 degrees of freedom). Selection of fast growing seedlots may not be accompanied by a desirable wood quality. The specific gravity-diameter growth correlation was also nil ($r = 0.003$) in Japanese larch.

The overall mean for the trunkwood specific gravity was 0.398, comparable to that observed for loblolly pine (Matziris and Zobel 1973). In both studies, juvenile wood was used as the study material. The range in seedlot means varied from 0.366 to 0.441--a difference of 20 percent. This is equivalent to 75 kg more wood/m^3 (4.68 lbs/ft^3). The between-tree range was from 0.311 to 0.525, a difference of 69 percent. The greater between-tree variation was expected; it has been found in eastern white pine and European black pine growing in the same area in Michigan. The larger the variation among individual trees, the faster and more efficient improvement can be made through selection.

Tracheid Length

There were no significant differences in tracheid length among the 22 Japanese larch seed sources.

Bannan (1965) studied fiber morphology for members of several coniferous genera and concluded that species or races growing on favorable sites in general had longer fibers. Although the tracheid-height and the

tracheid-diameter correlations were statistically significant (1 percent level) in Japanese larch, they are of little practical importance. Mean tracheid length of the five tallest seedlots was only 0.04 mm longer than that of the five shortest ones.

Overall mean tracheid length was 2.07 mm. The seedlot means ranged from 1.98 to 2.17 mm, a difference of only 10 percent. The between-tree range was from 1.75 to 2.39 mm, a difference of 37 percent. Both ranges were much narrower than those of specific gravity.

The tracheid length-specific gravity correlation was weak (r = 0.370). This was also the case with a number of pine species such as loblolly pine (Jackson and Strickland 1962, Matziris and Zobel 1973), eastern white pine (Lee 1974) and European black pine (Lee and Wright 1975). The two wood properties may be inherited independently.

PRACTICAL APPLICATION

Based on my observations, seed sources from Mt. Nantai in the northeastern species range should be recommended for planting in the Lake States area. They are tall and grow at least 5 percent taller than the plantation mean (647 cm at age 10 from planting). Trees from Mt. Nantai are efficient wood producers (table 2); they started growth early in the spring (the time of leafing not correlated with the latitude and altitude of seed source according to Yanagisawa 1961), tended to shed off their leaves early, and suffered little damage from winter cold (Farnsworth et al. 1972). Schönbach et al. (1966) indicated that the resistance to early autumnal frost was closely associated with time of growth cessation. Mt. Nantai is located at a northern latitude; thus, trees from that area lignify early which contributed to frost-damage being less than that found in trees from more southerly seed sources (Yanagisawa 1961). The wood quality of trees from Mt. Nantai is average. Genys (1972) reported that trees from Mt. Nantai are not as susceptible to larch sawfly and offer better stem quality (straightness) when compared to trees growing further south.

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TEN-YEAR PERFORMANCE OF DOUGLAS-FIR PROVENANCES IN EASTERN NEBRASKA

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ABSTRACT--Seedlings from 55 seed sources were established in a field test as 1+1+1 potted transplants on a silt loam soil near Plattsmouth, Nebraska. Mortality was 76 percent in the nursery the first year, increased to 89 percent for potted seedlings the second year, and reached 98 percent 1 year after field planting. All coastal types died and survival was low for north-central provenances. Arizona and New Mexico seed sources gave the best survival (20 percent). Height-latitude correlation was r = 0.81, southern Colorado, New Mexico, and Arizona seed sources grew best. Some north-central provenances have grown well in recent years. Spring growth flush is earlier in southern than in northern material. The pattern agrees with the spring frost pattern in Michigan: southern sources are damaged while northern sources are not. In Nebraska the southern material suffered fall frost damage perhaps as a result of delayed growth cessation. A Durango, Colorado, provenance is recommended for landscape, greenbelt, and Christmas tree plantings in eastern Nebraska. A Mt. Lemmon, Arizona, provenance is recommended for Christmas trees in eastern Nebraska sites protected from spring frosts and winter winds.

Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) is the most important commercial timber tree in the United States. In this paper, however, we speak of Douglas-fir not for timber purposes but as a tree for the enhancement of the environment and for use as Christmas trees. The Great Plains region of North America needs conifers for protection and for ornamental purposes, and Douglas-fir trees can help fulfill these needs in selected locations if the source of the seed is carefully chosen.

A study to identify better adapted seed origins of trees for planting in the central Great Plains is being conducted as part of the Cooperative Regional Tree Improvement Project (NC-99) of the North Central States Agricultural Experiment Stations. Credits go to Jonathan W. Wright, Professor of Forestry, Michigan State University for initiating the study

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and providing planting stock, and to Walter T. Bagley, Associate Professor, Department of Forestry, University of Nebraska, for cooperation in planting and maintaining the plantation.

PAST RESEARCH

Douglas-fir has been successfully introduced in Europe. The interior (Rocky Mountain) variety has been planted in mountainous areas with severe climates and the coastal form restricted to milder climates of England and parts of Germany (Frothingham 1909). In the United States, rangewide provenance tests east of the Rocky Mountains have consistently revealed that West Coast origins are highly susceptible to winter damage and that trees from southern Rocky Mountain origins grow fastest.

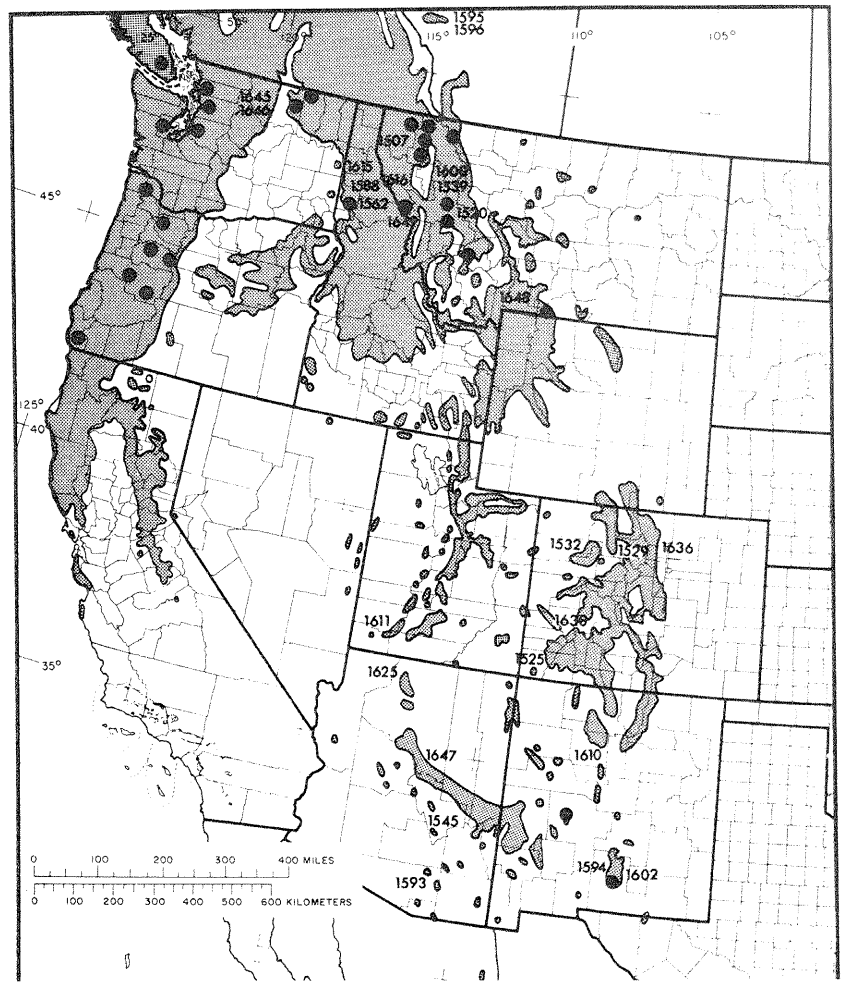

Figure 1.--Natural distribution of Douglas-fir, and provenances tested in eastern Nebraska. Origin numbers denote those that have survived; black circles, those that died in the first 3 years (Little 1971).

Geographic patterns of variation within the range of the species (fig. 1) have been recognized but there is some lack of agreement on them. Frothingham (1909) divided the range into five silvical regions: (1) North coast, (2) Sierra, (3) Northern Rockies, (4) Central Rockies, and (5) Southern Rockies. The first two comprise the area known as var. menziesii, and the other three are in the regions of var. glauca.

Wright et al. (1971) delineated 8 to 10 geographic areas based on performance in 3- to 8-year-old provenance plantations in Michigan and Nebraska. Some of these groups were similar to Frothingham's pattern and others were not. Arizona and New Mexico sources grew tallest and had bluer foliage at 8 years of age, but were damaged by winter cold in the Michigan plantings. Origins from west of the Cascades suffered extreme winter damage, and practically all of them died in the nursery.

Heit (1968) recognized three separate forms of Douglas-fir based on a New York nursery study of seedlings from range-wide sources. He referred to a Pacific Coast form viridis (now var. menziesii); a continental inland form caesia (presumably northern Idaho, Montana, and northern Wyoming); and a lower Rocky Mountain form glauca (remainder of range from central Rockies southward). Heit found, that of 11 interior origins, those from Coconino National Forest, Arizona, and the Carson National Forest, New Mexico, grew fastest and had the bluest foliage. A Montana origin of the Lewis and Clark National Forest grew slowest, and Colorado origins had average growth. He concluded that southern origins grew later into the summer when annual growth ceased for others, and that frost damage to these origins as seedlings was light and temporary because they grew normally the following season without any apparent leader deformity.

A Pennsylvania test of 19 origins from the Pacific Coast to the Rockies resulted in 55 percent mortality in western Washington and Oregon origins, compared to 21 percent for those from Colorado and New Mexico. Yet growth rate of surviving Pacific northwest trees slightly exceeded that of interior origins. Late spring frosts damaged interior origins, but they recovered rapidly and grew well (Byrnes et al. 1958).

Gerhold (1966) tested 67 of Wright's origins in a nursery near Potters Mills, Pennsylvania. West Coast origins were severely damaged by winter cold but the survivors were tallest of all origins at age 3. As in Wright's study, the Arizona and New Mexico origins outgrew other interior origins but suffered more winter injury.

Past studies all seem to indicate that the natural range of Douglas-fir in the United States is composed of the following groups: (1) Pacific Coast, (2) northern Rockies (and Wright's inland Empire), (3) central Rockies, and (4) southern Rockies. Moreover, all studies appear to indicate that the Pacific Coast variety does not survive east of the Rockies, and that the southern Rocky Mountain origins grow fastest.

The performance of Douglas-fir origins in eastern Nebraska reported here updates the first report by Wright et al. (1971) of the 55 origins tested in Nebraska, with 5 additional years' growth, and new information on spring growth flushing and winter damage to terminals.

METHODS

Seeds were collected from 128 natural stands throughout the range of the species in United States and Canada and sown in spring 1962 in a Michigan State University nursery near East Lansing. One year later, 30 to 60 seedlings each of 55 origins were sent to Lincoln, Nebraska, where they were lined-out in a holding bed (table 1). In spring 1964, the 1+1 transplants were dug, potted, and lined-out again at the same location to increase their size.

In spring 1965 they were field planted as 1+1+1 stock at the Horning State Farm near Plattsmouth, Nebraska. The plantation is located on a ridge top of silt loam derived from loess, at 41°N,96°W, and 1,100 feet elevation. Growing season averages 170 days and mean annual precipitation is 30 inches; 75 percent falls during the growing season. Seedlings were planted in one-tree plots at a spacing of 12 by 12 feet Eastern redcedar (*Juniperus virginiana*) fillers were planted for early protection between each Douglas-fir in the rows (but not between rows) to give a spacing of 6 by 12 feet.

The site was cultivated 1 year before planting, and Simazine 80W at 4 lbs per acre was sprayed on both sides of each tree row after planting to control weeds, and for 5 years thereafter. The plantation was mowed between rows during the growing season. The trees were checked several times each year for insects, diseases, and other injury. Heights were measured annually from 1966, except for 1972. The eastern redcedar fillers were removed in spring 1974 to prevent excessive crowding.

Trees were rated on two dates in spring 1974 and on four dates in spring 1975 as to the developmental stage of buds and growth of new shoots and needles. Each tree was given a phenology rating on a scale of 1 to 5, ranging from dormant buds to well advanced shoot and needle growth. An estimate of the sequence in which the different origins start spring growth was obtained by using the ratings of the one date each year that showed the greatest spread in values.

Average needle length was computed from five measurements of needles collected from lateral branches on the south side of each tree, and cone production was evaluated by counting all cones in August 1975.

Table 1.--Data on seed origin locations of Douglas-fir tested in eastern Nebraska

COAST VAR. MENZIESII

Michigan State Univ. origin No.	State or Province	Place	North latitude	West longitude	Elevation
			Degrees	Degrees	Feet
1634	VAN	Cowichan L.	48.8	124.0	600
1620	WA	Camano	48.2	122.3	50
1617	WA	Granite Falls	48.1	122.0	600
1623	WA	Enemclaw	47.2	122.0	1,308
1627	WA	Shelton	47.2	123.4	320
1624	OR	Jewell	45.8	123.4	700
1621	OR	Molalla	45.2	122.2	100
1618	OR	Cascadia	44.4	122.7	800
1585	OR	Sisters	44.3	121.8	3,500
1622	OR	Cottage Grove	43.8	123.0	675
1613	OR	Oakridge	43.7	122.5	3,000
1619	OR	Brookings	42.0	124.2	162
*1645	WA	Fish Lake	48.6	119.7	2,000
*1646	WA	Buck Mtn.	48.4	119.8	5,000
ROCKY MOUNTAIN VAR. GLAUCA (WEST OF CONTINENTAL DIVIDE)					
1556	WA	Curlew	48.9	118.8	4,100
1651	WA	Omak	48.6	119.5	2,500
*1615	ID	Coeur d'Alene	47.7	116.8	2,400
*1588	ID	Wallace	47.5	116.0	3,000
*1562	ID	Clarkia	47.0	116.1	4,500
1573	ID	Moscow	46.6	116.8	2,500
*1507	MT	Libby	48.4	115.5	3,800
1517	MT	Libby	48.4	115.2	4,000
1650	MT	Whitefish	48.5	114.7	3,500
1519	MT	Whitefish	48.4	114.7	4,000
1521	MT	Kalispell	48.2	114.5	3,000
*1600	MT	Spotted Bear RS	48.0	113.0	3,680
*1616	MT	St. Regis	47.5	115.2	4,000
1603	MT	St. Regis	47.2	114.8	5,000
*1649	MT	Missoula	47.0	114.0	3,500
1504	MT	Missoula	47.0	113.8	6,000
*1520	MT	Greenough	46.9	113.4	4,000
1506	MT	Salmon Lake	47.2	113.2	5,000
*1539	MT	Big Prairie RS	47.3	113.5	4,600
1518	MT	Stevensville	46.5	114.2	4,500
1606	MT	Butte	46.0	112.5	6,500

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Table 1. continued

Michigan State Univ. Origin No.	State or Province	Place	North latitude	West longitude	Elevation
			Degrees	Degrees	Feet
ROCKY MOUNTAIN VAR. GLAUCA (EAST OF CONTINENTAL DIVIDE)					
*1595	ALB	Kananaskis	51.0	115.0	4,500
*1596	ALB	Kananaskis	51.1	115.0	5,000
1513	MT	St. Mary	48.8	113.5	4,480
*1648	MT	Big Timber	45.5	110.0	6,000
1503	MT	Absarokee	45.5	109.4	5,600
ROCKY MOUNTAIN VAR. GLAUCA (CENTRAL AND SOUTHERN ROCKIES)					
*1636	CO	Boulder	40.2	105.5	8,650
*1529	CO	Kremmling	40.0	106.5	8,000
*1532	CO	Meeker	40.2	107.9	8,200
*1630	CO	Ouray	38.2	107.6	9,100
*1525	CO	Durango	37.5	107.8	8.500
*1610	NM	Jemez RD	35.5	106.8	8,500
1546	NM	Magdalena	34.2	107.2	8,200
*1594	NM	Cloudcroft	33.0	105.8	8,670
*1602	NM	Mayhill	32.9	105.4	7,000
1614	NM	Sacramento Mtns.	32.7	105.7	8,300
*1611	UT	Panguitch	37.6	112.5	8,250
*1625	az	Fredonia	37.0	112.5	9,000
*1647	AZ	Long Valley	34.7	111.0	7,000
*1545	AZ	Globe	33.3	110.7	7,800
*1593	AZ	Mt. Lemmon	32.4	110.8	8,400

* Origins for which subsequent data are given in table 2; all other origins failed.

RESULTS AND DISCUSSION

Survival

The number of surviving trees from many of the northern origins was only one or two; this naturally limits the confidence one can place on growth performance, and should be considered when the results are interpreted.

Mortality in the temporary lineout beds at Lincoln was 76 percent the first year. At the end of the second year as potted seedlings, total mortality had increased to 89 percent. All seedlings of Coastal western Washington and Oregon origins died during the first 2 years. Three years

after field planting in 1968, mortality had reached 98 percent. Only 2 percent of the seedlings from northern Idaho, western Montana, eastern Washington, and Alberta, Canada, origins survived after 3 years in the field (table 2).

Table 2.--Survival and height growth of Douglas-fir provenances in eastern Nebraska

Michigan State Univ. origin No.	Location	Surviving trees	Height growth: Mean ann. 1967-1974	Height growth: 10-year field	Height growth: Plantation mean
		No.	Feet	Feet	Percent
1539	Big Prairie, MT	1	0.3	2.7	25
1648	Big Timber, MT	1	0.3	2.9	27
1562	Clarkia, ID	2	0.4	3.3	30
1596	Kananaskis, ALB	1	0.4	3.9	36
1595	Kananaskis, ALB	1	0.5	4.6	42
1600	Spotted Bear, MT	1	0.6	5.1	47
1649	Missoula, MT	1	0.8	6.8	62
1636	Boulder, CO	7	0.8	7.1	65
1588	Wallace, ID	7	0.8	7.2	66
1529	Kremmling, CO	2	0.8	7.3	67
1615	Coeur d'Alene, ID	2	0.9	7.4	68
1520	Greenough, MT	1	0.9	7.6	70
1646	Buck Mtn. WA	1	1.0	8.0	73
1507	Libby, MT	1	1.0	8.0	73
1532	Meeker, CO	21	0.9	8.0	73
1611	Panguitch, UT	5	0.9	8.1	74
1616	St. Regis, MT	3	1.0	8.3	76
1645	Fish Lake, WA	4	1.0	8.8	81
1625	Fredonia, AZ	8	1.0	9.1	83
1630	Ouray, CO	1	1.1	9.4	86
1525	Durango, CO	8	1.0	10.8	99
1647	Long Valley, AZ	12	1.3	11.6	106
1610	Jemez, NM	28	1.5	12.9	118
1545	Globe, AZ	4	1.4	12.9	118
1594	Cloudcroft, NM	8	1.5	13.0	119
1602	Mayhill, NM	34	1.6	13.5	124
1593	Mt. Lemmon, AZ	13	1.8	15.8	145

Survival of central and southern Rocky Mountain origins was 20 percent. Arizona and New Mexico origins had the best survival.

The excessive mortality of coast and northern Rocky Mountain origins in the first 2 years was probably because they were smaller than stock normally planted in the Plains region. Douglas-fir planting stock is usually not transplanted until seedlings are 2 years old. They are then grown another 2 years as transplants before field planting. Past experience in field planting Colorado sources in the Plains has shown that much higher survivals can be obtained if stock is 2+2.

Height and Growth Rates

Heights and growth rates (table 2, figs. 2 and 3) were correlated with latitude; trees from the southern origins grew faster than those from northern origins. Regression analysis using individual trees as a basis indicated that latitude accounted for 21 percent of the variation in tree heights. Correlation of height and latitude using origin means was $r = -0.81$.

Growth curves grouped by geographic areas show that for 5 to 6 years after planting the central and northern Rocky Mountain origins (the seven lowest curves on fig. 3) grew very slowly, but have since shown a gradual acceleration in growth. This contrasts with the performance of various pine provenance tests at the Horning farm, which normally increase their growth rate in the third year. At first the Douglas-fir planting site was open and exposed, but as redcedar filler trees and adjacent species developed, the plantation was protected from wind during both winter and summer.

All New Mexico and Arizona origins have grown rapidly since planting. Trees from Globe, Arizona, (#1545) grew fastest through 1969, but since that time have suffered winter injury and repeated loss of terminals. Mt. Lemmon, Arizona, (#1593) trees surpassed the Globe source and remained the tallest origin to date, averaging 15.8 feet.

Foliage Characteristics and Form

Needle lengths varied among and within origins, but showed no correlation with rate of height growth or latitude (table 3). Several New Mexico and Arizona trees had bluish-green foliage, but none of these origins was consistently blue-green, as reported by other investigators. Northern Rocky Mountain trees had green foliage. Branch angles, foliage densities, and compactness of crowns varied among trees within the same origin, making it impossible to recommend any particular source for its inherently superior aesthetic value.

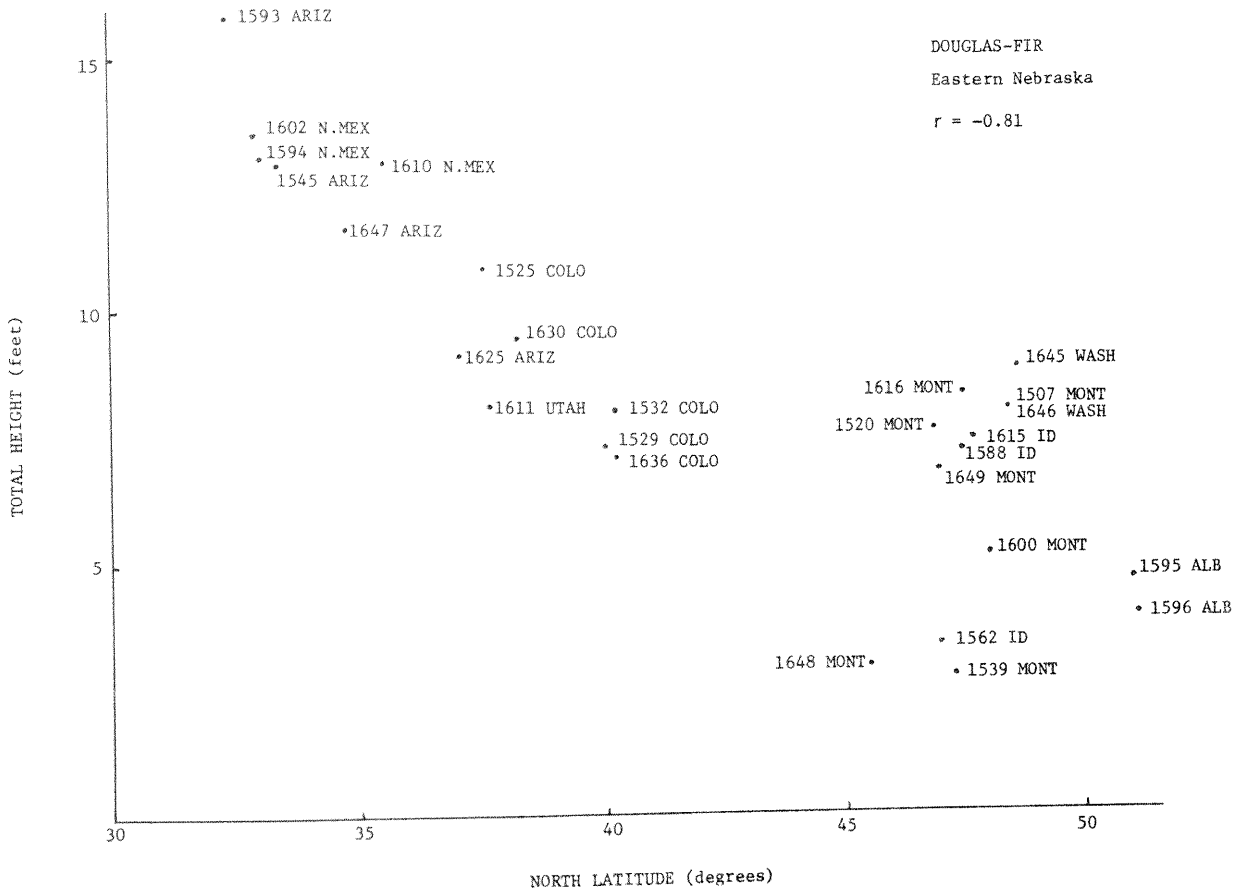

Figure 2--Correlation of 10-year field height with latitude of origin, in eastern Nebraska.

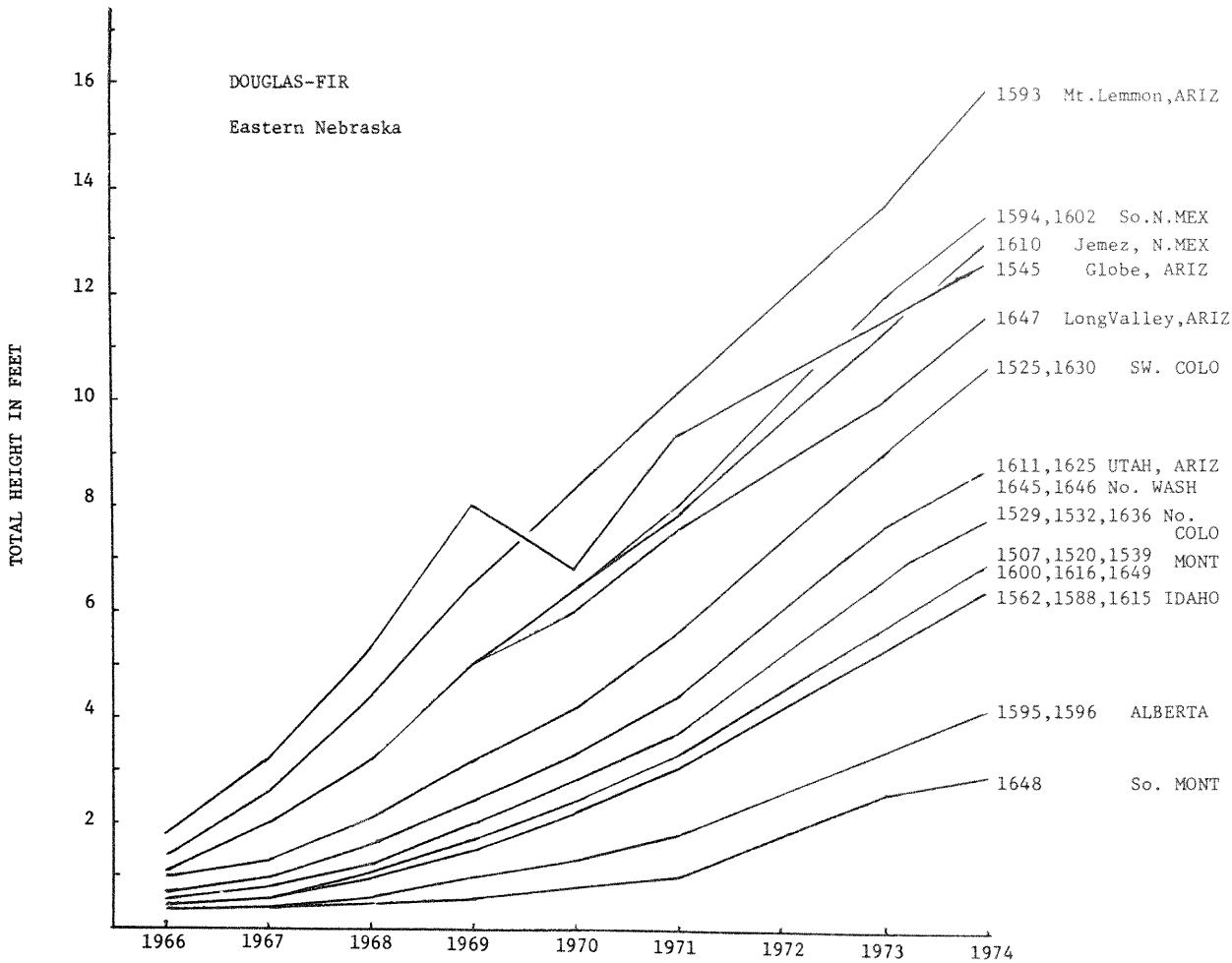

Figure 3.--Growth curves of origins (some grouped together) in the 10-year field test in eastern Nebraska.

Table 3.--Needle length, spring flushing, and terminal dieback on Douglas-fir provenances in eastern Nebraska

Michigan State Univ. origin No.	Location	Average needle length	Spring growth flushing May 9, 1974	Spring growth flushing May 12, 1975	Trees with terminal die back
		mm.	Code[1/]		Percent
1595	Alberta	26	2.0	1.5	
1596	Alberta	25	3.0	1.5	
1507	W. Montana	28	1.0	1.5	
1616	W. Montana	28	1.7	1.0	
1600	W. Montana	28	2.0	1.0	
1649	W. Montana	28	2.0	3.0	
1520	W. Montana	28	2.0	2.0	
1539	W. Montana	31	3.0	2.0	
1588	N. Idaho	32	2.0	1.5	
1615	N. Idaho	32	2.8	1.8	
1562	N. Idaho	28	2.8	1.5	
1648	S. Montana	25	3.0	2.0	
1645	NC.Washington	32	2.9	2.0	25
1646	NC.Washington	27	4.0	3.0	
1532	N. Colorado	27	3.9	2.8	5
1636	N. Colorado	25	4.3	2.7	
1529	N. Colorado	24	4.8	3.0	
1611	S. Utah	30	3.2	2.3	
1625	N. Arizona	27	3.2	2.2	25
1630	SW.Colorado	33	2.0	1.0	
1525	SW.Colorado	34	4.5	3.5	
1610	N. New Mexico	29	3.5	2.8	39
1602	S. New Mexico	30	3.5	2.6	30
1594	S. New Mexico	32	3.8	2.8	38
1647	NC.Arizona	32	4.6	3.2	58
1545	SC.Arizona	40	4.4	3.4	100
1593	S. Arizona	30	4.1	2.9	38

1/ 1.0 = latest; 5.0 = earliest.

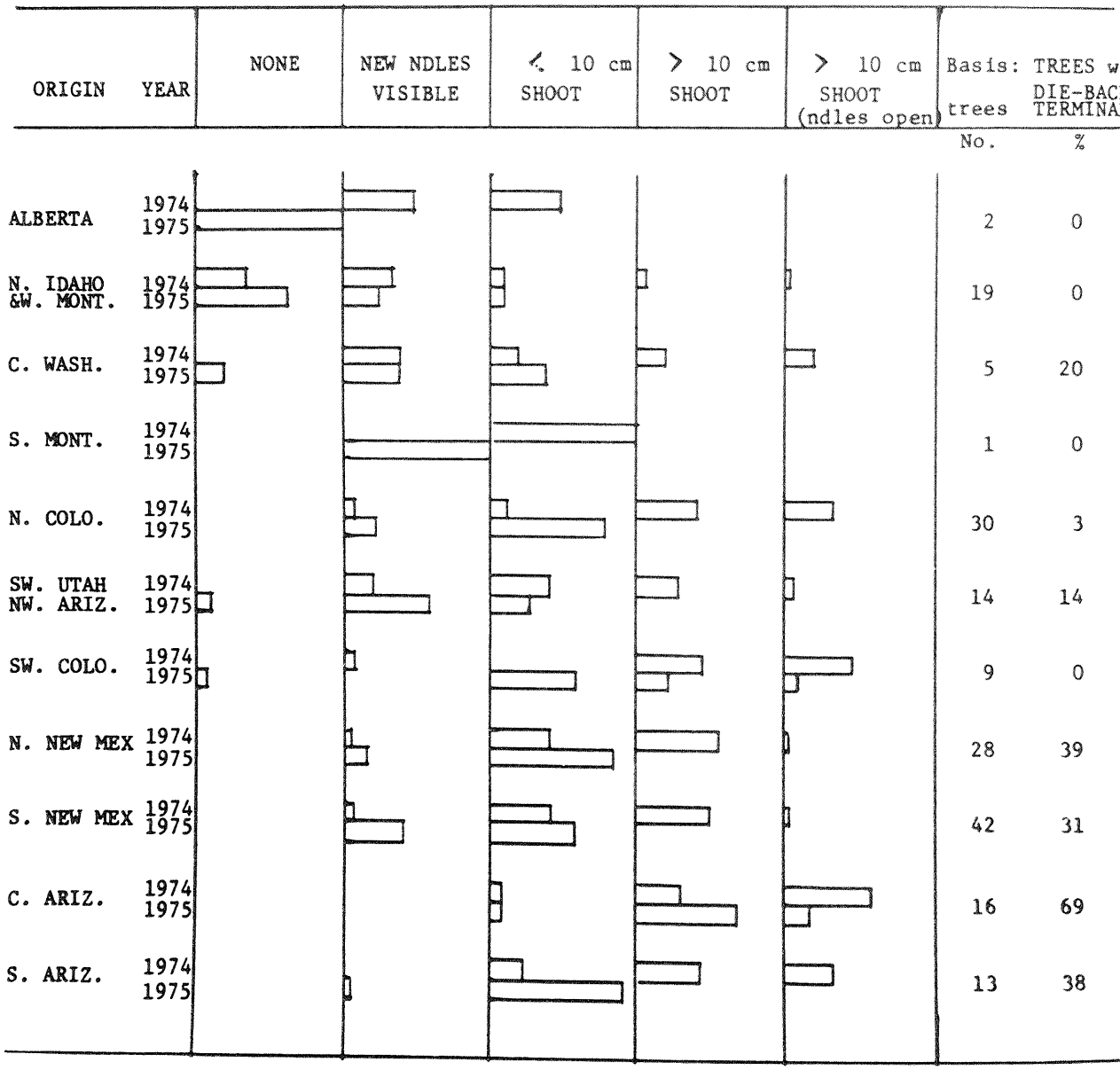

Figure 4.--Spring growth flushing 1974 and 1975 by origins and percent of trees affected by terminal dieback.

Spring Growth Flush

Bud bursting and subsequent shoot and needle development showed widest variation by origin on May 9, 1974, and on May 12, 1975. New shoot and needle development were well advanced on most Arizona and New Mexico origins, at the same time many of the northern origins were still dormant or just beginning to flush (table 3 and fig. 4). Colorado origins tended to be intermediate. This was consistent for the 2 years, 1974 and 1975, although different spring temperature patterns in 1975 resulted in a narrowing of the rating values compared to 1974.

Steiner and Wright (1975) found this same relation in a Kellogg, Michigan, plantation at 12 years of age. Arizona, New Mexico, and Colorado origins leafed out early and were highly susceptible to late spring frost, whereas origins from western Montana and northern Idaho leafed out a month later and were not susceptible.

Munger and Morris (1936) recorded the same bud bursting activity in 13 Coastal sources of Douglas-fir, west of the Cascade Range and extending over only 3-1/2° latitude from northern Washington to central Oregon. Bud bursting was earliest on the southerly and low elevation sources, and latest on those of northern Washington.

Another point of interest is the apparent similarity in phenology of the central Washington origins with those further south in the central Rocky Mountains, rather than with origins much closer in northern Idaho and western Montana (fig. 4). Frothingham's distribution of silvical regions shows the eastern side of the Cascades through Washington in the same silvical region as the central Rockies.

These observations indicate that Douglas-fir phenology at this Nebraska latitude (41°) as related to latitude of origin, is the reverse of ponderosa pine. In a central Nebraska nursery experiment, the northern origins of ponderosa seedlings from central and eastern Montana began spring growth several weeks before origins from New Mexico (Read 1975). Despite the early growth flush of southern origin Douglas-fir in Nebraska, no damage from late spring frost has yet occurred. This is because the ridge top plantation site is sufficiently exposed to delay extremely early bud bursting and to prevent frost pockets.

Terminal Dieback

Differences in time of bud set and cessation of terminal growth were not measured. However, a possible result of differences in time of growth cessation, has been dieback of the terminals on 44 trees (about 40 percent) of the southern origins. Every Arizona and New Mexico origin showed damage on some trees, ranging from 25 percent of #1625, Fredonia, Arizona, to 100 percent of #1545 Globe, Arizona, (table 3, figs. 3 and 4).

Trees of southern origins do not cease growth early enough to avoid frost damage in late fall. This agrees with Wright et al. (1971) who found that among interior origins growing in Michigan and Pennsylvania, southern origins set buds latest, and therefore were winter damaged, while northern origins set buds earliest and were not injured.

Campbell and Sorensen (1973) found this same relation among West Coast origins of Douglas-fir, covering only 5 degrees spread in latitude from southern Oregon to northern Washington.

It is interesting that terminal dieback did not occur until these trees were 5 to 6 years old and averaged around 7 feet in height. Increased exposure of tops to winter winds may increase susceptibility, as it was noted that dieback increased significantly during the first winter after removal of the filler trees. Winter dieback has not yet caused mortality despite its recurrence on the same trees in successive winters. Strong lateral branches grow into dominant terminals the following growing seasons (fig. 5).

Cone Production

First cones were observed in August 1975, after 11 years in the field. No measure of seed production and viability is yet available.

Six of 13 trees of the Mt. Lemmon, Arizona, source had cones ranging from 2 to 75 per tree, with a median of 7 to 8 cones. Two of 28 trees of the Jemez, New Mexico, source had 3 to 5 cones, and one of 4 trees of the Fish Lake, Washington, source had 13 cones. Initial cone production was strongly related to the tallest and largest crown trees in the plantation.

CONCLUSIONS AND RECOMMENDATIONS

This provenance test indicates that within the interior (Rocky Mountain) Douglas-fir var. glauca, there are large variations in survival, growth, and susceptibility to cold, which are strongly correlated with latitude of origin. Pacific Coast origins var. menziesii, cannot survive Nebraska winters. Northern Rocky Mountain origins have low survival rates and grow slowly. Therefore, northern Rockies and Pacific Coast origins of Douglas-fir are definitely not recommended for planting in Nebraska. Southern Rocky Mountain origins survive well and grow very fast, but individual trees suffer some cold injury. Central Rocky Mountain origins have average survival and growth, yet are not affected by cold temperatures.

Figure 5.--A #1647 central Arizona tree showing recovery from terminal dieback of the previous winter.

Douglas-fir can be grown with greatest success in eastern Nebraska, although with irrigation it probably can be grown further west in the State. Planting stock for maximum survival should have at least 8 to 12 inches top height and a fibrous root system of similar size; these will normally be 2+1 or 2+2 age class from the best nurseries. Younger potted stock may be satisfactory.

Major uses of this species in central Great Plains are for ornamental plantings and for Christmas trees. Therefore the slower growing, but winter hardy central Rocky Mountain origins may prove most successful in the long run. The Durango, Colorado, origin #1525, which has above average survival, medium growth, and no winter damage, is well adapted for landscape plantings, Christmas trees, greenbelts, and roadside parks. Central Rocky Mountain origins are not recommended for windbreaks because faster growing species of pine and juniper which give protection in fewer years, are available for this purpose.

Because of terminal dieback, the faster growing Arizona and New Mexico origins of Douglas-fir are the only ones recommended for Christmas trees. These short rotation tree crops should be a safe investment, because the winter killing of terminals in the Horning plantation did not occur until trees exceeded merchantable Christmas tree height. In planting these southern origins, it is essential to select plantation sites to avoid frost pockets where spring frost damage could occur, and wind swept areas where terminal dieback could be serious. The Mt. Lemmon, Arizona, source #1593 is recommended for Christmas tree growers in eastern Nebraska. These trees averaged 6.5 feet in height after only 5 years in the test plantation. Use of this fast growing origin will give Christmas tree growers a more rapid return on investment than slower growing trees.

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PROVENANCE AND FAMILY VARIATION IN BALSAM FIR FROM MICHIGAN AND WISCONSIN

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ABSTRACT.--Variation in height, branching, and flushing was measured for wind-pollinated families from six provenances at age 11 in from one to four plantations. Seed collected at one location in the Lower Peninsula of Michigan produced trees that were 20 percent taller, had 40 percent more lateral branches in the top whorl, and had a flushing score 25 percent later than average. Variation among families within provenances was between 30 and 40 percent of respective provenance means. Both provenance and family effects were significant, but provenance effects were generally much larger. Provenance selection clearly would be worthwhile in the Lake States.

Balsam fir (*Abies balsamea* L.), a species widely distributed in North America and a major component of the boreal forest, has received little attention from tree breeders and geneticists. Although commonly harvested for pulpwood, balsam fir is rarely planted except for ornamental uses. Principal interest, from a genetic point of view, has been in the taxonomic status of the species and the morphologically similar Fraser fir (*A. fraseri* (Pursh) Poir.) and subalpine fir (*A. lasiocarpa* (Hook) Nutt.) (Boivin 1959, Zavarin and Snajberk 1972).

To determine patterns of geographic variation, seeds were collected in 1960 by R. G. Hitt of the University of Wisconsin. Initial sampling was in Michigan and Wisconsin as a part of a regional project on geographic variation in many forest tree species. The study was expanded in 1962 to include sampling of the species range.

The results reported here represent early performance of provenances for which seed was maintained separately by maternal parent. These results are intended to supplement the picture of geographic variation being developed from measurements on provenance plantings from range-wide sampling.

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MATERIALS AND METHODS

Six balsam fir stands in Michigan and Wisconsin were sampled (fig. 1). At each location, cones were collected from 10 trees located at least 100 feet apart. Seeds from each tree were stored separately at 2°C until sowing in 1963. Seedlings were grown for 3 years at the Trout Lake State Forest Nursery in north-central Wisconsin, then distributed to cooperators who established four plantations (A, B, C, and D) (fig. 1). Plantations A and B trees were field planted as 3-2 stock in 1969 and plantation C and D trees were field planted as 3-3 stock in 1970. Plantations A and D are in areas averaging 150 or more frost-free days; plantations B and C average less than 120 frost-free days (fig. 1). Soils at all locations are loams and each plantation is on an upland site.

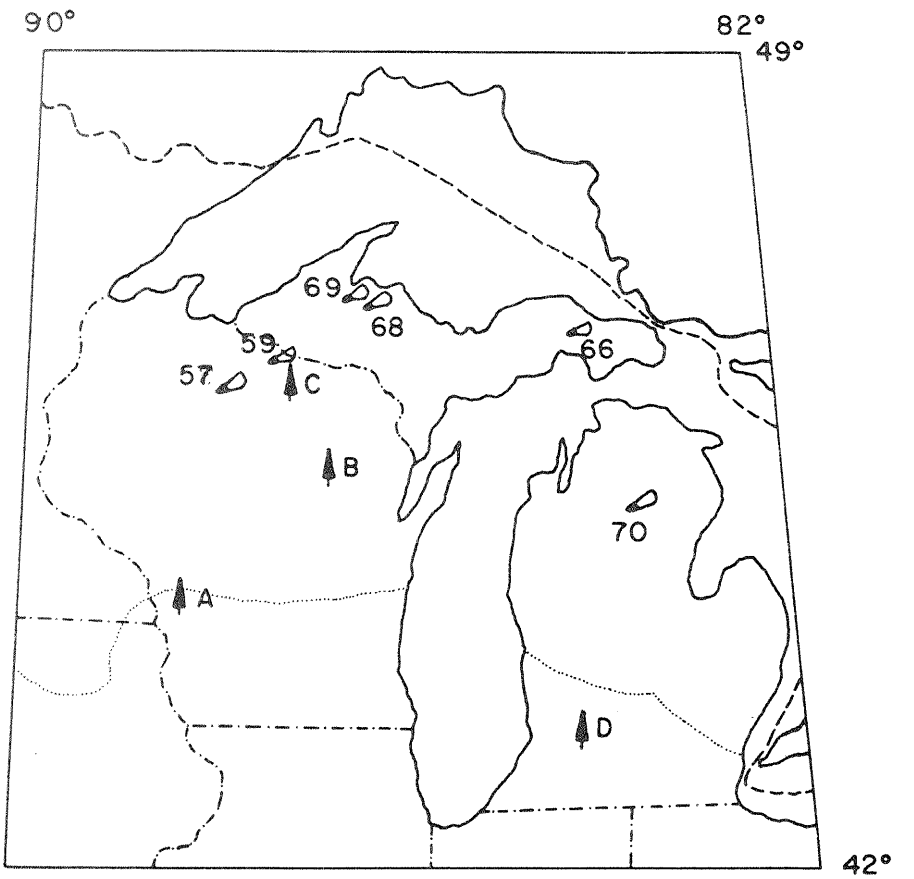

Figure 1.--Map of the study area with numbered provenance locations (◀) and plantation locations (▲). The southern boundary of the botanical range of balsam fir is indicated by a dotted line.

A call for data was issued to cooperators in 1974 and the most comprehensive data were standardized and analyzed for this summary of results.

Only a few families are represented in all plantations because numbers of seedlings in several families were small. Analyses of variance were based on randomized complete block designs using data only from families and numbers of blocks that were equally represented. A least-significant-difference was calculated following Waller and Duncan (1969). Components of variance were calculated using a random model.

RESULTS AND DISCUSSION

Means for total height show the superiority of Provenance 70 and the inferiority of Provenance 66 (table 1). The array of heights for this provenance sample from the Lake States is similar to the array from range-wide sampling except that some Quebec provenances had greater vigor and some Saskatchewan provenances had less.

Table 1.--Average performance of six balsam fir provenances for four traits at 11 years from seed (height was measured at plantations B, C, and D; lateral branch number at B and C; flushing score at C and D; and lateral bud number at C)

Provenance	Height	Lateral branches	Lateral buds	Flushing score[1/]
	Feet	Number	Number	
57	3.6	3.5	4.0	2.4
59	3.7	3.4	3.7	2.6
66	2.9	2.2	3.1	3.0
68	3.3	3.2	3.8	2.4
69	3.5	3.3	3.8	2.2
70	4.4	4.8	5.1	1.8
Least Significant Difference				
.05	0.2	0.4	0.2	0.5

1/ Scores range from 1 for least shoot growth initiation to 4 for most shoot growth initiation.

The number of lateral branches in the top whorl was chosen as a variable trait of some interest to Christmas tree growers. Although shearing can increase the density of crowns with few lateral branches, three or less laterals probably would reduce the quality of most Christmas trees. The range of variation again was similar to results from range-wide studies and Provenance 70 was the best.

The number of lateral buds in the top whorl also was variable. The average number of lateral buds is not directly comparable with average number of lateral branches because two plantations are represented in the former array and one in the latter. In plantation C, where both branches and buds were counted, an average of 0.4 bud per tree failed to develop in 1974.

Flushing scores for provenances showed a range of variation similar to that found for other traits. Ranking of provenance means was consistent in both plantations as well as in range-wide provenance data from nursery (Lester 1970) and field plantings. Flushing in Provenance 70 has been observed to be up to 10 days later than in most other provenances and frost damage has been rare. Conversely, Provenance 66 has been heavily frost-damaged in several years, presumably as the result of early flushing. In northeastern Wisconsin, a delay in flushing from May 5 to May 20 would reduce the probability of exposure to freezing temperatures by a factor of four.

When the four traits are viewed in aggregate, the provenances separate consistently into three groups. Provenance 70 is the most vigorous, forms the most lateral branches, and flushes latest. Provenance 66 is the converse of 70 and the other four provenances are intermediate to various degrees. These results raise the question whether differences in height and branching are a consequence of inherently different vigor or of the relation of early spring frost damage to variation in time of flushing. All plantations were damaged by frost in 1 or more years. Although joint effects of variation in vigor and in frost damage cannot be separated in these studies, patterns of shoot growth illustrated by periodic measurements in the nursery (Lester 1970) indicate that both inherent vigor and phenology of bud development are important in determining total height.

The effect of unseasonably low temperature on different stages of bud development needs further study. The loss of potential branches occurs at various stages from before exposure of new leaves to intermediate phases of branch elongation. Loss during early stages of branch elongation seems to be a direct response of succulent tissues to freezing and yet visible damage after frost often occurs only on some of the shoots that have elongated to the point of exposing most of their needles.

Among families within provenances, the range of variation for each trait was between 30 and 40 percent of the provenance mean. Families from Provenance 68 were the most variable for height (55 percent), number of buds (47 percent), and flushing score (54 percent). Families from Provenance 70 were least variable for height (21 percent) but highly variable for flushing score (48 percent).

Both provenance and family effects were almost always highly significant (table 2). Interactions were generally negligible and were relegated to the error term.

Table 2.--Summary of analyses of variance for height, number of lateral branches and buds in the top whorl, and flushing score for provenances and families of balsam fir

Source of variation	Plantation A				Plantation B			
	Height		Number of branches		Height		Number of branches	
	d.f.	F level	d.f.	F level	d.f.	F level	d.f.	F level
Blocks	8		5		8		6	
Provenances	2	**1/	2		5	**	5	**
Families/provenance	9	**	9	**	36	**	36	**
Error	87		55		326		240	

	Plantation C				
	Height		Number of branches	Number of buds	Flushing score
	d.f.	F level	F level	F level	F level
Blocks	9	**	**	**	
Provenances	5	**	**	**	**
Families/provenance	30	**	**	**	**
Error	315				

	Plantation D		
Blocks	2		
Provenances	5	**	**
Families/provenance	30	*	*
Error	68		

1/ Means differ at a probability of 99 percent (**) or 95 percent (*).

Components of variance (table 3) show that variation attributable to provenances was three to four times greater than that attributable to families within provenance. Provenance selection is thus the indicated first step for improving balsam fir in the Lake States.

Table 3.--Summary of analysis of variance with variance components for height (plantations B, C, and D) and number of lateral branches in the top whorl (plantations B and C) for balsam fir provenances and families

Source of variation	Height			Lateral Branches		
	d.f.	Significance level	Variance component	d.f.	Significance level	Variance component
Locations 1/	2	** 2/	27 3/	1	**	25
Provenances	5	**	20	5	**	23
Families/Provenance	18	**	7	18	**	6
Error	190		46	311		46

1/ Location effects may be confounded with effects attributable to stock age at time of planting due to different years of planting.

2/ Means differ at a probability of 99 percent (**) or 95 percent (*).

3/ Percentage of total variance.

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EASTERN WHITE PINE SEED SOURCE VARIATION IN THE NORTHEASTERN UNITED STATES: 16-YEAR RESULTS

Maurice E. Demeritt, Jr., and Harry C. Kettlewood[1]

ABSTRACT.--Twelve eastern white pine (*Pinus strobus* L.) provenance plantations in the northeastern United States were measured for 16-year height and diameter. Differences in height between northern and southern sources have diminished since the 10-year measurements. In general, the 16-year diameter measurements follow the same trends as do the 16-year-height measurements. Recommendations for selection and movement of seed from one region to another are discussed.

In 1955 the USDA Forest Service began a range-wide seed-source study of eastern white pine (*Pinus strobus* L.) to find the geographic variation in selected traits and to make recommendations for the movement of seed over the species' range. Sixteen-year results for total height and diameter measurements of 29 seed sources in 12 field plantings at 10 locations in the northeastern United States are reported here.

METHODS AND MATERIALS

Cones from 10 trees chosen at random in good stands of natural origin were collected. Cones, seeds, and resulting seedlings were kept separate by parent tree within source through the nursery phase of the study in the Northeast. Seedlings within sources were bulked when lifted from the nursery beds and then were randomly selected for field plots. The location of field plantings and seed-source origins are listed in tables 1 and 2. All seed sources were not represented in every field planting.

Plantations 7 through 11 were established in 1959 with 2-0 seedlings grown in the Maryland State Forest Nursery. Plantations 1 through 4 were established in 1960 with 3-0 seedlings grown in the New Jersey and New York State Forest Nurseries. A detailed description of plantation sites, site preparation, and care has been previously reported (Garrett *et al.* 1973).

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Table 1.--Location and design of plantations

Plantation No.	and location	Field design	Latitude	Longitude	Elevation
					(feet)
1	Orono, ME	III	44°53'N	68°39'W	100
2	Alfred, ME	I, II, III	43°32'N	70°40'W	300
3	Essex Junction, VT	III	44°28'N	73°09'W	327
4	Paul Smiths, NY	I	44°26'N	74°13'W	1,815
6	Warren, PA	I	41°50'N	79°15'W	1,180
7	Standing Stone, PA	III	40°37'N	78°55'W	960
8	Kennett Square, PA	I	39°52'N	75°41'W	400
9	Savage River State Forest, MD	III	39°40'N	79°15'W	2,740
10	Horseshoe Run, WV	I	39°11'N	79°35'W	1,720
11	Rison, MD	II	38°30'N	77°20'W	100

Plantations 7 through 11 were established in 1959 with 2-0 seedlings grown in the Maryland State Forest Nursery. Plantations 1 through 6 were established in 1960 with 3-0 seedlings grown in the New Jersey and New York State Forest Nurseries. A detailed description of plantation sites, site preparation, and care has been previously reported (Garrett *et al.* 1973).

Three field designs, all randomized complete blocks, are represented.

Design I.--One tree from each seed source randomly located in each of 24 blocks at a spacing of 10 by 10 feet.

Design II.--Two-tree-row plots from each seed source randomly located in each of 24 blocks. Seed-source trees were planted at a spacing of 7 feet in rows and 14 feet between seed-source rows. Additional commercial seedlings were planted between seed-source rows to obtain a spacing of 7 by 7 feet.

Design III.--Four-tree-row plots from each seed source randomly located in each of 12 blocks. Seed-source trees were planted at a spacing of 7 feet in rows and 14 feet between seed-source rows. Additional commercial seedlings were planted between seed-source rows to obtain a spacing of 7 by 7 feet.

The additional (nonstudy) trees were removed from all plantations 1 to 3 years before 16-year measurements.

Table 2.--Seed-source locations for white pine provenance study

Seedlot No.	and location	Latitude	Longitude	Elevation (feet)
1	Union County, GA	34°46'N	84°03'W	2,450
2	Transylvania County, NC	35°14'N	82°38'W	2,120
3	Greene County, TN	36°00'N	82°48'W	2,250
4	Garrett County, MD	39°39'N	78°45'W	2,460
5	Greenbrier County, WV	38°00'N	80°30'W	2,600
6	Monroe County, PA	41°05'N	75°25'W	1,800
7	Monroe County, PA	41°05'N	75°25'W	740
8	Clearfield County, PA	41°00'N	78°30'W	--
9	Clearfield County, PA	41°00'N	78°30'W	--
10	Ulster County, NY	41°45'N	74°15'W	--
11	Ulster County, NY	41°45'N	74°15'W	--
12	Franklin County, NY	44°25'N	74°15'W	1,600
13	Worcester County, MA	42°30'N	72°15'W	1,275
14	Penobscot County, ME	44°51'N	68°38'W	150
15	Allamakee County, IA	43°15'N	91°30'W	1,000
16	Ashland County, OH	40°45'N	82°15'W	1,000
18	Forest County, WI	45°30'N	88°30'W	1,500
19	Cass County, MN	47°30'N	94°30'W	1,300
20	Lunenburg County, Nova Scotia	44°25'N	64°35'W	150
21	Sunbury County, New Brunswick	46°00'N	66°15'W	200
22	Quebec County, Quebec	47°30'N	72°00'W	550
23	Pontiac County, Quebec	47°30'N	77°00'W	1,000
24	Norfolk County, Ontario	42°40'N	80°27'W	750
25	Algoma District, Ontario	46°10'N	82°37'W	650
27	Carroll County, NH	43°45'N	71°25'W	610
28	Lake County, MN	48°00'N	91°45'W	1,300
29	Houghton County, MI	47°00'N	88°30'W	625
30	Pulaski County, VA	37°00'N	80°50'W	2,400
31	Sauk County, WI	43°30'N	89°30'W	1,000

Total height and diameter were measured on each seed-source tree after the 1972 growing season (16 years from seed), except for the three plantations at Alfred, Maine. The Alfred plantations were measured after the 1973 growing season (17 years old), and the data were corrected to 1972.

Analyses of variance for diameter and total height, using plot means, were calculated for each plantation using a least-squares and maximum-likelihood general-purpose program. The program partitioned the total sum of squares into sum of squares for blocks, seed sources, and residual (Block x Seed Source Interaction plus Error).

At each plantation every seed-source mean for height and diameter was divided by the plantation mean and then multiplied by 100. With this statistic we were able to evaluate seed sources across all plantations (tables 3 and 4). It must be noted, however, that increasing height and diameter create a scale effect so equal relative differences do not imply equal actual differences.

RESULTS AND DISCUSSION

Seed-source differences were significant at the 1-percent probability level for total height and diameter at 16 years from seed at all 12 field plantations. This variability in growth traits among seed sources indicates that particular seed sources can be selected for use in certain geographic areas.

Blocks were significantly different at the 1-percent probability level except in plantations 9 and 10. The use of randomized complete block design was efficient at locations where blocks were significantly different because it removed site variation from seed-source variation, which gave us better information on the performance of the seed sources at those locations.

Height Growth

The relative differences in height growth between northern and southern sources of eastern white pine have diminished since the 10-year measurements were made, although actual differences have increased in all plantations (table 3, Garrett *et al.* 1973). At 10 years of age, southern sources were clearly superior. This same trend has been observed in the Central States in measurements at 10 and 15 years, although the magnitude of the relative differences is greater in the Central States (Funk *et al.* 1975).

In the study reported here, the differences between southern and northern sources were no greater in southern plantations with no or little weeviling (plantations 8 through 11) than they were in northerly plantations with previously good growth but heavy weeviling (plantations 6 and 7). Therefore, the decrease in superiority of southern sources in the Northeast compared to the Central States does not seem to be the result of the white pine weevil (*Pissodes strobi* Peck.) attacking and killing leaders of southern source trees.

Nor is the decreased superiority of southern sources caused by growing-space limitations. The southern-source trees did not grow any taller in Design I plantations at a 10 by 10 foot spacing than they did in Design II and III plantations at a 7 by 7 foot spacing before thinning. It may be that southern-source trees in the Northeast are good early growers, but lose their superiority with time. If this is the case, it will be difficult to evaluate seed sources in provenance tests at an early age.

Table 3.--Height at 16 years from seed of white pine seed-source plantations (seed source mean as percentage of plantation mean)

Seed Source No. and location	Plantation location											
	Cent. Maine 1[1]	Vt. 3[1]	N.Y. 4[2]	S. Maine 2[2]	S. Maine 2[3]	S. Maine 2[1]	N.W. Pa. 6[2]	Cent. Pa. 7[1]	S.E. Pa. 8[2]	W. Md. 9[1]	W. Va. 10[2]	S. Md. 11[3]
1 Georgia	85	107	88	95	89	97	113	118	113	115	110	114
2 North Carolina	89	99	79	94	94	98	102	114	100	106	105	103
3 Tennessee	89	103	99	99	97	103	118	120	120	122	108	121
30 Virginia	--	92	--	105	--	76	--	86	75	--	92	88
5 West Virginia	94	99	99	99	102	100	105	95	104	105	107	99
4 Maryland	86	97	98	101	97	95	107	110	96	115	98	100
16 Ohio	101	104	106	99	98	106	101	101	100	108	96	94
8 Pennsylvania	93	106	108	99	97	106	--	106	101	92	103	98
9 Pennsylvania	114	102	109	105	106	108	117	113	109	116	115	108
6 Pennsylvania	108	105	106	102	101	107	114	106	106	114	109	99
7 Pennsylvania	104	--	96	100	102	102	--	--	--	--	--	--
10 New York	104	103	108	106	103	106	104	113	101	107	102	99
11 New York	118	110	117	107	106	106	105	117	111	112	108	100
13 Massachusetts	110	106	105	108	104	109	109	100	106	104	108	102
24 Ontario	108	110	95	111	108	107	89	115	106	107	112	108
15 Iowa	95	94	99	91	93	94	92	91	95	--	98	95
31 Wisconsin	--	--	--	--	--	--	--	91	105	98	103	106
27 New Hampshire	101	105	--	103	99	108	--	105	107	--	--	109
12 New York	103	103	110	103	102	105	108	101	103	102	99	104
20 Nova Scotia	106	104	95	104	108	103	89	98	100	98	100	101
14 Maine	105	106	111	103	97	101	90	94	93	93	93	95
18 Wisconsin	101	93	103	95	99	97	106	83	95	--	--	92
25 Ontario	108	85	--	93	--	102	89	95	103	89	100	97
21 New Brunswick	99	98	88	97	97	91	100	93	81	84	87	97
29 Michigan	--	--	88	96	93	85	--	85	98	86	93	86
19 Minnesota	99	97	92	97	99	102	103	89	100	100	98	97
22 Quebec	80	86	89	97	84	75	68	74	85	66	78	87
23 Quebec	87	90	--	84	--	95	35	82	86	84	90	81
28 Minnesota	97	94	--	--	--	98	--	91	87	88	92	94
Plantation mean (in feet)	15.7	17.3	14.3	16.8	18.7	19.5	22.2	17.3	20.5	16.1	29.1	26.7

In general, seedlings of southern sources (Georgia, North Carolina, and Tennessee) grew as well as or better than those from most northerly sources in plantations as far north as central Pennsylvania. Garrett et al. (1973) found this same trend for 10-year-height measurements. Faster height growth has also been reported for trees from southern Appalachian sources in the Central States (Funk 1971, Lee 1974, Funk et al. 1975). Sluder and Dorman (1971) found that southern-source trees grew better in North Carolina, Georgia, and Virginia than did those from more northerly sources at 10 years of age. Southern Appalachian sources were no taller than slightly more northerly sources, but were taller than far northern sources at 7 years of age when grown in Ontario (Fowler and Heimburger 1969). Thor (1975) found in a number of tests in Tennessee with a different collection of seed sources that local and southerly sources grew better than more northerly sources from Virginia, West Virginia, and Pennsylvania.

Sources 6 and 9 from Pennsylvania, 11 from New York, 13 from Massachusetts, and 24 from Ontario grew well in all plantations, even when they were moved some distance south from their origin. Garrett et al. (1973) found this same trend for these five sources for 10-year-height measurements.

Responses varied between sources from the same county within a State. Sources 6 and 9 from Pennsylvania grew better at most locations than sources 7 and 8 from the same counties. Also, source 11 from New York grew better at all locations than source 10 from the same county. This indicates that only proven sources from a geographic region should be selected and collected.

The Central States plantations do not have as many of the sources as the Northeast plantations, thus it is difficult to make good comparisons between regions. Some good sources from the Northeast did well in Lower Michigan at age 15 (Lee 1974) and in the Central States (Funk 1971, Funk et al. 1975). Fowler and Heimburger (1969) recommend sources from the Pennsylvania area for planting in Ontario, based on 7-year-height data.

Diameter Growth

At 16 years of age the range of differences in seed-source diameters is equal to or greater than the range of differences in height at each plantation, except the northwestern Pennsylvania plantation (table 4). In general, the ranking of seed sources is the same whether height or diameter is used as the measurement. Wright (1970) found a strong relation between height and diameter at 11 years for 15 sources in southern Michigan.

Table 4.--Diameter at 16 years from seed of white pine seed-source plantations (seed source mean as percentage of plantation mean)

Seed source No. and location	Plantation location											
	Cent. Maine 1 1/	Vt. 3 1/	N. Y. 4 2/	S. Maine 2 2/	S. Maine 2 3/	S. Maine 2 1/	N.W. Pa. 6 2/	Cent. Pa. 7 1/	S.E. Pa. 8 2/	W. Md. 9 1/	W. Va. 10 2/	S. Md. 11 3/
1 Georgia	79	112	93	98	91	100	128	136	131	117	132	142
2 North Carolina	79	104	62	92	96	102	97	125	106	100	124	116
3 Tennessee	79	104	92	97	97	108	127	125	131	121	123	143
30 Virginia	--	85	--	105	--	73	--	96	64	--	88	86
5 West Virginia	85	90	96	94	97	99	102	94	103	107	106	104
4 Maryland	79	82	93	97	97	96	109	106	95	120	99	97
16 Ohio	101	109	102	101	101	113	94	98	94	105	97	90
8 Pennsylvania	95	112	108	102	96	104	--	107	99	87	109	101
9 Pennsylvania	115	103	103	109	106	113	124	118	112	120	128	111
6 Pennsylvania	117	112	105	102	104	111	119	114	113	116	120	102
7 Pennsylvania	116	--	91	104	101	97	--	--	--	--	--	--
10 New York	103	109	128	113	105	112	100	114	110	107	103	105
11 New York	120	110	123	109	109	106	102	118	117	116	112	103
13 Massachusetts	115	112	98	113	111	116	112	104	114	103	125	105
24 Ontario	100	115	100	108	109	105	83	122	107	103	119	117
15 Iowa	97	97	101	93	102	92	87	89	100	--	96	87
31 Wisconsin	--	--	--	--	--	--	--	91	107	98	104	108
27 New Hampshire	104	111	--	103	101	109	--	108	111	--	--	130
12 New York	107	105	106	107	103	106	113	93	107	108	98	105
20 Nova Scotia	110	109	96	107	110	100	81	92	92	90	85	92
14 Maine	111	102	109	106	98	101	84	94	88	95	86	88
18 Wisconsin	102	91	113	90	97	100	99	86	87	--	--	77
25 Ontario	113	88	--	94	--	101	84	91	101	96	88	93
21 New Brunswick	104	92	93	95	93	89	94	90	72	85	75	93
29 Michigan	--	--	84	97	90	84	--	80	97	84	81	74
19 Minnesota	98	95	90	99	102	99	95	84	102	97	93	84
22 Quebec	80	81	98	96	76	78	62	71	75	70	65	76
23 Quebec	80	82	--	74	--	87	55	69	74	74	78	69
28 Minnesota	100	92	--	--	--	95	--	79	79	72	79	81
Plantation mean (in inches)	2.70	3.67	3.04	4.21	4.32	4.54	4.64	3.25	4.53	2.98	5.89	4.14

1/ Design III; 2/ Design I; 3/ Design II.

CONCLUSIONS

1. The Georgia source (1) and the Tennessee source (3) should be selected for planting as far north as central Pennsylvania because of their above-average growth in height and diameter.

2. Pennsylvania sources 6 and 9, New York source 11, Massachusetts source 13, and Ontario source 24 should be selected for planting in the United States north and east of central Pennsylvania.

3. Only proven sources within seed-collection zones should be selected for seedling production.

4. Additional fast-growing sources from the regions outlined in conclusions 1 and 2 may be identified and added through further testing of sources.

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STAND, FAMILY, AND SITE EFFECTS IN UPPER OTTAWA VALLEY WHITE SPRUCE[1]

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ABSTRACT.--Forty-nine open-pollinated white spruce progenies from eight Upper Ottawa Valley white spruce stands were tested at three sites located within 10 miles of each other. Statistical analyses were limited to 42 families--6 from each of 7 of the stands. Performance was site dependent with nearly a two-fold difference between the best and the poorest. Differences due to stands were not important. The best family was 28 percent taller than the family mean height, but performance was not consistent from site to site in spite of a nonsignificant family-site interaction term. This probably was due to limitations imposed by the statistical design. Heritability estimates for heights were h_1^2 (individual tree heritability) = 0.10; h_2^2 (family heritability) = 0.39. Genetic gain in 10 year height through one cycle of simple mass selection was estimated to be 8.6 percent; establishing a clonal orchard with the best trees (4 percent selection intensity) from the best families (10 percent selection intensity) in the test boosts the estimated gain to 11 percent. The genetic parameters determined in this study are compared with previously published data.

White spruce (*Picea glauca* (Moench) Voss) provenances, have been shown to differ widely in growth and survival in field tests covering a broad spectrum of test sites in Canada and the United States (Nienstaedt 1969, Teich 1973). Among the provenances tested, the Beachburg source from the Upper Ottawa Valley performed consistently well in all field tests and showed the best height growth in most tests. These findings generated interest in the Upper Ottawa Valley white spruce populations and more intensive studies of these populations were initiated by the Petawawa Forest Experiment Station (P.F.E.S.).

The objective of the study reported here was to evaluate the significance of variation among and within white spruce stands of the

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Upper Ottawa Valley. Ten-year height growth and survival (6 years after planting) were studied at three test sites. Heritability of height growth was determined and expected genetic improvement in this trait through selection and breeding examined.

MATERIAL AND METHODS

Open-pollinated seeds were collected from 4 to seven trees in eight stands. A total of 49 seedlots (open-pollinated families) was obtained.

Seedlings of the 49 families were raised in the P.F.E.S. nursery and outplanted as 2-2 stock in spring 1969 at 3 sites identified as D-1, D-2, and D-3. The test sites were located within a distance of 10 miles of each other in Head Township, Renfrew County, but varied markedly in their productivity and previous vegetation (fig. 1). Site D-1 was a cutover hardwood stand where young unmerchantable trees remained in the overstory after planting. Its soil was stony with a well developed humus layer. Sites D-2 and D-3 were abandoned farms with a heavy sod overlying a loamy sand.

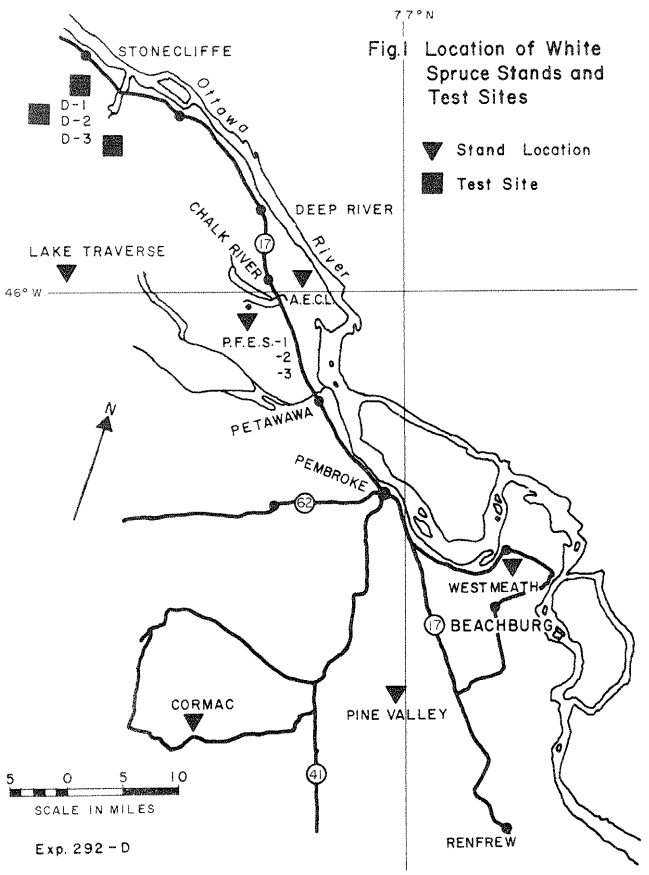

Figure 1.--Location of white spruce stands and test sites.

Planting was done at 1.2 by 1.2 m spacing. Scalping removed competing vegetation within about 20 cm of the planted trees. The experimental design consisted of two replications at each site. Twenty-five tree plots were used at sites D-2 and D-3, but at Site D-1, where lesser survival was anticipated because of stony soil, 40 tree plots were used.

The plantations were generally healthy and showed no serious damage by any pest. The plantation on site D-3, however, had suffered moderate frost damage in early years but appeared to have recovered. Ground competition was considered to be an integral part of the test environment and no attempt was made to control this after the field trials were successfully established.

STATISTICAL ANALYSIS

The data were summarized by standard statistical methods. Standard errors of site and stand means were calculated from the error mean sum of squares of the appropriate analysis of variance (Steel and Torrie 1960).

Analysis of variance for data combined from all the sites was done by using a balanced set of 42 families (6 families each from 7 stands). The analysis of variance format and expectation of the mean sums of squares, assuming a completely random model, are given in table 1. The variance components and their standard errors were determined using procedures described by Hanson (1955). All tests of statistical significance were made at the 5 percent probability level.

Table 1.--Analysis of variance format and expectation of the mean sums of squares

Source of variation	d.f.	E(M.S.S.)[1]
Sites	$\ell-1$	$\frac{\sigma_w^2}{k} + \sigma_e^2 + sf\sigma_{R/L}^2 + rsf\sigma_L^2$
Reps/sites	$\ell(f-1)$	$\frac{\sigma_w^2}{k} + \sigma_e^2 + sf\sigma_{R/L}^2$
Stands	$s-1$	$\frac{\sigma_w^2}{k} + \sigma_e^2 + \ell r\sigma_{F/S}^2 + \ell rf\sigma_S^2$
Families/stands	$s(f-1)$	$\frac{\sigma_w^2}{k} + \sigma_e^2 + \ell r\sigma_{F/S}^2$
Pooled error[2]	$(\ell rsf-sf-\ell r+1)$	$\frac{\sigma_w^2}{k} + \sigma_e^2$
Within plots	ℓrsf $\sum_{i=1}(n_i-1)$	σ_w^2

1 ℓ = No. of sites, r = No. of reps. in each site, s = No. of stands, f = No. of families per stand, n = No. of plants per plot, k = harmonic mean of number of plants per plot.

2 Contains sources of variation due to families x sites and families x reps/sites.

RESULTS

The performance of white spruce families was greatly influenced by test sites (table 2). Mean height on the best site (D-1) was nearly twice as much as on the poorest site (D-3). Site D-1 was characterized by a more favorable moisture regime, lack of severe grass competition, and protection from severe frosts. Poor survival on site D-1 was attributed to difficulty in planting among the stones and stumps on this site.

Table 2.--Mean height and survival of the families at three sites

Site	Height (cm) mean ± s.e.	Survival (percent) mean ± s.e.
D-1	151.1 ± 2.2	79.5 ± 1.2
D-2	120.3 ± 1.6	91.8 ± 0.9
D-3	77.3 ± 1.3	93.7 ± 1.0
All sites	116.2 ± 1.0	88.3 ± 0.6

Mean height of the stands ranged from 110.1 cm to 123.6 cm and mean survival from 82.9 percent to 91.2 percent (table 3). Westmeath showed the best performance among the eight stands studied. Its height growth and survival were respectively 6 percent and 3 percent better than the average (table 4). However, its superiority was not consistent on all sites, e.g., on site D-2 Lake Traverse showed the best height and P.F.E.S. -2 showed the best survival.

Table 3.--Mean performance of white spruce stand collections

Stand origin	Families	Height (mean ± s.e.)	Survival (mean ± s.e.)
	No.	cm	Percent
Westmeath	7	123.6 ± 2.7	91.2 ± 1.6
P.F.E.S. -3	6	118.9 ± 2.9	90.3 ± 1.7
Lake Traverse	4	117.1 ± 3.5	87.1 ± 2.1
Pine Valley	7	115.9 ± 2.7	89.6 ± 1.6
A.E.C.L. Reserve	7	115.4 ± 2.7	88.3 ± 1.6
P.F.E.S. -2	6	113.7 ± 2.9	88.5 ± 1.7
Cormac	6	113.4 ± 2.9	82.9 ± 1.7
P.F.E.S. -1	6	110.1 ± 2.9	87.4 ± 1.7

Table 4.--Performance of the best stand and family at each site as percent of the site mean

	Height			Survival			D-1,D-2,D-3 combined	
	D-1	D-2	D-3	D-1	D-2	D-3	Height	Survival
Stand	106	110	112	105	106	105	106	103
Family	128	138	132	118	109	107	128	108

Individual families varied more in height than they did in survival (table 4). Considering all sites, the tallest family was found to be 29 percent taller than the average. Mean height and survival of the families showed significant correlation for site D-2 ($r = 0.54$) and D-3 ($r = 0.46$) but not for site D-1 ($r = 0.19$).

Performance of individual families was generally inconsistent from site to site and the family ranks fluctuated considerably. This is illustrated by simple correlation coefficients of family means on different sites (table 5); correlations based on family ranks were very similar. Out of the 49 families studied, only 7 showed above average height growth on all test sites.

Table 5.--Simple correlation coefficients of family height and survival means among test sites

		D-2	D-3
D-1	Height	0.11	0.01
	Survival	0.02	0.16
D-2	Height	--	0.31*
	Survival	--	0.29*

* Significant at $p < 0.05$

The error mean squares of the analyses of variance were derived by pooling families x sites and families x replications in sites sums of squares because families-sites interactions were negligible for both height and survival (respective F-ratios 0.95 and 0.90) (table 6). Sites, replications in sites, and families in stands were significant sources contributing to the variability of height growth but percent survival was significantly influenced by sites and reps in sites only (table 6).

Table 6.--Mean squares in the analyses of variance and corresponding variance components and their standard errors

Source of variation	d.f.	M.S.S. Height	M.S.S. Survival	Component estimated	Variance components Height	Variance components Survival
Sites	2	113,354 1/	5,713 1/	σ_L^2	1281.5±1350.6	60.1±68.3
Reps/sites	3	5,711 1/	668 1/	$\sigma_{R/L}^2$	128.8± 111.0	13.4±13.0
Stands	6	686	273	σ_S^2	5.8± 11.5	4.1± 4.5
Families/stands	35	479 1/	125	$\sigma_{F/S}^2$	35.6± 19.7	4.0± 5.2
Pooled error	205	295	101	σ_e^2 2/	250.3± 29.2	100.7± 8.3
Within plots (k=25.08)	7,609	1,128	--	σ_w^2	1,128.3 18.3	--

1/ Significant at $p<0.05$

2/ For survival, variance component estimated is $\frac{\sigma_w^2}{k} + \sigma_e^2$.

Estimates of narrow sense heritability of tree height for individual plants (h_1^2) and family means (h_2^2) reference units were constructed from the variance components listed in table 6 (Comstock and Robinson 1948, Dudley and Moll 1969). It was assumed that the members of individual families were true half-sibs.

h_1^2 (individual tree heritability) $= \frac{\sigma_A^2}{\sigma_p^2} = \frac{142.4}{1,420.0} = 0.10$

h_2^2 (family heritability)[3] $= \frac{\sigma_{A'}^2}{\sigma_{\bar{p}}^2} = \frac{35.6}{90.6} = 0.39$

3 The correct numerator for computing family heritability can be either $\sigma_{A'}^2$, as used in the case illustrated above, or $2\,\sigma_{A'}^2$ depending upon the type of selection to be practiced (Dudley and Moll 1969).

Where[4]

$$\sigma_A^2 = 4 \quad \sigma_{F/S}^2 \quad \text{(additive genetic variance of individual trees)}$$

$$\sigma_p^2 = \sigma_w^2 + \sigma_e^2 + \sigma_{F/S}^2 + \sigma_S^2 \quad \text{(phenotypic variance of individual trees)}$$

$$\sigma_{A'}^2 = \sigma_{F/S}^2 \quad \text{(additive genetic variance of half-sib families)}$$

$$\sigma_{\bar{p}}^2 = \frac{\sigma_w^2}{\ell rk} + \frac{\sigma_e^2}{\ell r} + \sigma_{F/S}^2 + \sigma_S^2 \quad \text{(phenotypic variance of half-sib families)}$$

Heritability of survival percentage was not calculated because the effects of families in stands for this trait were not significant. Because stand origin effects were also nonsignificant, it can be assumed that variability in the surviving ability of the families was purely environmental in origin.

DISCUSSION

Genetic differences in 10-year height of local white spruce in the Upper Ottawa Valley were primarily due to mother trees as shown by the performance of their progenies. Differences attributable to stand origin were not important, which suggests a lack of genetic differentiation among local white spruce populations. Similar findings were reported by Yeatman (1975) for early height growth of the Ottawa Valley jack pine progenies. Substantial variation among open-pollinated progenies of white spruce was reported in earlier studies by Holst and Teich (1969) and Jeffers (1969). These workers, however, sampled material from wider geographic areas and tested it at only one location.

Site had a dominating influence on performance of the families but family-site interactions were statistically nonsignificant. Absence of family-site interaction usually implies a good agreement in the ranking of families on different sites, but this was not true for the results of this study. This was largely due to inconsistency in the performance of families among replications on individual sites. It was, therefore, felt that the experimental design used in this study provided a poor control of site heterogeneity. Smaller plots and three or more replications per site would have increased the efficiency of the tests.

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4 See tables 1 and 6 for definition of variance components and subscripts.

The single tree heritability value of 0.10 for 10-year height, reported here, is similar to a value of 0.145 obtained by Yeatman (1975) for 6-year height in the Ottawa Valley jack pine in a similar experimental design and reference environment. Heritability values for 8- and 11-year heights of Ontario white spruce ranged from 0.06 to 0.18 on an individual tree basis and from 0.75 to 0.91 on a family basis, in the studies reported by Holst and Teich (1969). However, the procedure used by these authors for computing heritability differs from the one used in the present study so the results are not comparable.[5]

Heritability values should always be interpreted with caution because such values are applicable only to the defined base population, reference selection unit, and reference test environment (Hanson 1963, Dudley and Moll 1969). Furthermore, heritability estimates obtained from genetic parameters estimated by open-pollinated progeny tests, assuming families to be true half-sibs, are expected to be biased and tend to be inflated (Namkoong 1966, Squillace 1974).

Genetic parameters estimated in this study can be used to derive the expected genetic gain through selection and breeding (Namkoong et al. 1966, Shelbourne 1969). Genetic gain in 10-year height of the Upper Ottawa Valley white spruce through one cycle of simple mass selection was estimated to be 8.6 percent. This is an appreciable gain and particularly noteworthy in view of the poor heritability of this trait reported by this study. Genetic gain resulting from the seed produced in a clonal seed orchard, established with scions collected from the tallest tree in each plot (4 percent selection intensity) of the top five families (10 percent selection intensity), was estimated to be 11 percent.

The heritability of early height found in this study is less than the value of this parameter suggested by white spruce progeny tests in the Lake States (Jeffers 1969, Mohn et al., these proceedings). As pointed out earlier, experimental plantations used by this study suffered several shortcomings and it is likely that field experiments emphasizing better design and more uniform treatment of experimental units will show higher heritability for this trait of local white spruce in the Upper Ottawa Valley.

SUMMARY AND CONCLUSIONS

1. Open-pollinated progenies of the Upper Ottawa Valley white spruce stands (provenances) were studied for early height growth and field survival at three test sites.

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5 Method used by Holst and Teich (1969) in their heritability calculations does not take into account "within plot" variance. This method is appropriate for one-way classification experiments but not for multi-way classification experiments. Also for family heritability calculations they used $2\sigma_A^2$ in the numerator. The formulae used by Holst and Teich (1969), give the following values with my data: single tree heritability = 0.06; family heritability = 0.63.

2. The stands showed similar performance indicating lack of genetic differentiation among them for the traits studied. It appears that the superior white spruce sources of Beachburg and Douglas identified in earlier provenance tests are part of a larger population that extends beyond the original collection area of these sources. Further studies with more intensive sampling of stands and better field design are needed to delineate the boundaries of this population complex.

3. Site had a dominating influence on progeny performance, which was very inconsistent from site to site.

4. Substantial variation was found for height growth among families but their ability to survive in the field was primarily determined by the site factors.

5. Heritability of individual plant height was low (0.10) but appreciable genetic gain (8.6 percent) was expected to result from one cycle of simple mass selection at 1 percent selection intensity. Overall benefits resulting from white spruce breeding in the Upper Ottawa Valley, using multistage and multitrait selection procedures, can be expected to be larger than the 8.6 percent gain reported for simple mass selection.

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A WHITE SPRUCE PROGENY TEST--SEEDLING SEED ORCHARD: 12TH YEAR PROGRESS REPORT[1]

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ABSTRACT-- Two hundred thirty-nine open-pollinated progenies of white spruce were established in a combination progeny test-seedling seed orchard near Grand Rapids, Minnesota. Characteristics evaluated included, total heights at 2-0 and 2-2 in the nursery and 9 and 12 years from seed (in the field); field survival 9 years from seed was included. Survival was just over 77 percent. Family mean heights were from 60 to 167 percent of the nursery test mean at 2-0 and decreased in variation to from 52 to 125 percent of the plantation mean at age 12. Narrow sense heritabilities (computed as four times the interclass correlation) were 0.27 at age 9 and 0.35 at age 12 and are comparable to earlier results with white spruce in Wisconsin. They exceed Canadian results probably because Canadian material represented a more narrow geographic base. Data suggest a possible relation between growth rates and climatic seed collection zones. Pending further study, nurserymen should not collect seed in the extreme climatic zones in northeastern Minnesota. Plans for conversion of the test to a seed orchard are described. Possible genetic gains of from 15 to 20 percent are predicted on the basis of present genetic parameters.

In 1962 a cooperative improvement project for white spruce (*Picea glauca* (Moench) Voss) was initiated by Blandin Paper Company of Grand Rapids, Minnesota, and the College of Forestry, University of Minnesota. Objectives included the establishment of a small commercial seed orchard to produce improved materials for use in a limited area of north-central

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Minnesota, the development of information concerning genetic variation in the species, and the identification of materials for use in future breeding work.

We decided to run a combination one-parent progeny test and seedling-seed orchard similar to that described by Wright and Bull (1963) for red pine in Michigan. Both the theoretical and applied aspects of such an approach are controversial. However, the choice was dictated by the objectives of the cooperators, the limited physical and human resources available, and our knowledge of white spruce genetics in the early 1960's. This report reviews the establishment of the project, presents a preliminary analysis of data collected through age 12, and outlines plans for future developments.

MATERIALS AND METHODS

Seed Collection

All seeds were collected in 1962. The goal was 200 to 250 individual tree collections from the range of white spruce in Minnesota. To meet this goal, assistance was requested from foresters with the Minnesota Department of Natural Resources and other organizations.

Collectors were instructed to obtain 25 to 30 cones per selected tree, package single-tree collections separately, and mail the cones to a central point along with a legal description of the mother tree's location. Selection criteria for mother trees were not stringent: cooperators were to confine their selections to wild stands, avoid isolated specimens, and choose trees "above average in health and vigor when compared to trees of the same age in the immediate area."

The response by Minnesota foresters was outstanding and 281 usable single-tree collections were assembled. The collections represented the efforts of at least 35 individuals employed by several organizations.

Nursery Phase

Cleaned and dewinged seeds were sown in October of 1962 in the Blandin Nursery near Grand Rapids, Minnesota. The sowing was made in 3 blocks; each block contained 1 plot of each of the 281 seed lots. A plot was a 4-foot row across the nursery beds containing 110 to 150 seeds. Spacing of 6 inches between rows was maintained by using planting boards.

The sowing produced 249 open-pollinated progenies in each of which there were 20 or more healthy seedlings. The seedlings were transplanted in May of 1965 into a randomized complete block design with four replications.

Row plots of 12 trees each were used at a spacing of 4 by 6 inches. Low numbers of seedlings resulted in 33 of the families being transplanted to only 2 or 3 of the 4 plots.

In April of 1967 the 2-2 stock was labeled and lifted. At the conclusion of the nursery phase the original 281 seed lots had yielded 239 open-pollinated progenies with adequate numbers of plantable seedlings (fig. 1).

Outplanting

Seedlings were hand planted in May of 1967 near Grand Rapids, Minnesota, on a gently rolling, old-field site with a sandy-loam soil. Before planting the site was plowed, disked, and divided into 30 contiguous 70 by 80 foot blocks. Spacing of 5 by 5 feet gave 240 positions per block and 1 seedling from each of the 239 families was randomly assigned to each position. The 240th position was filled with surplus seedlings from progenies with more than 30 seedlings as were voids caused by families having less than 30 seedlings. This resulted in the number of trees in a progeny varying from 15 to 38.

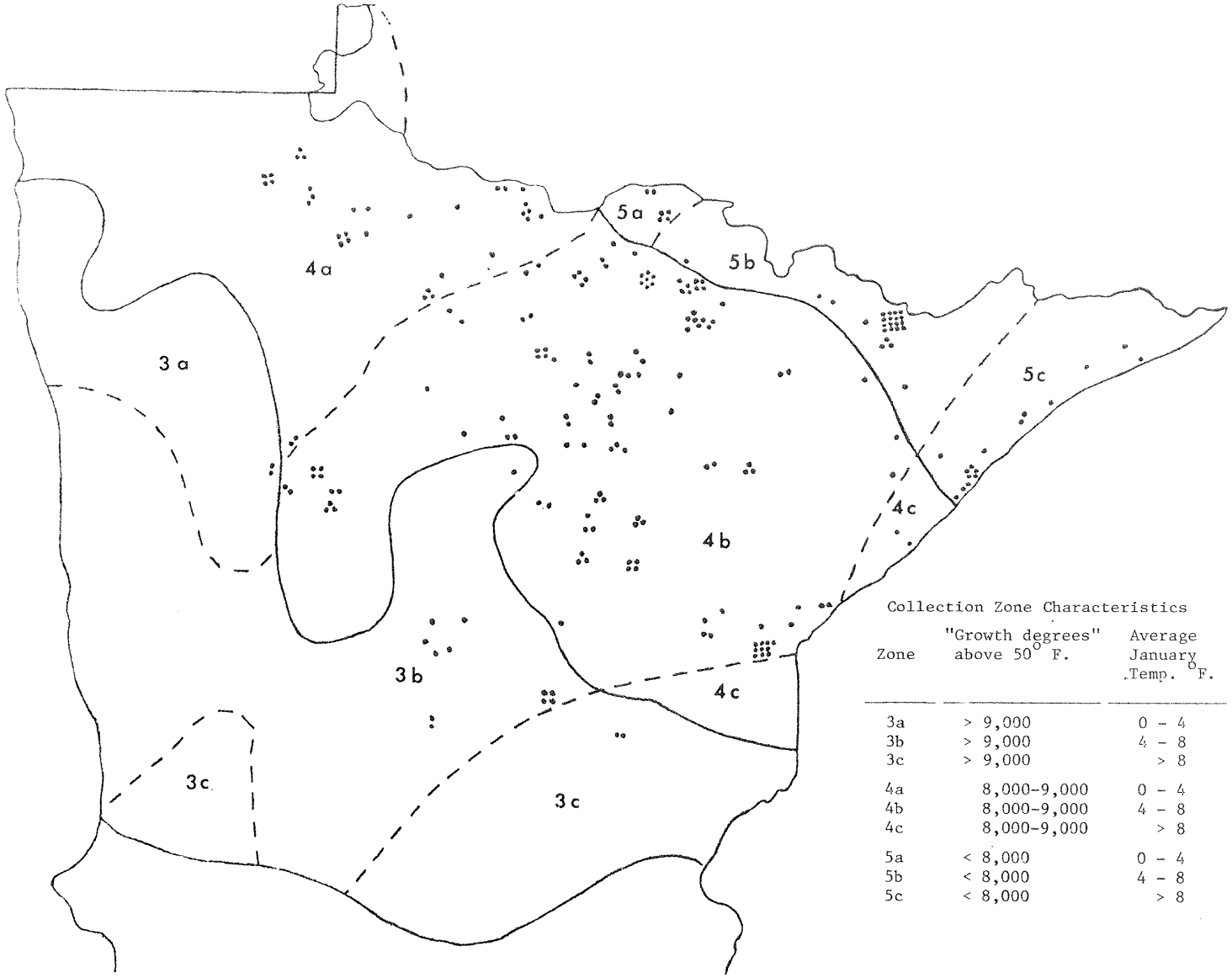
Post planting care include replacing 587 dead plants in 1968, treating an area around each plant with an herbicide in 1968, and spraying with an insecticide to control yellow-headed spruce sawfly each year.

MEASUREMENT AND ANALYSIS

Nursery measurements included the determination of plot mean heights for 2-0 seedlings in 1965 and the 2-2 transplants in 1967 to the nearest centimeter. Mean family heights were computed using these data and compared by analysis of variance, assuming a randomized complete-block design.

Field heights were measured after five and eight growing seasons in the field (in 1971 and 1974). Data from these measurements were examined on the basis of a completely random design because of the large number of missing plots.

Narrow-sense heritabilities for individual differences were calculated as four times the interclass correlation (Falconer 1960, Kung 1972). Further analysis of 12th year height data included computing the means and variances for families from six collection zones. The zones, modified from Rudolf (1956), were defined by average January temperature and the annual sums of normal average daily temperatures per year above 50°F (fig. 1).



Collection Zone Characteristics

Zone	"Growth degrees" above 50° F.	Average January Temp. °F.
3a	> 9,000	0 - 4
3b	> 9,000	4 - 8
3c	> 9,000	> 8
4a	8,000-9,000	0 - 4
4b	8,000-9,000	4 - 8
4c	8,000-9,000	> 8
5a	< 8,000	0 - 4
5b	< 8,000	4 - 8
5c	< 8,000	> 8

Figure 1.--Location of mother trees and seed collection zones for white spruce seedling seed orchard-progeny test.

Simple correlation coefficients among family means for height at the four measurement ages and survival in 1971 (age 9) were calculated.

Significance levels for all tests were set at 0.05.

RESULTS

Field Survival

All the field mortality occurred during the first 5 years after planting. By 1974, eight growing seasons after field planting, survival was just over 77 percent. On a family basis, survival ranged from 39 to 100 percent. The average family had 23.5 living plants and only 11 of the 239 families were represented by less than 15 trees. Mortality was strongly related to location in the planting with losses in the 30 blocks ranging from less than 1 percent to 55 percent.

Height Growth

Growth in the nursery and during the first 5 years in the field was slow (table 1). However, from 1972 to 1974 (10th-12th years) it averaged 28 cm per year. Much variation in individual tree height was shown by the field data. The standard deviations of individual tree heights were equal to 36 percent and 34 percent of the plantation mean in the 9th and 12th years, respectively.

Table 1.--Summary of nursery and field height measurements

Year	Years materials in: Seed-bed	Transplant bed	Field	Families	Test mean	Standard deviation (indiv.tree)	Range of family means: Actual	Test mean
				(No.)	(cm)	(cm)	(cm)	(percent)
1964	2	--	--	237 1/	8.8	--	5.3-14.7	60-167
1966	2	2	--	237 1/	17.2	--	8.3-24.8	48-144
1971	2	2	5	239	101	36	57-129	56-128
1974	2	2	8	239	184	62	96-230	52-125

1/ Two families omitted from nursery data because of missing plots.

Differences among open-pollinated families were significant for all height measurements (fig. 2). The range of family means was largest for the 2-0 measurements which ran from 60 to 167 percent of the plantation mean. Although the range of family means decreased over time (table 1), at age 12 it still ranged from 52 to 125 percent of the plantation mean.

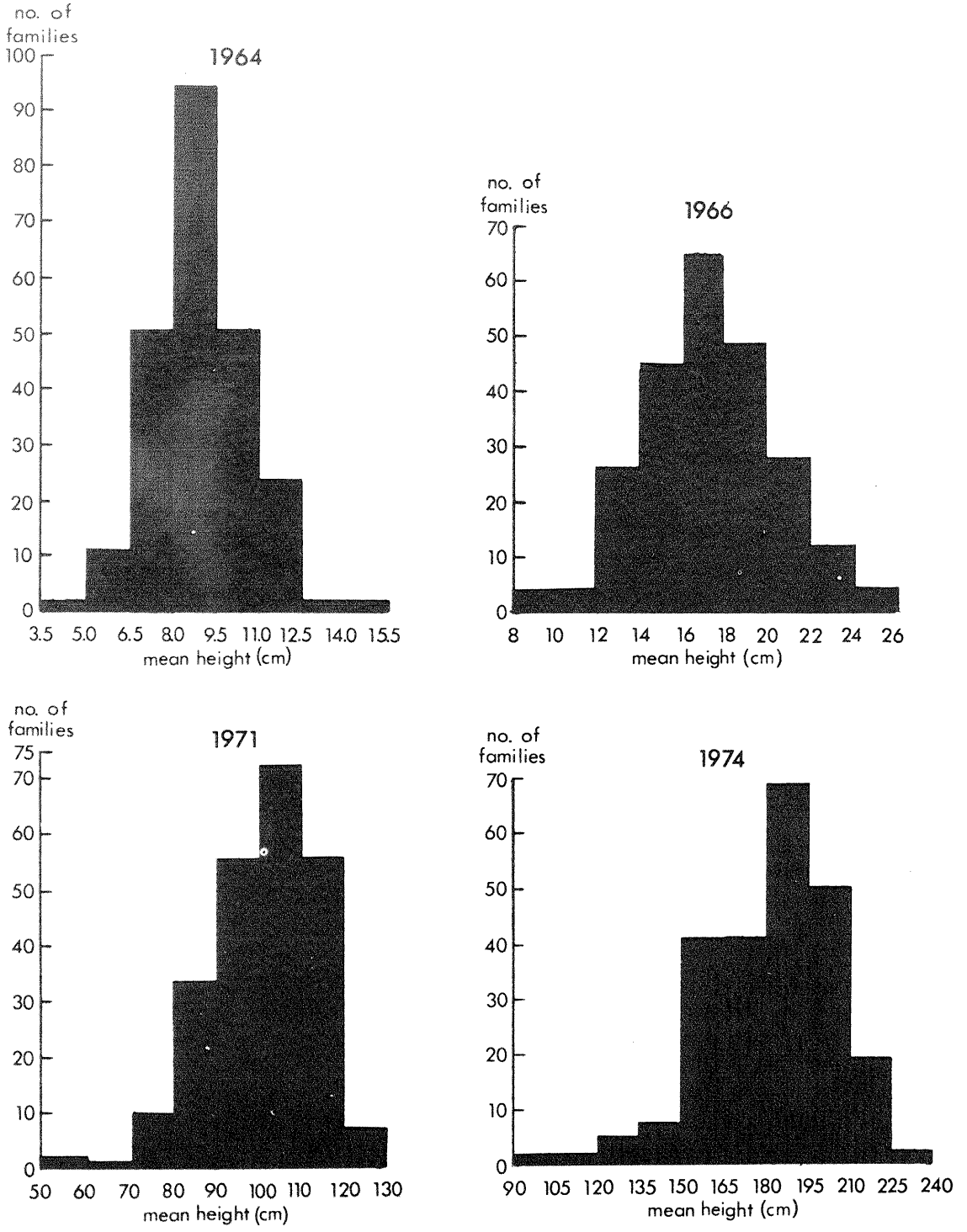


Figure 2.--Distributions of white spruce open-pollinated family means for height.

Narrow-sense heritabilities (individual tree basis) were 0.27 at age 9 and 0.35 at age 12.

The mean 12th-year heights for families from the six collection zones in which six or more individual trees were sampled are given in fig. 1, table 2. Bartlett's test indicated that the variances for the zones exhibited heterogeneity and the data were not subjected to further analysis (Steel and Torrie 1960).

Table 2.--Mean 12th-year heights for families in six collection zones

Seed collection zone[1/]	Families in sample	Mean height of families	Family variance	Standard error of mean
	(No.)	(cm)		
3b	12	193.2	8.58	4.57
4a	34	188.4	7.24	2.44
4b	138	183.8	15.36	1.83
5a	6	179.2	17.69	9.45
5b	24	177.4	12.06	3.96
5c	16	152.4	25.59	6.71

1/ Collection zone shown in figure 1.

The simple correlation coefficients of family mean heights for the four measurement dates and mean 9th year survival are given in table 3. All correlations between these variables were statistically significant.

Table 3.--Simple correlation coefficients (r)[1/] for heights and 1971 survival

	2-0 height	2-2 height	9th year height	12th year height
1971 survival	.29	.48	.40	.48
2-0 height		.54	.44	.43
2-2 height			.58	.60
9th year height				.90

1/ r values are significant at the 5 percent level if they exceed 0.14.

DISCUSSION

Heritabilities from similar tests with white spruce were converted to a common basis using the procedures outlined by Kung (1972). Jeffers' (1969) data for the height of 28 open-pollinated families at age 4 gave a value close to those obtained in this study (0.34 vs. 0.27 and 0.35). In contrast, heritabilities established from 8th or 11th year data from 4 Ontario tests with 8 to 18 open-pollinated progenies ranged from 0.03 to 0.09 (Holst and Teich 1969).

It should be noted that the Minnesota and Wisconsin test materials came from a wide geographic area, but three of the four Ontario tests sampled a very limited area. The data summarized by table 2, although not evaluated statistically, suggest differences among materials from the several seed collection zones. A trend toward greater heights with increasing annual sums of normal average daily temperatures of 50°F or above and with lower average January temperatures may exist. Given these results, a reduction in variation would be anticipated when working with geographically restricted populations.

The possible relation of growth rates to collection zones suggested by the data also has implications for seed collection in Minnesota. Pending further study, nurserymen would be advised to avoid the northeastern region of the State; i.e., zones 5a, 5b, and particularly zone 5c (fig. 1).

Correlations between nursery and field height, although positive and statistically significant are only moderately high (table 3). Nursery-field correlations do not suggest that nursery data can form the primary base for selection. However, they are strong enough to indicate that culling in the nursery could improve cost/gain ratios in white spruce improvement programs.

Predictions of genetic gain based on the results of these data are encouraging. Given the heritabilities and magnitude of phenotypic variation observed for 9th and 12th year heights, conversion of the progeny test to a seed orchard by removing 90 percent of the trees would give an expected genetic gain in height growth of 15 to 20 percent.

Such predictions should be viewed with caution, however. The height data are for a maximum age of 12, which is far from rotation age. At least some culling of the materials must be done on the basis of early growth and, unless juvenile-mature correlations are high, predicted gains will be reduced. However, there is evidence of juvenile growth superiority of provenances persisting 29 years after field planting (Nienstaedt 1969), and the observed 9th-12th year correlation coefficient of 0.9 is encouraging.

It is also important to realize that testing was limited to a single site. Estimates of additive genetic variance and consequent predicted gains are biased upward by the failure to estimate the genotype-environment interaction component. The impact of this bias is unknown, however, it can be expected to be greatest on sites that differ most from the test site.

Despite these limiations, conversion of the progeny test site to a seed orchard appears worthwhile. Seeds produced by the orchard will be used in the area near the test location and this should reduce the impact of single site evaluation. In addition, much of the selection will be based on 20th-year or later measurements, so the probability of high selection age-rotation age correlation will be increased. The current plan for conversion to a seed orchard can be summarized as follows:

Activity	Year	Status at end of activity (Number)		
		Trees per acre	Total trees	Families
Measurement	1974	1,347	5,562	239
1st thinning	1975	870	3,600	228
Measurement, 2nd thinning	1982	435	1,800	50+
Measurement, final thinning	?	109	450	50+

The timing of the initial thinning is essentially an economic matter. Cutting and disposing of a large number of stems is cheaper when they are small. Although retaining all trees is theoretically advantageous, it is assumed that the low intensity of selection will have minimal impact on final gain.

The first thinning will be based on index values derived from the height of an individual tree, mean height of its family, and number of living trees in its family (Falconer 1960). Given the 12th-year estimate of heritability, the predicted response from this form of selection is superior to that expected from selecting only on the basis of a tree's phenotype (mass selection) and far superior to using the family as a selection unit. The thinning will eliminate only 11 of the 239 families and 125 of the remaining families will have 15 or more progeny.

The second thinning will occur at about 20 years from seed when trees begin flowering consistently. It will maintain the plantation's vigor and provide access for seed collection. This thinning will be of moderate intensity, leaving one tree per 100 square feet. The final

thinning to about 8 percent of the stems now living is planned for the early 1990's. Because the objective is to improve the genetic quality of seeds being used as quickly as possible, the final thinning will be earlier if age-age correlations are high.

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REPORT ON THE CROSS *PINUS RESIONSA* X *P. TROPICALIS*[1]

Richard B. Hall, David F. Karnosky, and Donald P. Fowler[2]

ABSTRACT.-- Interspecific hybridization was attempted between *Pinus resinosa* and *P. tropicalis*, the only two New World members of the *Pinus* group Sylvestres. Thirteen *P. resinosa* trees at Madison, Wisconsin, and nine trees near Fredericton, New Brunswick, were used as female parents in crosses with *P. tropicalis* pollen. A total of 504 pollinated strobili yielded 115 mature cones, but only 2 putative hybrid seeds contained filled embryos as determined by X-ray photography. The two seeds failed to germinate.

Probably no other commercially important tree species has less intraspecific variability and is more difficult to hybridize with other species than red pine (*Pinus resinosa* Ait.). Fowler and Lester (1970) summarized the small differences among provenances that have been reported for several characteristics of red pine, and they point out that the induction of greater genetic variation through interspecific hybridization has been almost impossible to obtain. Although some genetic gains can probably be made through inter- and intraprovenance selection and breeding, the rate of progress will be much slower than for other species. However, despite past disappointments in interspecific hybridization with red pine, the full range of possible crosses has yet to be adequately tried.

Red pine belongs to the *Pinus* subsection called Sylvestres (Critchfield and Little 1966) or Lariciones (Shaw 1914). All the other members of this taxon are restricted to the Eastern Hemisphere except *Pinus tropicalis* Morelet (tropical pine) which is restricted to two small areas in the Caribbean, the low mountains of western Cuba, and the Isle of Pines (Critchfield and Little 1966).

Much effort has been expended in attempting to cross red pine with other Sylvestres pines (Fowler and Lester 1970). One of the repeated attempts to cross Austrian pine (*Pinus nigra* Arnold) with red pine

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resulted in a few confirmed hybrid seedlings (Critchfield 1963); no success has been achieved with other species. Apparently there have been no previous attempts at crossing red pine with Pinus tropicalis.

Saylor's (1964) karyotype study of the Sylvestres group indicates that the chromosomes of P. resinosa resemble those of P. tropicalis more than they do any other pine. The most striking evidence for this is the similarity in arm ratios for the two smallest chromosomes in each species. This observation, coupled with the lack of other geographically close relatives, suggests the cross with P. tropicalis as a logical step in trying to hybridize red pine.

Little information is recorded on the biology of Pinus tropicalis, but this species must be adapted to a different set of climatic conditions than is red pine. P. resinosa x P. tropicalis hybrids, if produced, would probably not be hardy within the natural range of red pine, but might be directly useful in more southerly latitudes. However, the main advantage would be the introduction of new germplasm that could eventually be incorporated into a hardy organism by back crossing to red pine or by further hybridization with temperate-zone pines of the Sylvestres group.

P. tropicalis has long needles (up to two times longer than P. resinosa), distinctively large needle resin ducts (Shaw 1914), and a seedling "grass" stage (Mirov 1967). These traits should be helpful in hybrid identification. The presence of a grass stage in this species also leads to speculation on possible past hybridization of P. tropicalis with other southern and tropical pines that might make it a particularly rich species in terms of germplasm diversity (Mirov 1967). Aside from the applied tree improvement benefits that the cross P. resinosa x P. tropicalis might yield, it should provide some insight to the evolution of the Sylvestres subsection.

MATERIAL AND METHODS

Pinus tropicalis pollen was obtained from plantations in South Africa and distributed in the United States and Canada by Dr. P. W. Garrett of the USDA Forest Service.

In May 1972, 13 red pine trees growing at the University of Wisconsin Arboretum at Madison and 9 trees growing near Fredericton, New Brunswick, were used as female parents for crosses with P. tropicalis and red pine. An unpollinated control was also included. Cones were harvested in the fall of 1973, the seed extracted, and X-ray photographs taken to determine which seeds were filled and carried a normally developed embryo. Filled seeds were germinated without any pretreatment.

In May 1974, an additional set of five red pine trees at the Holst State Forest in central Iowa were used as female parents. The timing on these pollinations was somewhat delayed, and the peak of strobili

receptivity had passed by the time pollen was applied. Subsequent cone development was observed in the fall of 1974 and the summer of 1975. Cones that mature will be harvested in the fall of 1975.

RESULTS

No hybrid seedlings have been produced to date. A reasonably good cone set resulted from the *P. tropicalis* pollinations, although the number of seeds formed per cone was less than for strobili pollinated with red pine pollen (table 1). In Wisconsin a total of 276 seeds was produced from *P. tropicalis* pollinations, but X-ray analysis revealed that only one of these seeds contained a morphologically normal embryo. All other seeds were empty. The one normal "hybrid" seed failed to germinate.

Table 1.--Results of 1972 red pine pollinations in Wisconsin and New Brunswick

Item	Location	Pollen applied	
		P. tropicalis	*P. resinosa*
Strobili pollinated[1] (No.)	Wisconsin	71	12
	New Brunswick	433	117
Mature cones produced (No.)	Wisconsin	37	9
	New Brunswick	78	50
Seeds per cone			
Range	Wisconsin	1-38	34-58
Average	Wisconsin	7.5	39.8
Average	New Brunswick	5.2	21.5
Seeds produced (Total No.)	Wisconsin	276	358
	New Brunswick	409	1,075
Filled seeds[2] (No.)	Wisconsin	1	304
	New Brunswick	1 + 103[3]	954
Germination (%)	Wisconsin	0	70
	New Brunswick	0 + 98[3]	94

[1] Excluding strobili lost to insect damage or breakage.
[2] Determined from X-ray photographs.
[3] Two cones in one pollination bag produced 103 full seeds. These seeds proved to be nonhybrids in subsequent tests (see text).

In New Brunswick, pollinations with *P. tropicalis* yielded 409 seeds, of which 104 were full. However, 103 of these full seeds were obtained from 2 cones in a single pollination bag and their hybrid authenticity was dubious. Those putative hybrid seeds, along with suitable control seeds of red pine and *P. tropicalis*, were germinated and transplanted into a greenhouse test. In August 1974 the surviving putative hybrids were examined and compared to the parent species. They were all nonhybrids and indistinguishable from red pine. It was concluded that they resulted

from use of a defective pollination bag or the inclusion of male strobili in the pollination bag. The one supposedly "legitimate" hybrid seed failed to germinate.

The 1974 pollinations performed in Iowa have even less promise of success based on the percent of pollinated strobili that were still maturing as cones in midsummer of 1975. Only 3 of 126 pollinated strobili were continuing to develop, and those were somewhat smaller than the open-pollinated cones on the same trees.

DISCUSSION

The results observed to date are not encouraging. *P. tropicalis* pollen can stimulate conelet and seed coat development in red pine, but it appears that embryogenesis occurs rarely, if at all. Based on a sample of two crossing trials, one in Wisconsin and one in New Brunswick, the viability of the hybrid is also questionable. However, the possibility that red pine can be crossed with *P. tropicalis* should not be abandoned on the basis of this work alone. In these trials only one source of 1-year-old *P. tropicalis* pollen was used and although this pollen showed "normal" germination in distilled water, this is not conclusive evidence that it had retained the ability to function normally *in vivo*.

McWilliam (1959) studied the course of events during the breakdown of seed formation in incompatible pine hybridizations. His work focused on the cross *P. nigra* x *P. resinosa*. In most cases of incompatibility, he observed some penetration of the nucellus by the foreign pollen tubes. In some cases this pollen tube growth halted within the first few months and rapid breakdown of the megaspore followed. Cone abortion was common. When initial pollen tube growth was vigorous, development of the nucellus and megaspore proceeded normally through the first 12 months. But in all cases studied, normal pollen tube growth did not resume in the second spring leading to deterioration of the gametophyte and empty seeds. The failure to complete fertilization was attributed to an adverse metabolic environment for foreign pollen tube growth.

The results of the *P. tropicalis* pollinations indicated that many pollen grains are functioning during the first season, but nearly all fail to complete development the second spring. It was observed that some female parents yielded a higher percentage of matured cones and seeds per cone. For example, 1 female in the 1972 Wisconsin pollinations provided only 4 of the strobili that were pollinated (out of a total of 71), but all 4 strobili matured and yielded an average of 20+ seeds per cone including the 1 filled seed. Much tree-to-tree variation in seed set was also observed among the New Brunswick trees. Such tree- to-tree differences favor the "metabolic imbalance" hypothesis. It is also possible that failure of the *P. tropicalis* pollen to fertilize red pine results from the inability of the germinated pollen to survive the rigorous climates of Wisconsin and New Brunswick.

The fact that the percent of pollinated strobili to continue development in the second year was much lower in the Iowa study is probably more a result of delayed pollination than of tree-to-tree variation or climatic differences.

At this point in the effort to hybridize red pine with tropical pine, several approaches remain to be explored, including: (1) repeating the cross *P. resinosa* x *P. tropicalis* using pollen from South African plantations or preferably from other sources as these become available, and pollinating as many female parents as possible (manipulation of male parentage is not so easy to arrange because all pollen has to be supplied by foreign cooperators); (2) concentrating further effort on those female parents that have previously given good cone and seed set when pollinated with *P. tropicalis* pollen; (3) taking steps to reduce the harsh, overwinter environment that the *P. tropicalis* pollen tubes are exposed to (e.g., using grafted red pine females grown in the greenhouse); (4) making the reciprocal cross on either the native *P. tropicalis* stands or the South African plantations; and (5) using *P. resinosa* - *P. tropicalis* pollen mixes to pollinate either *P. tropicalis* or *P. resinosa*.

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GENETIC VARIATION IN THE HEIGHT-DIAMETER RATIO IN SCOTCH PINE

Jonanthan W. Wright[1/]

ABSTRACT.--A range-wide provenance test including seed from 110 parts of the species' natural range was established in 1961 in three Michigan plantations. The trees were measured in 1973-1974, shortly after crown closure. At that time the plantation in the Upper Peninsula averaged 12.7 ft. tall and 3.3 in. diameter-at-1-foot; the two plantations in the Lower Peninsula averaged 23.9 and 23.2 ft. tall and 6.0 and 6.2 in. diameter, respectively. The average height/diameter ratio (feet/feet) was 54:1 in all three plantations. The six tallest seedlots (30 percent taller than average) were from Belgium, northern France, West Germany, and eastern Czechoslovakia. Their height/diameter ratio varied from 50:1 to 54:1 (differences not significant); all six were among the eight largest in diameter. Thus, selection for rapid volume growth can be done on the basis of either height or diameter. Trees from the north (northern Sweden, Siberia, and the Ural Mountains) grew at very slow to moderate rates (40 to 90 percent of average). Such trees were more slender than average, having height/diameter ratios of 56:1 to 58:1. The stockiest trees (i.e., lowest height/diameter ratios) were from Spain, Greece, Turkey, and northern Italy (average height/diameter ratios of 44:1, 49:1, 50:1, and 50:1, respectively). Those races grew at moderate rates (80 to 100 percent of average) and are among the best for Christmas tree production from the standpoints of foliage color and needle length.

Traditionally in genetic experiments either height or diameter have been measured and separately reported. Sometimes both have been used as the basis for volume estimates but rarely has there been a critical analysis to show the exact relation between the two. This relation concerns the general question of whether growth is controlled by general "growth rate" genes, by specific genes for height growth and for diameter growth, or by a combination of the two types. The

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objective of the study is to answer this important question.

If "growth rate" genes predominate, tree breeding efficiency would improve in several ways: (1) selection of plus-trees for high yield would be simplified; (2) roguing of progeny test seedling seed orchards would be made easier; (3) measurements when trees are taller would be much less costly because only diameters would need to be measured, and (4) the prediction of future genetic differences would be more accurate.

PREVIOUS WORK

A few generalizations can be made from the extensive silvicultural research on diameter growth and stem form. Diameter growth is usually greatest in young trees, primarily because they suffer the least severe competition. In humid regions, rate of diameter growth may be nearly constant until crown closure and then decrease rapidly. It may then increase after thinning, to decrease again as growing space becomes limiting. In young trees the rate of diameter growth is approximately the same at different heights, resulting in a conical bole. With increasing age, diameter growth tends to be greatest near the base of the live crown. Hence, in old forest-grown trees the lower bole may be almost cylindrical.

Genetically, height and/or diameter have been measured more frequently than any other trait. Large differences among seedlots have been found in both traits, but the degree of independence between them has rarely been investigated. One exception is La Farge[2/] who worked with 10 to 12-year-old provenance and with half-sib progeny tests. The average ratio of total height to diameter-breast-high varied from 25:1 to 29:1 (ft/ft) in ponderosa pine, 67:1 to 73:1 in eastern white pine, and 36:1 to 38:1 in Scotch pine. In all three species most of the variation was associated with region of origin, little with differences among stands located a few miles from each other or with differences among the offspring of different trees within the same stand. In ponderosa and eastern white pines the fattest trees (i.e., the lowest ratios) were of southern origin; in Scotch pine the fattest trees were of northern origin. La Farge also studied the ratio between diameter at midheight to that at breastheight. Particularly in ponderosa pine, there were interesting differences in this ratio. It varied from 0.53 to 0.58 (conical stem form) in trees from the interior, and from 0.62 to 0.65 (cylindrical lower boles) in western trees.

2/ La Farge, Timothy. 1971. Inheritance and evolution of stem form in three northern species. Michigan State University Ph.D. thesis, 332 p.

Although much smaller than the differences in either height or diameter, the differences in these ratios were statistically significant and large enough to be of practical importance. La Farge interpreted some of the ratio-differences to be due to differences in cold hardiness; in the northern test area extreme winter cold may have affected height growth more than diameter growth with the result that the least hardy southern trees had the fattest stems. Some he interpreted as due to the presence of separate genes for height and diameter growth.

MATERIAL AND METHODS

The present study is based upon three provenance-test plantations established with 2-0 stock in 1961 as part of the NC-99 regional tree improvement project. The total experiment includes 122 seedlots representing 106 natural stands well distributed over the species' natural range in Eurasia. In the three plantations, 72, 108, and 110 of the seedlots were represented.

The seedlings were planted at a spacing of 8 by 8 ft, with 4-tree plots and 7 to 10 replications per plantation. Necessary weed control was practiced the first 2 years. Survival averaged 90+ percent at each site, but growth was much inferior in northern Michigan (table 1).

Table 1.--Mean heights, diameters, and height/diameter ratios in three Michigan test plantings of Scotch pine

Plantation location	Year measured	Average		
		Height	Diameter at 1 ft	Height/diameter ratio
		ft	inches	ft/ft
Kellogg Forest, southern Michigan	1974	23.9	6.0	54.6
Rose Lake, central Michigan	1974	23.2	6.4	53.4
Dunbar Forest, northern Michigan	1973	12.7	3.3	53.6

Crowns closed between 1971 and 1973 in the two fastest growing southern Michigan plantations. These plantations were thinned in the winter of 1973-1974 to leave the three best (tallest and/or straightest trees per plot and the trees were pruned to leave six whorls per tree. Crowns have not closed in the northern Michigan plantation, which has been pruned but not thinned.

Total height and diameter, as well as other traits, were measured in the summers of 1973 and 1974 on the two tallest trees per plot. These were generally straight-stemmed trees in which growth rate had not been affected by insect or bird damage. Measurement of the two

tallest trees per plot results in inflated estimates of height and diameter but the values of one seedlot to another are rarely affected by more than 1 percent. Diameter was measured at a height of 1 foot above the ground (or 15 inches if there was a branch whorl). Diameter measurements near the ground were better for biological purposes than diameter measurements at breast height because one plantation was twice as tall as another, and some seedlots were four times as tall as others in the same plantation.

Wright *et al.*[3/] describe variation in other traits and give general recommendations for the choice of Scotch pine planting stock.

An analysis of variance was performed on the height, diameter, and height/diameter ratio separately for each plantation and for all plantations combined.

RESULTS

Mortality averaged less than 10 percent in each of the three plantations. Most deaths occurred the first 2 years after field planting, primarily because of poor planting or weed competition.

The growth data are summarized by variety in table 2. In most varieties, both height and diameter were 70 to 100 percent greater in the Lower Peninsula than in the Upper Peninsula plantation.

Varieties *haguenensis* and *carpatica* from north-central Europe grew fastest at all locations. In the Lower Peninsula they averaged 28 ft tall 13 years after planting and grew at the rate of 2.5 ft per year during the previous 5 years. The six fastest growing seedlots, all from the same two varieties, grew 2.6 ft per year. Thus, they compare favorably with native pine species.

Varieties from northern Eurasia grew only 50 to 80 percent as fast, whether planted in the Upper or Lower Peninsula. Because of their slow growth and because they turn yellow during the winter, they are not planted extensively for either timber or Christmas trees.

Western and southern varieties are preferred for Christmas tree planting because they remain green during the winter and have short needles (table 2). Most grew 75 to 90 percent as fast as the timber varieties. Var. *iberica* from Spain, which remains the greenest during winter and is the favorite of many southern Michigan growers, suffered severe winter injury and consequently grew very slowly in the Upper Peninsula.

3/ Wright, Jonathan W., W. A. Lemmien, J. N. Bright, M. W. Day, and R. L. Sajdak. 1975. Scotch pine varieties for Christmas tree and forest planting in Michigan. Michigan Agric. Exp. Stn. Res. Rep. (In press.)

Table 2.--Height, diameter, and height/diameter ratios of 18 Scotch pine varieties grown in 3 Michigan plantations (data from the 2 Lower Michigan plantations were similar so they were combined)

Variety and place of origin[1/]	Height		Diameter		Height/diameter ratio	
	L.P.[2/]	U.P.	L.P.	U.P.	L.P.	U.P.
	Feet		Inches		Feet	
VARIETIES FROM NORTHERN EURASIA						
lapponica N SWE N FIN	14.1	6.7	3.4	1.9	56	45
septentrionalis SWE	19.9	10.5	4.8	2.8	55	50
rigensis S SWE LAT	22.9	14.1	5.8	3.5	55	54
mongolica SIB	20.9	12.2	4.7	2.7	57	53
uralensis URA	23.9	12.3	5.4	3.2	58	55
VARIETIES FROM CENTRAL EUROPE						
polonica POL	27.4	15.5	7.0	3.7	54	55
hercynica W GER W CZE E GER	27.1	15.6	6.7	3.9	54	54
carpatica E CZE	27.6	16.2	6.9	4.0	54	55
haguenensis BEL N FRA W GER	28.3	15.8	7.4	4.0	51	53
pannonica HUN	26.4	12.6	7.1	3.3	51	51
VARIETIES FROM WESTERN AND SOUTHERN EURASIA						
"E. Anglia' ENG	27.1	15.7	7.1	3.8	52	56
scotica SCO	22.7	11.5	5.9	3.0	52	52
iberica SPA	21.6	6.0	6.3	2.1	46	42
aquitana S FRA	22.9	11.6	5.9	3.1	53	54
subillyrica N ITA	24.2	12.4	5.9	3.8	50	51
illyrica YUG	25.3	13.2	6.3	3.4	54	51
rhodopaea GRE	24.0	12.8	6.3	3.3	50	47
armena TUR GEO	24.5	11.9	6.2	3.2	50	51

1/ BELgium, CZEchoslovakia, ENGland, FINland, FRAnce, GEOrgian SSR, GREece, GERmany, HUNgary, ITAly, LATvian SSR, POLand, SCOtland, SIBeria, SPAin, SWEden, TURkey, URAl Mountains, YUGoslavia.

2/ L.P. = Lower Peninsula, Michigan; U.P. = Upper Peninsula, Michigan.

Despite the large differences in growth rate between the Upper and Lower Peninsula plantations, bole form was similar. The height/diameter ratio averaged 54 ft/ft at all three test sites. Northern varieties were the most cylindrical, i.e., had the highest height/diameter ratios. Southern varieties were generally the most conical, having the lowest ratios. Spanish var. iberica was especially noteworthy, with ratios of 46 and 42 in the Lower and Upper Peninsula plantations, respectively. The very low ratio of 42 in the Upper Peninsula plantation was related to the severe winter injury suffered by Spanish trees there. Because of repeated top dieback, they made little height growth but continued diameter growth.

In both height and diameter, differences among varieties accounted for three-fourths of the total genetic variance (table 3). Interactions accounted for most of the remainder. Much of the interaction was caused by the very different performances of var. iberica and var. lapponica in the northern and southern test sites.

Table 3.--Statistical significance of the differences in height, diameter, and height/diameter ratio

Source of variation	Degrees of freedom	Trait: Height	Trait: Diameter	Trait: Height/diameter ratio
		Mean squares		
Variety	16	16,564**	6,482**	6,546**
Seedlot within variety	92	215	137**	667**
Variety x plantation	32	720**	416**	1,983**
Seedlot within variety x plantation	150	184**	68	301
Replication within plantation	14	2,086**	864**	1,526**
Error (= seedlot within variety x replication within plantation)	1,271	94	58	259
		Variance components, as percent of total genetic variance		
Variety		79	74	32
Seedlot within variety		1	6	21
Variety x plantation		9	12	40
Seedlot within variety x plantation		10	8	7

** = statistically significant at 1 percent level.

In the height/diameter ratio, differences among varieties were important but not overwhelming. About 20 percent of the total genetic variance was due to differences among seedlots within the same variety. For example, this ratio varied from 49 to 54 within the fastest growing variety, haguenensis, and from 53 to 56 within the second fastest growing variety, carpatica. Also, there was some interaction in the ratio, due primarily to the different behavior of the slow growing var. lapponica and the cold sensitive var. iberica in the two peninsulas.

POSSIBLE CAUSES OF DIFFERENCES IN HEIGHT/DIAMETER RATIOS

Measurement Technique

For the best estimates of a tree's productivity, measure diameter at ground level and total height. Measuring diameter at a higher point will result in an increase in height/diameter ratios and the effect will be much larger for small than for large trees.

The present data are based upon diameters measured 1 foot above the ground, thus eliminating butt swell. The 1 foot was an appreciable portion of total height only for the slowest growing seedlots planted in the Upper Peninsula. The fact that height/diameter ratios were low indicates that the results were not influenced appreciably by measurement technique.

Competition

The measurements were timed to provide information on bole form of trees grown under competition-free conditions. Actually, crown closure in the two Lower Peninsula plantations had begun in 1971 and was practically complete by the time the measurements were made. Thus, there was some opportunity for competition-induced inflation of height/diameter ratios. However, judging from the stumps of trees cut in thinning and by the similarity of ratios between the plantations which differed markedly in growth rate, competition had not affected rate of diameter growth appreciably at the time of measurement.

Winter Hardiness

Lack of cold hardiness had a demonstrable effect on the height/diameter ratio of var. iberica in the Dunbar Forest plantation. Nearly every winter most trees of this variety suffered dieback of branches or main stem and as a consequence grew little in height. However, most retained live branches below the snow line and continued diameter growth.

Other southern varieties did not suffer visible winter injury in Michigan, but in European experiments have not withstood extreme winter cold. There is a possibility that their growth processes are upset during Michigan winters, and that this upset is apparent only in a

slight reduction in rate of height growth the subsequent season. However, this cannot be demonstrated by a difference in ratio for most southern varieties grown in the Upper versus the Lower Peninsula.

Separate Genes for Height and Diameter Growth

The main purpose of this experiment was to determine whether there are general genes for growth rate or separate genes for height growth and for diameter growth. The results indicate that both types of genes are present. A strong correlation exists between height and diameter, indicating that some genes influence both. But differences in the height/diameter ratio sufficient to cause 10 percent differences in diameter growth among varieties having the same rates of height growth indicate that some genes affect mainly height growth or mainly diameter growth.

PRACTICAL SIGNIFICANCE

Timber Production

Good stem form and high volume production are the primary considerations in timber production. The ideal Scotch pines are those that grow most rapidly in height and in diameter. From the practical standpoint, the most important data on height/diameter ratios are those that concern the fastest growing seedlots. The height/diameter ratio did not differ enough among these seedlots to be of practical importance at the present time. The fastest growing variety, haguenensis, was tallest and had the largest diameter and would, therefore, be selected for planting no matter what the criterion. Of the 122 individual seedlots, those which ranked 1, 2, 3, 4, 5, and 6 in rate of height growth ranked 7, 3, 1, 8, 2, and 6 in rate of diameter growth; they did not differ significantly in either height or diameter. Having both height and diameter data would improve the estimates of genetic gain in volume production, but would not materially change the amount of such genetic gain.

Christmas Tree Production

Christmas tree growers are interested primarily in Scotch pines that retain green foliage color during the winter, have short needles, and grow slowly enough to require a minimum of shearing. For those reasons, southern and western varieties are preferred.

The height/diameter ratio is also of interest to growers who ship long distances, because shipping costs are highest for heavy-boled trees. Several growers have reported that their shipping costs were extraordinarily high for Spanish trees. The present data show that this is true because the Spanish var. iberica has the lowest height/diameter ratio. For trees of equal height, bole diameter is 10 percent greater and bole volume is 20 percent greater for Spanish than for most other Christmas tree varieties.

Volume Production

Some data on volume production of Scotch pine over long rotations in Michigan are already available. They are based on 30- to 40-year-old plantations, probably of German (var. hercynica) origin. For estimates of volume production with genetically improved trees, we must rely on comparatively young experiments such as described here.

To do so requires extrapolation. Under Lower Peninsula conditions, the six fastest growing seedlots (var. haguenensis and carpatica) averaged 29.1 ft tall and are growing at the rate of 2.6 ft per year; 11 percent superior to var. hercynica. Up to age 14 there has been very little change in growth rates of various seedlots, and it is safe to assume that the long term genetic gain in rate of height growth is 11 percent.

For those six seedlots the present genetic gain in diameter growth is also 11 percent or 0.7 inches. However, future diameter growth will be governed more by spacing than by genetic potential because crowns have closed. If these six seedlots were planted for saw logs and subjected to frequent thinning, a continuation of the 11 percent advantage in rate of height growth and rate of diameter growth could be assumed, at least until age 25 or 30. In that case, there would be an approximate 37 percent gain in rate of volume production per tree. If, however, plantations of the improved stock were managed so as to maintain a closed crown canopy, the 0.7 in. diameter advantage by age 14 would be maintained, but future rate of diameter growth would be similar to that already found for plantations of var. hercynica. In that case, volume estimates for the improved Scotch pine should assume 11 percent greater height and 0.7 in. greater diameter for all trees after age 14.

Future Measurement of Test Plantations

The three test plantations described here are easy to walk through. They have been pruned, and diameter measurements are easily made. Early height measurements were not much of a problem but those made at age 14 were time consuming. By age 20, the tallest varieties will exceed 40 ft and individual trees will be 50 ft tall.

Yet such experiments as this must be maintained and may produce their most valuable information after age 20. Growth data will be an important part of that information. The easiest solution will be to measure diameter only and to assume a constant height-diameter relation. That would be moderately satisfactory as regards choosing those seedlots with the greatest average growth rates but would be totally unsatisfactory in comparing future growth rates of northern and southern varieties, in view of the large racial differences in the height/diameter ratio.

SELECTION AND BREEDING EASTERN COTTONWOOD FOR RESISTANCE TO FOLIAGE DISEASES[1/]

J. J. Jokela and W. R. Lovett[2/]

ABSTRACT.--The incidence and effects of *Melampsora* leaf rust and *Marssonina* leaf spot on eastern cottonwood in the central United States are discussed. Yield at age 15 in an Illinois plantation was related to means of leaf-rust scores observed in September of the second and third growing season. An increase of 1 rust score class on a 5-point scale (1=light infection....5=severe infection) reduced yield by about 20 percent. The need for selection and breeding for resistance is stressed and methods for attaining resistance are presented.

Intensive culture of eastern cottonwood, *Populus deltoides* Bartr., is becoming commonplace on the Mississippi Delta and is viewed with increasing interest in the Midwest and elsewhere in the United States. Early results have been sufficiently encouraging to uphold the promise of high yields over short rotations, such as are attained in Europe with selected clones. Nevertheless, it is unlikely that expectations will be fully realized with our natural species--most of which do not warrant intensive culture at wide spacings mainly because of their susceptibility to numerous stem and foliage diseases.

Schreiner's (1963) assessment that "poplars, if not the most pest-ridden of the world's important timber species, certainly rank high in this respect" has been largely unheeded in our quest for superior clones of eastern cottonwood. Resistance to specific diseases must be a primary goal rather than a fortuitous result of breeding and selection.

IMPORTANT FOLIAGE DISEASES

Two diseases meriting primary consideration in the central United States are leaf rust caused by *Melampsora medusae* Thum. and leaf spot caused by *Marssonina brunnea* (Ell. et Ev.) P. Magn. Although the

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1/ Study was supported in part by the North Central Regional Project NC-99, "Improvement of Forest Trees through Selection and Breeding," and the Illinois Agricultural Experiment Station.

2/ Associate Professor and former Graduate Research Assistant, University of Illinois.

effects of the latter disease are obscured by and have been confused with the effects of the better known leaf rust, the two diseases are easily distinguished. Leaf rust is recognized by the presence of yellow-to-orange pustules of urediospores on the upper and often on the lower surfaces of infected leaves. Irregular, dark brown necrotic areas, which may include the entire leaf, and rumpling of the leaf blade are characteristic of leaf-rust infection. Leaf-spot infection is manifest within a few weeks after leafing as discrete reddish brown to purple discolorations on both leaf surfaces. These develop into dark brown lesions that are seldom over 1 mm in diameter. The release of light-colored macroconidia from acervuli in the middle of a dark lesion produces a characteristic ringlike structure. Elongated dark lesions on the veins and petioles are also characteristic. Infected leaves turn yellow to bronze in color and remain flattened in contrast to rusted leaves that darken and rumple. Both diseases progress acropetally in the crown and normally do not reach the tip of the tree until late in the growing season.

INCIDENCE AND EFFECT

Melampsora leaf rust occurs throughout the natural range of eastern cottonwood. The incidence of *Marssonina* leaf rust is less well known. Recent studies suggest that it is particularly severe in the central United States.

The incidence and severity of *Melampsora* leaf rust and *Marssonina* leaf spot on unselected clones of eastern cottonwood of varying geographic origin has been studied in an east-central Illinois plantation on Drummer silty clay loam (Typic Haplaquoll). Rust infection was scored on the following rating system (Jokela 1966):

- Class 1. Pustules not apparent or rare.
- Class 2. Pustules present but not prevalent; little or no leaf drop.
- Class 3. Pustules prevalent on most leaves but not abundant; some leaf drop.
- Class 4. Pustules prevalent and abundant on many leaves; considerable leaf necrosis and leaf drop in the lower portion of the crown.
- Class 5. Pustules so abundant as to appear coalesced; over 50 percent leaf drop.

Leaf spot was scored on a similar rating system except that it was based on the presence of lesions rather than pustules and on the yellowing or bronzing rather than necrosis of the foliage.

Trees were scored on September 24, 1974 (table 1). Each plotted point above the blocks on the diagonal or equality line represents a clone more severely attacked by leaf spot than by leaf rust. Most clones of

Table 1.--Incidence of *Melampsora* leaf rust and *Marssonina* leaf spot on clones of eastern cottonwood of southern, central, and northern seed origins in a 5-year-old plantation, September 24, 1974, Urbana, Illinois

Leaf-spot score	Mississippi (*)						So. Illinois (•) and Missouri (+)						No. Illinois (x) and Minn.-Wisc. (0)					
	Leaf-rust score					Percent of clones	Leaf-rust score					Percent of clones	Leaf-rust score					Percent of clones
	1	2	3	4	5		1	2	3	4	5		1	2	3	4	5	
5						-						-						39
4						28						21						49
3						43						51						12
2						29						28						-
1						-						-						-
Percent of clones	28	43	29	-	-	100	7	12	39	16	26	100	5	29	41	20	5	100
Basis	7 clones (11 trees)						43 clones (67 trees)						41 clones (67 trees)					

northern origins are in this category, whereas most trees of central origin are more severely infected with leaf rust. Observations made over several years have shown that clones of southern origin are not severely attacked by either pathogen until late in the growing season.

The primary effect of either disease is premature defoliation. In Illinois this may occur 2 months before the end of the normal growing season and is sufficiently extensive and severe to virtually preclude the development of yellow autumn coloration on eastern cottonwood. Premature defoliation caused by leaf rust has been associated with loss in vigor, lessened winter hardness in colder climates (Nagel 1949, Meiden and Vloten 1958, Peace 1952), a predisposition to attack by other pathogens (Nagel 1949, Schreiner 1959), and root starvation.[3/]

The economic impact of foliage diseases has not yet been thoroughly evaluated in American plantations because until recently there has been little interest in intensive culture of poplars. The contention that leaf rust infection occurs too late in the growing season to affect growth is unfounded. Wilcox and Farmer (1967) found high negative correlations between first-year rust reaction and second-year growth in height and diameter in a Mississippi plantation of eastern cottonwood. Chiba and Nagata (1973) found similar correlations in *P. maximowiczii* between rust scores made in the nursery and height at 8 years of age.

The cumulative effect of annual rust infections on growth and survival is being studied in a clonal and seedling plantation established in 1960 on a bottomland site in east-central Illinois for the purpose of studying heritable variation in eastern cottonwood. Only results obtained in the seedling planting are reported here. This plantation was established with 20 1-year-old seedlings selected at random from each of 22 progenies of southern Illinois origin, 38 progenies of west-central Illinois origin, and 32 progenies of east-central Illinois origin.

All trees were scored for rust infection in early September of the second growing season and again in late September of the third growing season. Subsequent growth and survival measurements have been correlated with the means of these two rust scores. Significant differences in height and survival that were related to mean rust scores were already present at 7 years of age when the first height and survival measurements following rust ratings were made. These differences have increased with age and have been most pronounced on the trees of southern Illinois origin and least pronounced on trees of east-central Illinois or local origin.

3/ Personal communication with C. W. S. van Kraayenoord.

The relation between mean rust score and diameter squared x height, a relative measure of tree volume, at 15 years of age is shown in figure 1. The solid line shows the relation for trees that survived through the 15th growing season following planting. The relation shown by the broken line is a better indicator of yield because it accounts for mortality. Accumulated mortality to age 15, which averaged about 15 percent of the trees in the lower three rust score classes, increased to 33 percent of the trees in rust class 4 and to 50 percent in rust class 5. The essentially linear relation between D^2H and rust score suggests that an increase in one rust score class reduced yield at age 15 by about 20 percent.

Information on the economic impact of Marssonia disease on eastern cottonwood in American plantations is not presently available. Premature defoliation caused by this disease occurs earlier and is at least as extensive in the central United States as that caused by leaf rust, hence there is reason to expect that economic losses may be as serious as those caused by leaf rust. Marssonina leaf diseases have caused severe economic losses on euramericana poplars in Europe (Meiden 1962, Castellani and Cellerino 1964).

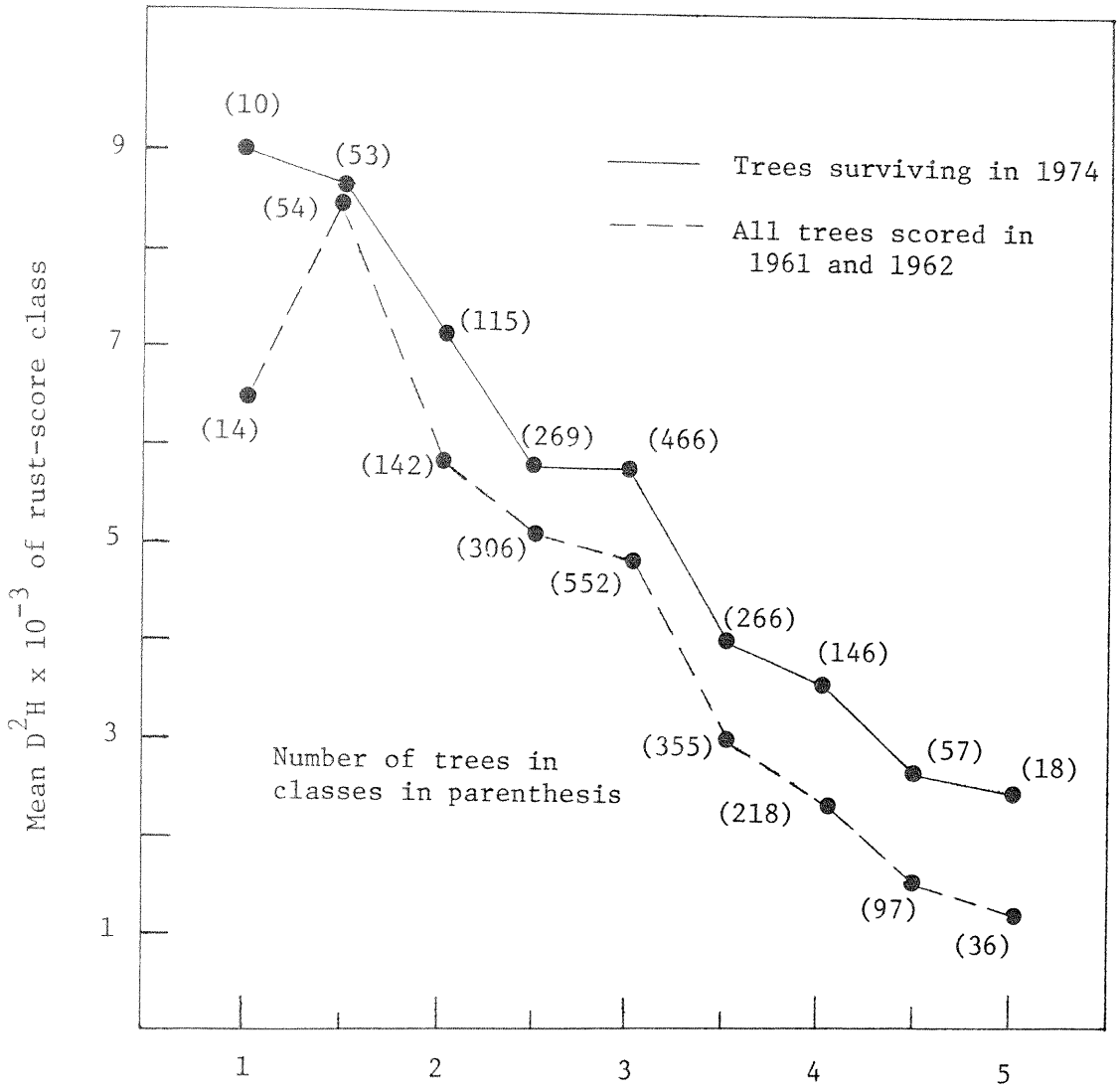
SELECTION AND BREEDING

The wide range of resistance to Melampsora leaf rust among geographic origins, progenies, and clones of eastern cottonwood, and the reliable and rapid assessment of rust reaction argues well for the development of resistant clones and varieties.

Geographic Origin Tests

Evidence from NC-99 tests suggests that utilization of geographic variation is one approach to obtaining rust resistance. In the Ohio test (Thielges and Adams 1975), trees of Missouri origin were more resistant than were trees of local or other midwestern origins. In the Illinois test most clones of Louisiana or Mississippi origin remained practically rust free until very late in the growing season. Correspondence with world-wide recipients of eastern cottonwood seed distributed by the Poplar Council suggests that seed from certain origins has yielded large numbers of resistant trees. This type of uniform resistance appears to be due to an interaction between seed origin and environment and it may be specific to the locations of the planting. Northward movement of seed enhances rust resistance but incurs the risk of planting trees where they are not winter hardy. Because a reliable assessment of winter hardiness requires many years. NC-99 test plantings, which are already 10 years old, are a logical place to search for this type of resistance. Resistance to Marssonina might be selected for simultaneously. We have selected a number of clones of Mississippi and southern Illinois origins that are field

Figure 1.--Relation between *Melampsora* leaf-rust score and diameter squared x height of trees of Illinois seed origin in a 15-year-old plantation in central Illinois



Rust-score class. Mean of scorings made in September of second and third growing season.

resistant to both diseases, are rapid growers, and appear to be hardy in central Illinois.

Progeny Tests

Observations of numerous open-pollinated progenies do not support the idea that seed collections from untested, rust-resistant wild trees will necessarily yield rust-resistant progenies. This is not to say that differences among open-pollinated progenies do not exist. On the contrary, we have found progenies that are mostly field resistant but we have never been able to demonstrate a correlation between the resistance of the female parents and the progeny means.

Results of a test of open-pollinated progenies are given in table 2. All progenies are of southern Illinois origin with the exception of those from seed collections made in the central Illinois counties of Vermilion, Henderson, and Hancock. Most trees in Progeny 73-002, which had the lowest mean rust scores on both dates that scorings were made, were field resistant (i.e., rust scores of 1 or 2 on September 6). More than 50 seedlings with a rust score of 1 could have been selected in this progeny and Progeny 73-001, which had the next lowest mean rust score. Less than 20 rust-resistant seedlings were found in the remaining 20 progenies.

The tendency for progenies to be either lightly or heavily rusted suggests that progeny screening tests conducted in the nursery would be useful in selecting parents that yield a high proportion of resistant individuals. Fifty to 100 seedlings per progeny, with 3 replications, would be sufficient to identify resistant progenies. On the basis of observations reported in table 2, one might expect 1 to 3 percent of the progenies to contain sufficient numbers of resistant individuals to warrant large seed collections from their parents in subsequent years.

A small to moderate size program such as this should yield hundreds of resistant individuals for establishing clonal tests to assess growth and other traits. A major drawback, however, is that Marssonina infections appear to be too light in the nursery or in 1 or 2-year-old plantings on new sites to allow reliable assessment of resistance to this disease.

Controlled Pollination

Hybridization studies begun by Muhle-Larsen (1970) and continued by Steenackers (1972a, 1972b) show great promise for obtaining resistance to several diseases.

Table 2.--Mean rust scores on September 6 and October 4, 1973, of open-pollinated progenies sown in Union State Tree Nursery, Jonesboro, Illinois, on June 20, 1973

Progeny (UIFG No. 73-)	Seed origin: Nearest town	Seed origin: County	Mean rust score[1/]: Sept. 6	Mean rust score[1/]: Oct. 4
001	Golconda	Pope	3.0	3.4
002	Golconda	Pope	1.0	1.4
003	Joppa	Massac	2.8	3.0
005	Grand Chain	Pulaski	3.0	3.6
006	Grand Chain	Pulaski	3.5	4.6
008	Thebes	Alexander	3.8	4.8
011	Georgetown	Vermillion	1.5	2.4
012	Oquawka	Henderson	3.0	4.1
013[2/]	Carthage	Hancock	3.5	4.1
014	Brookyln	Schuyler	3.0	4.7
016	Chester	Randolph	3.0	3.4
017	Rockwood	Randolph	3.0	4.1
018	Jones Ridge	Jackson	3.2	4.0
019	Valmeyer	Monroe	3.5	5.0
020	Valmeyer	Monroe	2.8	3.1
021	Rockwood	Randolph	3.8	4.4
022	Rockwood	Randolph	3.2	4.0
024	Grand Tower	Jackson	3.0	4.1
026	Ware	Union	3.5	4.0
027	Reynoldsville	Unioon	3.2	4.3
028	McClure	Alexander	3.8	4.4
029	Cache	Alexander	4.0	4.7
All sources			3.1	3.9

1/ Means for September 6 and October 4 are averages of 4 and 7 plot means, respectively, 50 to 200 trees per plot.

2/ UIFG-68-J18-00. Selected in 1968 for resistance to *Melampsora* and *Marssonina*.

Assortative matings of *Melampsora* resistant and susceptible eastern cottonwood were studied by Lovett (1975). He found significant differences among mating types. However, these differences were not as large or in the direction one would expect if resistance was largely controlled by additive genes. The results supported Jokela's (1966) heritability studies, which suggested that much of the genetic variation in susceptibility to *Melampsora* within eastern cottonwood was nonadditive. Controlled pollinations will be useful and necessary in

combining resistance and other desirable traits. Until more is known about the inheritance of specific traits and of general and specific combining abilities, however, selection of wild types appears to be a more practical approach.

Selection of Resistant Phenotypes

The most promising approach to obtaining resistant clones for immediate use is to select phenotypes from natural stands in midfall just before the first killing frost. At this time trees highly resistant to both Melampsora and Marssonina diseases are strikingly conspicuous because only they will have a full complement of healthy green foliage. This method also allows simultaneous selection for tree form and, to a limited extent, growth rate.

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GENETIC CONTROL OF RESISTANCE TO HYPOXYLON INFECTION AND CANKER DEVELOPMENT IN *POPULUS TREMULOIDES*[1/]

Fredrick A. Valentine, Paul D. Manion and Kathleen E. Moore[2/]

ABSTRACT.--The responses of 24 families of *P. tremuloides* (6 groups of 4 maternal half-sibling families each) to 4 sources of *Hypoxylon mammatum* were observed. Three mechanisms of resistance to the disease were studied: (1) callus formation, (2) branch death, and (3) resistance through retardation of canker growth. Resistance by callous formation is due to a hypersensitive response of the host to the pathogen. Little variation exists in the nature or time of the host response, but the pathogen's ability to elicit the host response, expressed as incidence per inoculum, varies from about 3 to 35 percent. Evidence suggests that a few major genes control this trait and are the basis for Mendelian ratios within families and discrete differences between groups of half-sibling families. Resistance by branch death occurs at a low incidence (8.3 percent) and death is due to the canker encircling the branch. Heritability estimates are low, 0.075 or less in response to the four inocula and 0.027 for all data. These are probably underestimates because all potentially resistant phenotypes most likely have not been expressed 4 months after inoculation. The third form of resistance, retardation of the spread of the pathogen, is measured as canker length. h^2 is low for three inocula ($\leq$ 0.074), but reasonably high (0.254) for the fourth source.

The best breeding methods for disease-resistant forest trees may be those concerned with breeding for "nonspecific" or horizontal resistance. This type of resistance functions against all pathogenic races or biotypes and is generally considered polygenic. "Specific resistance" functions against individual races of a pathogen and is also called major gene resistance, hypersensitivity, and vertical resistance. It is controlled by single or a small number of genes. A knowledge of the genetic control of resistance is necessary if we are to design an effective tree breeding program.

1/ Miss Moore completed the Veldman computer analysis of canker lengths as an undergraduate research problem.

2/ College of environmental Science and Forestry, State University of New York, Syracuse.

Preliminary results (Valentine and Manion 1972, Manion and Valentine 1974) suggest that resistance to Hypoxylon canker in trembling aspen (Populus tremuloides Michx.) and bigtooth aspen (P. grandidentata Michx.) is polygenic. In this report we show that much genetic variation exists in factors affecting (1) infection of the host by artificial inoculation with Hypoxylon mammatum (Wahl.) Mill. (syn. H. pruinatum (Klot.) Che), (2) prevention of the establishment by rapid death of host tissue, or (3) isolation through callousing and retarding canker enlargement.

MATERIAL AND METHODS

We used 7-year-old P. tremuloides at the Tully Genetic Field Station, Tully, New York (fig. 1). Following Comstock and Robinson's Experiment 1, each of six pistillate-flowered trees was crossed to a random sample of four staminate-flowered trees to give six maternal half-sibling groups of four families each. One-year-old seedlings were planted in 1968 in a randomized complete block design with three blocks and nine trees per family per block.

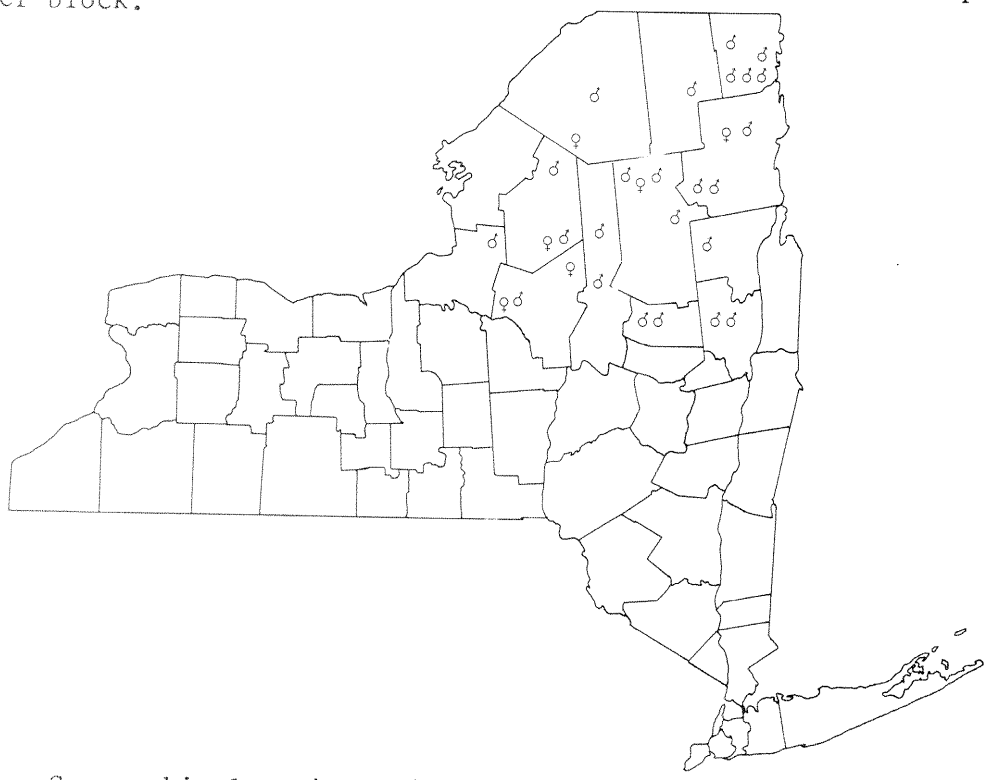

Figure 1.--Geographic locations of the six trees serving as female parents (♀) and the 24 trees as male parents (♂) in the northern New York population of P. tremuloides.

In June 1974, four 2-year-old branches, approximately 1.5 m from the ground, were inoculated with H. mammatum mycellum grown for 2 weeks at 30°C on moist sterilized wheat. Six-mm circular patches of bark were removed with a cork borer and one piece of H. mammatum-infested grain placed in each wound. The inoculations were wrapped with parafilm "M". Four pathogen sources were used: (1) a "Baldwinsville, New York" culture (French and Manion 1975, isolate 208 M), (2) a "Tully" culture originating from a single ascospore (Hsu 1975, isolate 4B19 #5 set 1), (3) a "Shiawassee County, Michigan" culture, and (4) a "Heiberg Forest" culture (French and Manion 1975, isolate 608 M). The four strains were used to inoculate branches with north, east, south, and west exposures, respectively. Some trees and branches were missing so that there were unequal numbers of inoculations in the families. Four months later the lengths of the resulting cankers were measured. Cankers surrounded or covered by callous tissues and cankers on branches that had died were noted. Cankers on broken branches (8.5 percent) were counted but not measured.

We used the method of Robertson and Lerner (1949) to estimate the heritability of resistance by callousing and by branch death (also see Dempster and Lerner 1950). The method requires the calculation of the average genetic relation (r^G) of the members of each family making up each half-sibling group. For this we used the method of Osbourne (1957).

Heritability estimates for canker length were based upon a three-way ANOVA (Veldman 1967). The methods of King and Henderson (1954) and Becker (1975) were used to calculate the coefficients for partitioning variance when there are unequal numbers of observations per family. These were then applied to the ANOVA results to estimate the covariance of maternal half-sibling groups and the phenotypic variance.

RESULTS

Resistance Through Callous Formation

Genetic factors control all three mechanisms of resistance to cankering

The results of Chi-square Tests of Homogeneity for the responses of the six half-sibling groups to each of the four inocula are statistically nonsignificant except for the Tully data, table 1, therefore shows group means only. In the Tully results, three of the six means are outside the range of the $\bar{x} \pm 2s$. Some differences were also evident in the Baldwinsville data, with two mean values outside the $\bar{x} \pm 2s$, but the probability value for the Chi-square test is greater than 0.20.

Table 1.--Incidence of resistance by callousing among maternal half-sibling aspen families inoculated with four sources of *Hypoxylon mammatum*

(In percent)

Item	Source of inoculum			
	Baldwinsville, New York	Tully New York	Shiawassee County, Michigan	Heiberg Forest, New York
All families	12.6±1.4	3.3±0.8	15.7±1.5	34.9±2.0
Total number of inoculations	557	552	573	567

The heritability estimates were calculated but the values are small with large standard errors or are negative, so they have not been given. The estimate for the Tully data is the largest, but is only 0.068 and its standard error 0.067.

Male effects within maternal half-sibling groups were considered for the Heiberg data because they include the highest incidences of callousing. If segregations for one or more "major" genes for callous formation occur in the population, Mendelian ratios may be inferred from these data (table 2). The four families in each group separate into two or three different frequencies. Groups CA107, HA313, and ES107 exhibit two levels, with the lower incidence, ranging from about 20 to 30 percent, about half that of the higher incidences that range from about 40 to 50 percent. In the other three groups, one or more families occur with either a "low" or a "high" incidence of callousing, but in addition, families with a frequency intermediate to these also occur. Minor variations also can be seen among the half-sib groups, with the SL209 group lower than the others.

Resistance Through Branch Death

Responses to the Baldwinsville and Tully inocula are large, ranging from 0 to 13.4 percent for Baldwinsville and from 6.8 to 19.5 percent for the Tully isolate (table 3). In the test for homogeneity the Chi-square values are statistically significant. The variation in the other two inocula is much less, and the Chi-square results are not significant.

The mean frequency of branch death for the four inocula ranges from 4.6 percent for the Heiberg source to 11.6 percent for the Tully source. The tests for homogeneity, however, suggest that there are no real differences in the responses of the other half-sibling groups to the four pathogen sources (except for the SL209 group). The data for the four inocula for each half-sib group were then pooled and a Chi-square test used to analyze the variation in the mean incidences among the six groups. Maternal parents SL209 and LE208 appear to contribute genes

Table 2.--Incidence of calloused cankers in response to the Heiberg inoculum in each of the four half-sibling families constituting each of the six maternal groups (percent incidence is shown by the position of the male parent tree designation along the vertical axis

Calloused cankers (percent)	Maternal groups of half-sibling families (common female parent)					
	SL209	LE208	OA107	OA201	HA313	ES107
55						
50						CL202
				HE202		
					ES111	
45						
		HA312			FU111	
	OS109	CL106				
			CL104			
40			SL112			
			HA308			
	ES201			SA113		
35		CL306				
				CL110		
30						
						HA209
	SL111	WA105			ES202	OA102
25					LE108	
			FU109	FR212		
						HE109
20						
	LE201					
15						

for resistance, especially in response to the Baldwinsville and Tully inocula, and the HA313 parent appears to lack these genes because its progeny exhibit a low incidence of branch death.

Table 3.--Incidence and heritability of resistance by branch death among maternal half-sibling aspen families inoculated with four sources of *Hypoxylon mammatum*

(In percent)

Item	Source of inoculum: Baldwinsville, New York	Tully New York	Shiawassee County Michigan	Heiberg Forest New York	Half-sib mean incidence	Test of homogeneity: χ^2 (d.f.=3)	Probability
Half-sib Family							
SL209	12.2±3.6	19.5±4.3	7.8	1.6	11.0±1.7	13.130	<0.01
LE208	13.4±3.8	18.9±4.1	9.8	5.0	12.4±1.9	7.193	>.05
CA107	5.7±2.5	7.4±2.7	7.9	3.0	6.2±1.3	2.374 1/	>.95
CA201	8.0±2.9	8.0±2.9	8.9	8.2	8.2±1.5	0.085	>.99
HA313	0	6.8±2.9	4.2	5.8	4.1±1.2	5.396 1/	>.10
ES107	7.6±3.0	9.0±2.9	7.1	4.5	7.0±1.4	1.609 1/	>.70
All families	8.0±1.2	11.6±1.4	7.7±1.2	4.6±1.1	8.3±0.6		
Test of Homogeneity							
value of χ^2 (d.f.=5)	11.864	15.155	1.905	1/ 4.115	17.934		
Probability	<0.05	<0.01	>0.80	>0.50	<0.01		
Heritability	0.005±0.066	0.075±0.076	Negative value	Negative value	0.027±0.026		

1/ Yates adjustment was applied to these data due to expected values of 5 or less.

The variability among half-sib families could represent random sampling variation. This, however, does not appear to be the case because the callousing frequency in 20 of the 24 families is outside the range, p ± 2s (30.9 to 38.9 percent), for the Heiberg results. The three families that do occur within this range are "intermediates" in their groups, and the fourth, SL209 x ES206, is a high incidence family. The observed distribution is bimodal, with the "high" incidence families comprising one modal group and the "low" incidence families, the other.

The heritability estimates for the Baldwinsville and Tully strains are low and have large standard errors, and are negative for the Michigan and Heiberg data (table 3).

Resistance Through Retardation of Canker Growth

The size of cankers varies with the four pathogen sources and among the six maternal half-sibling groups (tables 4, 5, and 6). The ranking of the four inocula based on canker size is very consistent. The mean is smallest for Heiberg (6.02 cm), followed by Baldwinsville (6.34 cm), Tully (7.34 cm) and Michigan (7.76 cm). With only three exceptions this ranking is also the same for the six individual half-sib groups (table 4). The differences among maternal half-sibling groups is also large and is statistically significant for all inocula except Heiberg ("Females" in tables 5 and 6).

Table 4.--Mean canker length 4 months after inoculation for each of the four sources of pathogen (each family mean for each inoculum is based on between 55 to 85 cankers)

Half-sib	Source of inoculum: Baldwinsville, New York	Tully New York	Shiawassee County Michigan	Heiberg Forest New York	Pooled data: Mean	No.
	Centimeters					
SL209	6.28	7.99	7.81	6.09	7.16	285
LE208	6.22	7.68	7.87	6.50	7.16	300
OA107	5.94	6.43	7.10	6.10	6.43	308
OA201	6.16	6.47	7.32	5.99	6.48	300
HA313	6.13	7.52	7.81	5.43	6.86	257
ES107	7.32	7.94	8.59	6.00	7.58	305
Mean	6.34	7.34	7.76	6.02	6.91	1,755
No.	443	475	457	380		

Table 5.--ANOVA for canker lengths 4 months after inoculation and the heritability estimates based on the combined data for the four sources of inoculum

Source of variation	d.f.	Mean square
Between families	23	22.705**
Females	5	56.565**
Males W/N females	18	13.299**
Blocks	2	16.964*
Inoculum sources	3	260.198**
Families X blocks	46	6.323
Families X inocula	69	6.723*
Blocks X inocula	6	15.965**
Families X blocks X inocula	138	4.466
Within families (error)	1,468	5.107
Weighted coefficient for variance components:		
t	5.92	
Estimates of heritability:		
With the inocula effects included in σ_p^2	0.083	
Without the inocula effects and the interactions with other main effects in σ_p^2	0.109	

* Statistically significant at the 5 percent level of probability
** Statistically significant at the 1 percent level of probability

Table 6.--ANOVA results for canker lengths 4 months after inoculation and estimates of heritabilities for each of the inoculum sources

Source of variation	d.f.	Source of inoculum (mean square): Baldwinsville, New York	Tully New York	Shiawassee County Michigan	Heiberg Forest New York
Blocks	2	21.162**	12.629	24.001*	7.217
Females	5	16.206**	36.002**	17.891*	6.485
Males W/N females	18	9.313**	7.969	15.474**	2.666
F-M X Blocks	46	5.398	5.661	5.719	3.306
Within families d.f.		371	403	385	308
m.s.		4.521	5.061	6.824	4.357
Weighted coefficients of variance components:					
k_1		18.168	19.513	18.840	15.611
k_2		19.274	20.597	19.604	16.461
k_3		73.574	78.993	75.967	63.159
Estimates of heritability		0.074±0.119	0.254±0.224	0.015±0.090	0.058±0.062

* Statistically significant at the 5 percent level of probability
** Statistically significant at the 1 percent level of probability

Other significant results include block effects, the interaction between blocks and inocula, and the interaction between families and inocula. Because the variation in canker sizes associated with each pathogen source is similar in each of the half-sib groups, it is not likely that a Female and Inoculum interaction is an important part of the Families X Inocula interaction.

We used the weighted coefficients of the variance components to determine the heritability of canker length (bottom tables 5 and 6) (King and Henderson 1954, Becker 1975). The difference between the two heritability estimates in table 5, $h^2 = 0.083$ and $h^2 = 0.109$, is the consequence of including the variance components due to differences in inocula sources and its interactions only in the first estimated phenotypic variance. All of the heritability values are small except for the Tully inoculum (table 6) which is 0.254. All also have large standard errors.

DISCUSSION

Three mechanisms confer resistance to hypoxylon cankering in the northern New York trembling aspen population: (1) callous formation, (2) branch death, and (3) resistance through retardation of canker growth. On the basis of individual inocula the estimates of the heritabilities for all three traits are low: 0.064 or less for callous formation, 0.076 or less for branch death, and 0.074 or less for retardation of canker growth. For the pooled data of all four inoculations on each tree, h^2 are 0.027 and 0.083 for branch death and retardation of canker growth, respectively, but $h^2 = 0.109$ for canker growth retardation if variation due to inocula and its interactions with other main effects are not included in the estimate of the total phenotypic variance.

Callous formation is rapid and similar to a hypersensitive reaction, occurring before a measurable canker developed in 314 (83.5 percent) of the 376 calloused wounds or cankers. The "late" calloused cankers ranged up to 7 cm in length, with the incidence of callousing decreasing with an increase in length of the canker. The frequency distribution according to canker size when calloused, however, does not fit a Poisson distribution ($\chi 2 = \infty$, d.f. = 4., $P<0.001$). This type of early response of the host is characteristic of hypersensitive reaction, generally controlled by one or a few genes (Williams 1975).

The incidence of callousing differs markedly for the four sources of inoculum (table 1), which suggests that variation is due to the pathogen and not the host. Differences in the incidence of callousing is small and not significant (except the Tully inoculum). (Tully data are limited--only 18 calloused cankers of 552.) This suggests that the host genotypes for a gene or genes controlling the callousing response to the fungus are essentially alike. This limited variation is also responsible for the low heritability values because they are dependent upon the variance between half-sib groups.

The variations in the callousing response of each half-sib group to each of the four inocula are large and highly significant, with a high incidence exhibited by all six groups in response to the Heiberg inoculum, an intermediate incidence in response to the Michigan and Baldwinsville source, and a low incidence in response to the Tully inoculum. The greatest variation, therefore, exists in the pathogenicity of the fungus, with very little variation in the host reaction to a particular pathogen phenotype (table 1).

The sizes of calloused cankers also suggest that the host response to the pathogen is the same regardless of the source of the inoculum. The frequencies of "early" (calloused canker length = 2 cm) and "late" (>3 cm) callousing were tested for homogeneity using the Chi-square method. The "late" cankers in the 3 to 7 cm length classes were pooled. The results are nonsignificant ($\chi 2 = 7.402$, d.f. = 3; $0.10 > P > 0.05$). The Chi-square

value is large and primarily represents the contribution of the limited data for the Tully inoculum (11 "early" and 7 "late" cankers). If we include only the data for the other three inocula, the variation is small ($\chi 2 = 1.159$, d.f. = 2, $P > 0.50$). Therefore, the host response to the pathogen is the same regardless of the overall incidence of the callousing response, with the frequencies of "early" and "late" responses 84.6 percent and 15.4 percent, respectively.

The results support the hypothesis that callous formation is controlled by a few gene loci that are "turned on" in host tissue by the presence of pathogen genotypes with a given level of virulence. Clear-cut Mendelian ratios will not necessarily be observed, however, because this appears to be a threshold-type response. Nevertheless, sharp, distinct differences in the frequencies of callousing among the four families composing a given half-sib group would be expected. The two or three different frequencies in response to the Heiberg inoculum support this (table 2). The 11 families comprising the high incidence level of callousing in the 6 groups may represent the expectations from a testcross, namely a 1:1 Mendelian ratio. The nine families exhibiting the low level could represent an incidence of 25 percent callousing, the expectation if callousing is a recessive trait and the parents are both heterozygous. We tested the goodness of fit of the observed results to that expected for each of the 11 "high frequency" and the 9 "low frequency" families if they represent expected Mendelian ratios of 1:1 and 1:3, respectively, (calloused to noncalloused); all were nonsignificant. We also compared the observed incidence for each family to the range $p \pm 2s$, for each of the two Mendelian expectations. In all cases, the observed incidence was within the expected range. These results clearly support the hypothesis that callousing is due to a single gene in the "high" and "low" incidence families.

The intermediate frequencies exhibited by the four families in the SL209, LE208, and OA201 groups, cannot be explained as easily. It appears that resistance by callousing is not always a simple Mendelian trait, but rather, it is controlled by a more complex genetic system. Nevertheless, sharp, distinct differences that recur among related families are most easily explained by the segregation of major genes contributed by the common parent, in this case the female parent. Confirmation of this hypothesis must await further experimentation.

We expected heritability estimates to be high; instead, they were low. This is because the estimates were not based upon individuals or even family means for estimating additive genetic variance, but upon the variation among half-sibling group means around the population mean. The group mean, in turn, represented the average incidence in the four half-sibling families comprising that group. Because variation among mean values was low, heritability values were low.

Resistance by death of the infected branch does not appear to be a hypersensitive host reaction, but rather the response resulting from the spread of the canker to girdle the branch. The response is not as rapid as callousing. Only 13 (8.4 percent) of the 155 branches that died, did so before a measurable canker developed, i.e., greater than 2 cm in length. The incidence is so low that little information on the size of cankers at the time of branch death or the frequency distribution can be gleaned from the individual family data, the half-sibling group data in response to each inoculum source, or even the pooled data for each half-sib group in response to all four inocula. Data pooled for all half-sib groups for each inoculum or for all data approximate the normal distribution, with the modal class almost always including the average length of all living cankers (table 4). More branches were being girdled when these results were obtained so later field scoring should provide information on how long after infection this type of resistance can act to prevent disease development.

The variation in branch death among the six half-sibling family groups in response to the Baldwinsville and Tully inocula is statistically significant. There is little variation in response to the other two sources. Correspondingly, the largest h^2 values, 0.075 and 0.055, were obtained in response to Tully and Baldwinsville sources, with negative values for both the Michigan and Heiberg sources. The incidence in response to the Heiberg source was very low, only 17 among 369 cankered branches. Therefore, even though the Yates' adjustment was applied, the results may not be reliable.

Though small, differences probably do exist among pathogen sources (table 3). This is supported by the pattern in the frequencies of branch death in response to the four inoculum sources by each of the half-sibling groups. The lowest incidence in all groups except one, HA313, is in response to the Heiberg source. The incidences in response to Baldwinsville and Michigan are generally intermediate; and to the Tully source, the highest for four of the six half-sib groups. The lack of statistically significant results in the Chi-square Tests of Homogeneity for all but SL209 probably is a consequence of three factors: (1) the low incidence of this form of resistance, (2) a small variation in response, and (3) the numbers of cankered branches in each half-sib group for each pathogen source. Though these numbers range from 52 to 100, they are not large enough to show a difference among inoculum sources. The numbers for the branch death data are smaller than for callousing because calloused cankers are not considered candidates for branch death. This also accounts for the lowest numbers of cankers for the Heiberg data because that inoculum resulted in the highest callousing frequencies. Another point regarding pathogen variation should be made: if the pathogen variation is real, the heritability estimate based upon the pooled data (table 3) would be meaningless.

Although not as convincing as the callousing data, the branch death results appear to support the hypothesis that this is a polygenic control system with a low heritability. If the trait is controlled by many loci, a small amount of variation in incidence of branch death is expected among the four half-sib families. Sharp differences, as were found in the callousing results, would not be expected. Branch death in resistant trees generally occurs in all families with the exceptions almost entirely in either the HA313 families in response to all four inocula or among the families in all six half-sib groups in response to the Heiberg inoculum. In these cases, however, the incidences of branch death are low and the absence of branch death is probably due to chance variation. The highest frequencies of branch death, such as the SL209 and LE208 in response to the Baldwinsville and Tully inocula, are evenly scattered among the four half-sib families, but they do exhibit small variations. The heritability estimates obtained in this study are probably underestimates of the true values because it is not likely that all branches had died when these observations were made at 4 months. Results from further field observations should resolve this point.

The differences in canker lengths 4 months after inoculation appear to represent true differences among the six groups of maternal half-sibling families (tables 4, 5, and 6). If short canker length can be equated to resistance by the host and long cankers to susceptibility, the HA303 groups appear the most resistant, the OA107 and OA201 groups range from intermediate to resistant, SL209 and LE208 are generally intermediate but tend toward susceptibility and ES107 is the most susceptible. This order of ranking is similar for the pooled data for each maternal half-sib group and for the mean values for each inoculum. There is little doubt that this trait is controlled by a number of genes that exhibit a small amount of additive genetic variance. It exhibits general combining ability, but the experiment gives no information on specific combining ability. The heritability estimates suggest that slow gains could be made in the host's ability to retard spread of the pathogen by a selection program.

The variation in canker length associated with inoculum sources reflects real differences and appears to be a predictable response of the host to each inoculum (table 5). Apparently the Michigan and Tully inocula exhibit greater virulence than the other two pathogen sources. The four inocula rank from most virulent Michigan, Tully next, Baldwinsville third, and Heiberg the least virulent (table 4). The repeatability of their levels of virulence suggests that the differences are genetic. No information is available on the genetic control system.

Again, pathogen virulence is not related to resistance heritability. The Michigan inoculum exhibits the greatest virulence and results in cankers with considerable variation in size, but the heritability estimate for canker length is the smallest. Most of the variation occurs in the males within females sources of variation, with a smaller part due to females. This, combined with the large within families mean square, is the cause of the low heritability value.

As in the case of callous formation and branch death, the largest heritability for canker length ($h^2 = 0.254$) was obtained with the Tully inoculum. The most plausible explantation for the greater predictability probably is the genetic uniformity of this inoculum, which was derived from a single ascospore. The other three inocula, in contrast, were derived from mycelia extracted from diseased bark. Greater variation could occur in each of these cultures because not only could a heterokaryotic condition be present with nuclei of two or more origins, but two or more genetically different mycelia originating from multiple infections are also possible. If the pathogen as well as the host varies, the predictability of the host response would be less and heritability low. If this hypothesis is correct, the heritability estimates from the Tully data would most clearly reflect the host's ability to respond to the pathogen and would be the most reliable estimates of the predictability of the host responses. Further experiments are planned to test this hypothesis. If this is correct, one would expect more rapid improvement in resistance than our results suggest now.

The slow spread of the disease in the host is frequently referred to as a tolerance reaction of the host. If slow enough, the pathogen presumably could persist for years in a rapidly growing tree without killing the host because trees die when their stems are girdled. Selection for tolerant genotypes is feasible in an aspen breeding program, and is highly desirable because this mode of resistance would not exert strong selection pressure on the pathogen for more virulent forms. This might be especially useful in breeding trees for short rotation croppings (Einspahr 1972).

The tolerance reaction depends upon a slow growth of the fungus whereas fast canker growth is required in the branch death response because the branch has to be killed before the fungus can reach the main stem. Therefore, the characters would be expected to be negatively correlated: groups with short cankers should exhibit a low frequency of branch death, and vice versa. A comparison of mean canker length and incidence of branch death in maternal half-sib groups (tables 3 and 4), however, does not reveal any obvious relations. Rank correlations (Snedecor 1946) were also calculated, based upon maternal half-sib group means and the rank of the 24 families. Though negative correlation coefficients were obtained for the Baldwinsville and Tully results for maternal half-sib group means for the Baldwinsville, Tully, and Michigan results for the family data, none of the correlations are statistically significant. If a negative relation does exist, it may have been obscured by the effect of branch diameter (at the site of the inoculation) on the earliness of branch death. This will be taken into consideration in future studies to test this hypothesis.

Although this study considered only three mechanisms of resistance, there are undoubtedly more. Artificial inoculations with a mass of mycelial inoculum precludes genetic evaluation of resistance mechanisms affecting spore germination and initial infection. Future studies will

attempt to determine the genetic control of resistance to natural infections and the correlation of these results with that from artificial inoculations.

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NUCLEAR PROTEINS OF DRY AND GERMINATING CONIFER SEEDS

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ABSTRACT.--The proteins of the nuclear sap, the histones, and the nonhistone chromosomal proteins (NHCP) were extracted from a number of dry and germinating seeds and their composition was examined by polyacrylamide gel electrophoresis. The nuclear fraction was isolated and washed extensively with several buffer mixtures. The chromosomal material was then solubilized in a high salt-high urea buffer. After removal of the DNA by ultracentrifugation, the chromosomal proteins were passed through a QAE-Sephadex column to separate the histones from the NHCP. Gel patterns of the NHCP varied quantitatively during the early germination of jack pine and minor qualitative differences in protein complement were also detected. Differences in the profiles of the NHCP were found among species of the Pinaceae. Histones from coniferous seeds compared favorably with pea histones in classification and electrophoretic mobilities. The changes in histones in profiles from different species and with different stages of germination were due mainly to the heterogeneous FI fraction. The methods are suitable for studies of nuclear protein metabolism and genetic regulation and expression in tree improvement programs. Biochemical techniques for extracting and characterizing nuclear proteins are summarized.

The control of transcription (suppression or induction of selected genes) is now being pursued by many scientists. The structure and function of the nuclear proteins are important in this control, and polyacrylamide gel electrophoresis of proteins can become a useful diagnostic tool when dealing with quantitative and qualitative changes in genetic expression. For example, Bekhor et al. (1974a) suggested that the nonhistone chromosomal proteins (NHCP) could be fingerprinted to study chromosomal aberrations once functions are assigned to each of these proteins.

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In this report we summarize methods to extract and characterize the nuclear proteins and present the known biological functions of proteins, especially those dealing with genetic expression. It is hoped that this study will eventually lead to methods to predict genetic expression (i.e., before it can be expressed visually) and to improve the genetic quality of conifers.

METHODS

Extracting and Characterizing Conifer Nuclear Proteins

To study the nuclear proteins in tissue, species specificity, and gene regulation, the extracted proteins should be pure, intact, and free from aggregation. We selected and modified methods to obtain high yields for extracting nuclear proteins. We avoided the use of denaturants, increased the rapidity of extraction, inhibited degradative enzymes, avoided contamination, and retained as much of the biological activity of these proteins as possible. Because nuclear proteins from storage tissues are especially difficult to purify (Bonner *et al.* 1968a, Grellet and Guitton 1973, Pitel and Durzan 1974), various combinations and extensions of the existing methodology were tried.

Numerous reports indicate the presence of protease in *chromatin preparations* and in the cytoplasm which can degrade histones (Paik and Lee 1970, Kincade and Cole 1966). Selective tissue-specific proteases confuse the estimates of qualitative and quantitative differences in composition (Hnilica 1972). Several protease inhibitors decrease the activity of these enzymes. These include 0.05M sodium bisulfite (Panyim *et al.* 1971), diisopropyl fluorophosphate (Hnilica 1972), $CdSO_4$ (Vaughan and Comings 1973), and phenylmethylsulfonyl fluoride (Wintersberger *et al.* 1973, Towill and Nooden 1973).

With the conifers we routinely used sodium bisulfite as a protease inhibitor. This was found to be especially needed for the germinating seeds. However, sodium bisulfite should be used with caution because base (pyrimidine) modification of chromatin RNA may occur. In some cells it may reduce the yield of nuclei and chromatin (Towill and Nooden 1973). To further decrease the activity of nucleases and proteases the buffers were at pH 8.0 (Jockusch and Walker 1974). The reagents we used were of the highest purity and were made fresh to prevent changes in the buffers that could affect the properties of the extracted proteins. For example, aged urea solutions form cyanate that could then react with amino acid sulfhydryl groups of proteins (Graziano and Huang 1971).

To prevent aggregation of the histone F3 molecules, 2-mercaptoethanol was included in the buffer during electrophoresis. Mercaptoethanol is also useful in reducing the binding of the histones to the NHCP (Levy *et al.* 1972).

EDTA, which can lead to losses of the arginine-rich histones (Stein and Borun 1972), was not needed nor used.

Chemicals that could irreversibly denature the proteins were avoided. Acid treatment to extract the histones from nuclei or chromatin before NHCP separation affects the structural properties of the NHCP (Graziano and Huang 1971). Phenol has been used to extract the nonhistone proteins (Teng et al. 1971); however, this reagent can irreversibly affect the native structure in some of the NHCP. Studies by Mischke and Ward (1975) showed that acid treatment or phenol changed the position of some of the NHCP during electrophoresis. Ionic detergents bind strongly to proteins and thus are not useful except for electrophoretic studies (Shirey and Huang 1969).

To help the dispersal of extra-nuclear material, without damaging the nuclei, Triton X-100 (nonionic detergent) was added at low concentrations (0.2 to 0.5 percent) (Sadowski and Steiner 1968, Panyim et al. 1971). Higher concentrations break nuclei and may cause partial solubilization and loss of nuclear proteins (D'Allesio and Trim 1968). Lower concentrations remove the outer nuclear membrane.

We used sodium deoxychloate (bile-salt anion) to remove cytoplasmic contaminants. It has detergentlike properties and solubilizes many subcellular components (Hadler et al. 1971) including the outer nuclear membrane (Monahan and Hall 1973). Low concentrations of about 0.001 M are needed because concentrations in the range 0.005 to 0.1 M can selectively extract the histones (Smart and Bonner 1971a, 1971b) and higher concentrations can cause partial lysis of nuclei.

The nuclear preparation should be washed with buffered media to remove contaminating proteins originating from the nuclear sap and cytoplasmic proteins that become adsorbed during isolation to the nuclei and chromatin. Generally, washing is done with buffered isotonic (0.14M) saline and with varying concentrations of Tris-HCl buffers. Jockusch and Walker (1974) found that increasing the ionic strength of the buffer from 0.001M to 0.01M Tris increased the amount of NHCP extracted. Huang and Huang (1969) decreased the ionic strength at pH 8.0 during purification of their nuclear preparation. Stronger saline (0.3M) improved the removal of cytoplasmic contaminants but losses of NHCP can still occur (Hnilica 1972). At saline concentrations of 0.35M, some NHCP are extracted together with cytoplasmic proteins (Comings and Tack 1973). Kostraba et al. (1975) found that the 0.35M NaCl wash contained proteins similar to the cytoplasmic and nuclear sap proteins. This fraction included loosely bound NHCP that, after four washings, contained 43 percent of the total NHCP. Goodwin and Johns (1973) reported a possibility of some histone degradation products in the 0.35M NaCl extract. At higher salt concentrations more NHCP is removed; e.g., at 2.0M NaCl, 81 percent is extracted.

We also used, with varying degress of success, extractions at low pH for the removal of cytoplasmic-contaminating proteins. Dick (1968) used pH 2.8 for purification of the histone fraction. We also included citric acid at 0.01M (pH 2.6) and aqueous ethanol.

The ratio of the nucleus to the cytoplasm is important. If this ratio is as low as 1:500 by volume there are greater chances of contamination (Evans and Ozaki 1973). Most methods use dense sucrose for purification of the chromatin preparations. The chromatin can be centrifuged through 1.7M homogeneous sucrose or through discontinuous sucrose (Bonner et al. 1968a, 1968b; Panyim et al. 1970). In our studies with conifer seed tissue, we repeated this process to ensure greater removal of the contaminants. In addition to sucrose purification, prolonged stirring of the crude chromatin in Tris-CHl buffers solubilizes some contaminating material (Bonner et al. 1968a).

To avoid denaturing conditions for the extraction of the chromatin proteins, we separated them by dissociating the nuclear preparation in a high salt-high urea buffer. Essentially all chromatin proteins can be separated from DNA by dissociation in a 2.0M NaCl-5.0M urea buffer (Bekhor et al. 1974a, 1974b; Kleinman and Huang 1972). DNA can be removed by centrifugation for 18 hr at 60,000 RPM, by column chromatography (such as Bio-Gel A-50 as described by Graziano and Huang 1971), or if desired, by denaturing conditions such as selective hydrolysis, by partitioning with phenol (Shelton and Neelin 1971), or with DNase. In our studies we used ultracentrifugation to remove the DNA followed by separation of the histones from the NHCP with QAE-Sephadex. The use of ion-exchange to separate these two classes of proteins has resulted in preparations with less than 1 percent cross-contamination between the two (Levy et al. 1972). One can also use columns of Bio-Rex 70 (Levy et al. 1972), SP-Sephadex C-25 (Graziano and Huang 1971), hydroxylapatite in high salt and urea (MacGillivray and Rickwood 1974), and electrophoresis on SDS (sodium dodecyl sulfate). Our studies with SDS electrophoresis showed negligible contamination of the histones and NHCP with each other. If needed, 2 percent SDS can be used to solubilize the chromatin components.

Numerous other combinations can be used for solubilization such as 6M Urea-0.4M GuCl (Levy et al. 1972), urea alone (Mischke and Ward 1975, Pitel and Durzan 1974), 1M$CaCl_2$ (Mohberg and Rusch 1969), and NaCl alone (Busch 1968, Wang 1967). However, several reports have shown that not all NHCP are removed with 2.0M NaCl (Levy et al. 1972, Kostraba et al. 1975). In the latter study, 19 percent of the NHCP could not be removed from DNA with 2.0M NaCl (buffered). Most of these proteins were released with 3M NaCl-7M urea. A method using cations to condense the chromatin was found better than the use of 2M NaCl (Flavell and Kemble 1974). The latter had more contaminating RNA. Methods using shearing of the chromatin help in further dissociation (Levy et al. 1972).

Numerous methods selectively extract the histones from nuclear preparations. Variations occur in pH (Hnilica 1972); sodium deoxycholate (Smart and Bonner 1971a); SDS; mixtures of urea, NaCl, and ethanol (Bolund and Johns 1973); various salt concentrations (Hnilica 1972, Bolund and Johns 1973); removal of F1 with 5 percent TCA or perchloric acid, ethanolic-HCl; tRNA and double-stranded DNA (Ilyin et al. 1972), and others. The use of urea in combination with NaCl appears to be a superior method for both dissolution of the chromosomal proteins and for retention of their biological functions.

Nuclear proteins from storage tissues are hard to purify. Fambrough et al. (1968) found significant amounts of contaminants in histone preparation from pea cotyledons. These had low electrophoretic mobility and were said to be acidic ribosomal proteins and NHCP. Histones can be contaminated by proteins from the mitochondria and nucleoli (Mohberg and Rusch 1970). Histones prepared by acid or salt dissociation may have small quantities of ribo- and deoxyribonucleotides (Greenaway and Murray 1973). Direct acid extraction of the nuclei or chromatin to obtain the histones may result in some contamination by the NHCP (Sadgopal and Bonner 1970a, 1970b; Wilhelm et al. 1971). High molecular weight NHCP have been reported to contaminate certain histone preparations (Levy et al. 1972, Graziano and Huang 1971).

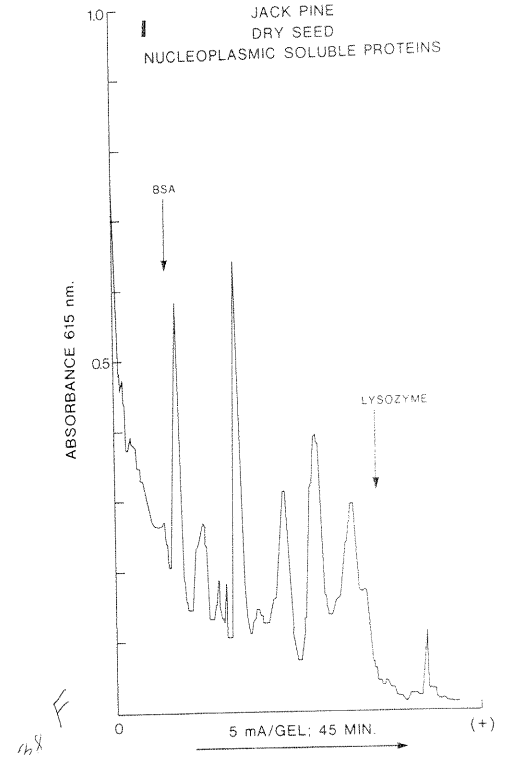
The extracted NHCP fraction can be contaminated from several sources. For example, Goodwin and Johns (1972; 1973) and Johns and Forrester (1969) found contamination of the NHCP by the nonspecific adsorption of cytoplasmic and nuclear sap proteins to the chromatin during isolation. Hill et al. (1971) said that the NHCP may be in equilibrium with the soluble nuclear sap proteins under physiological conditions, e.g., NHCP turnover. It was difficult to determine precisely what belonged to the NHCP or to the nuclear sap. The NHCP were also found to be contaminated by the acidic nuclear membrane proteins (Harlow et al. 1972) to the extent of 11 percent (Suria and Liew 1974).

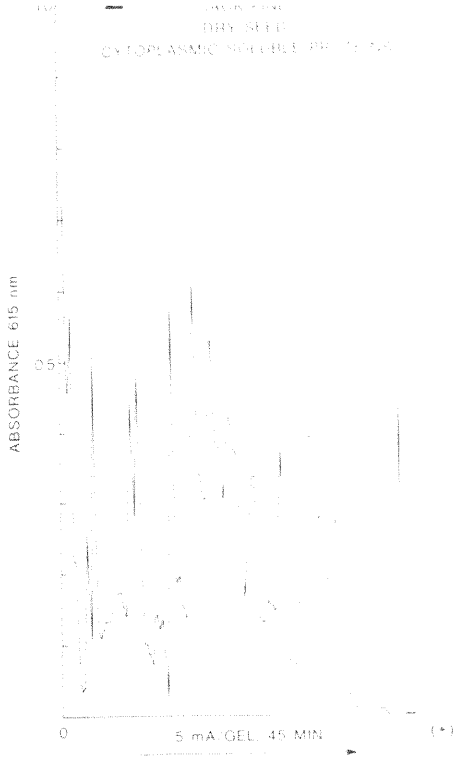
By considering these observations and introducing the modifications for coniferous tissues we have prepared the nuclear and cytoplasmic proteins in figures 1 to 4.

RESULTS AND DISCUSSION

Our results show that the nuclear proteins (histones, NHCP, and nuclear sap proteins) can be extracted from conifers and characterized by polyacrylamide gel electrophoresis. The purity and resolution of these proteins are comparable to those described for other plants and animals.

Problems of identification of proteins often arise by contamination, degradation, selective extraction, and aggregation. Some of these proteins may be in equilibrium with each other (e.g., NHCP and nuclear sap proteins) and thus may be hard to define precisely as belonging to one class or





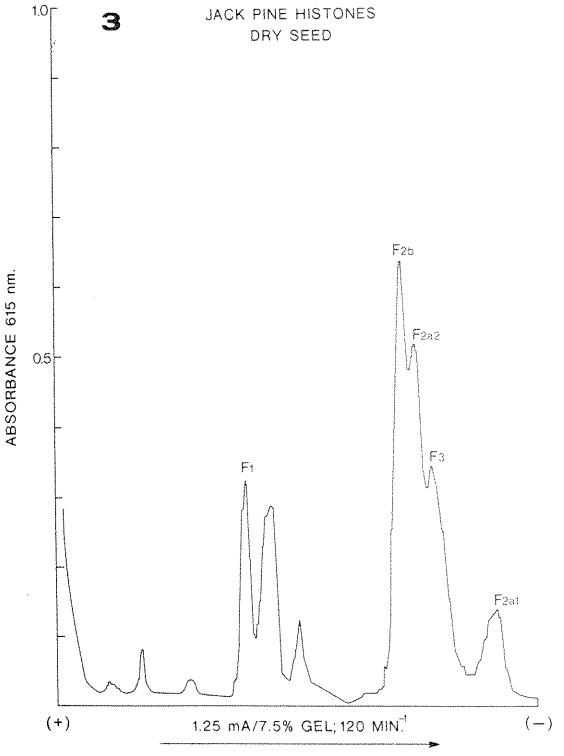


Fig. 1-4. Protein characterization of dry jack pine seed.

another. Also some can be transported between the cytoplasm and nucleus (Stein and Borun 1972). In our studies we examined the proteins of the cytoplasm, nucleoplasm, and the chromosomes and first compared them with each other to more precisely show that most belonged to separate classes and can be separated as such.

The cytoplasmic and nucleoplasmic soluble proteins had characteristic electrophoretic profiles that were significantly different from each other (figures 1 and 2). Differences in composition were found during germination (dry seed vs. 48 hr) for some of the bands for each of the classes. Studies with the cytoplasmic proteins, besides being useful to determine contamination with the different classes of proteins, are important because some of them may be gene regulators (Vaughan and Comings 1973), and may be able to bind to DNA (Choe and Rose 1974).

With jack pine we found that the soluble nuclear proteins were markedly different from those of the corresponding chromatin NHCP, although the two patterns showed some similar bands (figures 1 and 4). Similar results were found by others (Comings and Tack 1973, MacGillivray and Rickwood 1974). Also, the nuclear sap proteins can bind to DNA (Vaughan and Comings 1973) and may have some gene regulatory function (Comings and Tack 1973).

Histones were extracted from various stages of germination of jack pine and found to vary quantitatively after 24 hours of germination (fig. 3). The profiles for the dry seed and for 12 hrs imbibed seed were identical. DNA synthesis is difficult to detect during early stages of germination (Pitel and Durzan 1975). Because histone synthesis is associated with DNA synthesis (Hnilica 1972), the lack of any major change in the histone profile is consistent with previous results. Changes in the histone fraction after 24 hrs imbibition were due mainly to the very lysine-rich (F1) fraction. The protease inhibitor, sodium bisulfite, was especially useful for the germinated seeds. Extraction of 4-day-old seeds in the absence of sodium bisulfite gave a 50 percent reduction in the content of the F1 histone and produced several additional bands in the F1 region and gave one band with a mobility between F3 and F2a1.

Examination of the histones from several members of the Pinaceae revealed very little species specificity.

After passage of the conifer chromatin, soluble in 3M NaCl-5M urea, through a QAE-Sephadex column, the histone and NHCP fractions were analyzed by SDS electrophoresis and found to be essentially free of contamination from each other. By contrast, direct acid extraction of the nuclear preparation for histones gave numerous contaminating bands that could include proteins of the ribosomes (Mohberg and Rusch 1970, Fambrough *et al.* 1968) and NHCP (Graziano and Huang 1971, Grellet and Guitton 1973).

The NHCP were extracted using different concentrations of NaCl, various combinations of urea and NaCl, and urea alone. Profiles from all cases were largely similar on a qualitative basis. Most differences were found with the 0.14M NaCl. This extraction only removes 10 to 15 percent NHCP and is used mainly for removing nuclear sap proteins from the nuclei. Although this extraction contains the normal population of NHCP it also has proteins that resemble those from the nucleoplasm.

We compared the NHCP profile from dry jack pine seeds, where the chromatin is repressed, to that of the 48 hrs imbibed seed where gene activity was substantially greater. The major bands varied quantitatively. Unique bands were found with the minor proteins in germinated seeds. Although Coomassie Brilliant Blue was used for some studies it was not used to compare quantitative differences as the dye deviates from Beer's Law at higher protein concentrations (Chrambach *et al*. 1967). Caution must also be used with Amido Black, as it stains metachromatically (Johns 1967).

Limited species specificity was found when comparing the NHCP profiles of several *Pinus* spp. More qualitative and quantitative differences were found when comparing the *Pinus* spp. with those from *Abies* or *Picea*. The further interpretation of these results from a genetic and physiological viewpoint is in progress.

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THE POTENTIAL FOR CLONING WHITE SPRUCE VIA TISSUE CULTURE

Robert A. Campbell and Donald J. Durzan[1]

ABSTRACT.-- When hypocotyl segments of white spruce were placed with their apical ends in an agar medium containing 10^{-5} M 1-naphthaleneacetic acid (NAA) but no 6-benzylaminopurine (BAP), 50 percent formed roots. Almost all segments placed with their basal end in a medium containing 10^{-5} M BAP with 10^{-7} M NAA formed scalelike organs. When explants with the scalelike organs were transferred to media containing neither BAP nor NAA, the organs grew into needles, buds developed, and elongated, branched shoots were obtained from these buds. A number of shoots have been obtained from a single hypocotyl segment. One such shoot has rooted. These results strengthen the hypothesis that a small explant could be used to mass propagate a superior tree.

There will probably be a massive shortage of wood in the world market within 20-25 years (Keays 1974)[2,3] For this reason there is an urgent need to increase the productivity of our forests.

One means of doing this is by reforesting with superior genotypes that grow faster, larger, straighter, are responsive to silvicultural practices, and are resistant to diseases and pests. The question is, "How to get the superior genotypic material for planting?"

One way is through breeding, as has been done successfully for many agricultural crops. Unfortunately, the long life cycle of trees makes obtaining superior varieties a lengthy process. In Ontario, 30 million white spruce seedlings are outplanted each year but only 10 percent are from seed collected in seed orchards and production areas. Because the

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[2] Hair, D. 1975. Address to the Canadian Pulp and Paper Association, Montreal. March 25.

[3] Jones, P. 1975. Address to the Canadian Pulp and Paper Association, Montreal. March 25.

trees in the present orchards are not the progeny of controlled breeding programs and have not been tested, the genetic gain in using seed from them is probably small. Thus it will be some time before genetically improved and proven trees are available in sufficient numbers and are mature enough to produce useful quantities of improved seed.

Another way to reforest with superior genotypes is to take advantage of the variation in the natural population and vegetatively propagate superior specimens. This could allow immediate productivity gains. The problem here is that by the time a tree is old enough to demonstrate that it is superior, it is difficult to propagate it vegetatively by rooting cuttings. As the age of the ortet of spruce increases beyond 10 years, rooting ability decreases rapidly. "Percent rooting, speed of rooting, root length and number, survival, and growth in and after the year of rooting, all decrease with increasing age of the parent tree" (Girouard 1974). Another problem is that rooted lateral shoots must undergo a transition from plagiotropic to orthotropic growth and this occurs less readily with increasing ortet age (Girouard 1974). A final problem is that cuttings may grow slower than seedlings. For the first 5 years at least, the stem volume growth of *Pinus radiata* cuttings was considerably less than that of seedlings (Sweet and Wells 1974, Shelbourne and Thulin 1974, Libby 1974). If these differences continue beyond 5 years, they are probably associated with meristem aging. This could cancel out gains expected from the use of cuttings rather than seedlings from superior genotypes.

In an attempt to bypass some of the problems mentioned above, we have been investigating the use of tissue culture for the vegetative propagation of forest tree species.

Having obtained suitable media for the continuous culture of callus and suspensions of white spruce, jack pine, and American elm, we have recently begun attempts to induce differentiation in our cultures. The present work utilizes aseptic hypocotyl segments from 6 to 12-day-old white spruce seedlings (fig. 1). This is a model system because practical application would require the use of tissue from trees old enough to have demonstrated superior qualities. But the model system is useful at this stage for several reasons: (1) it is easier to obtain large numbers of sterile explants by surface-sterilizing seeds and then germinating them aseptically than it is to sterilize tissue from trees in the field; (2) there are no seasonal variations in the starting material because seeds can be germinated in a growth chamber any time; and (3) tissue from juvenile plants is often easier to induce to differentiate, so it makes more sense to start here and proceed to the more difficult later.

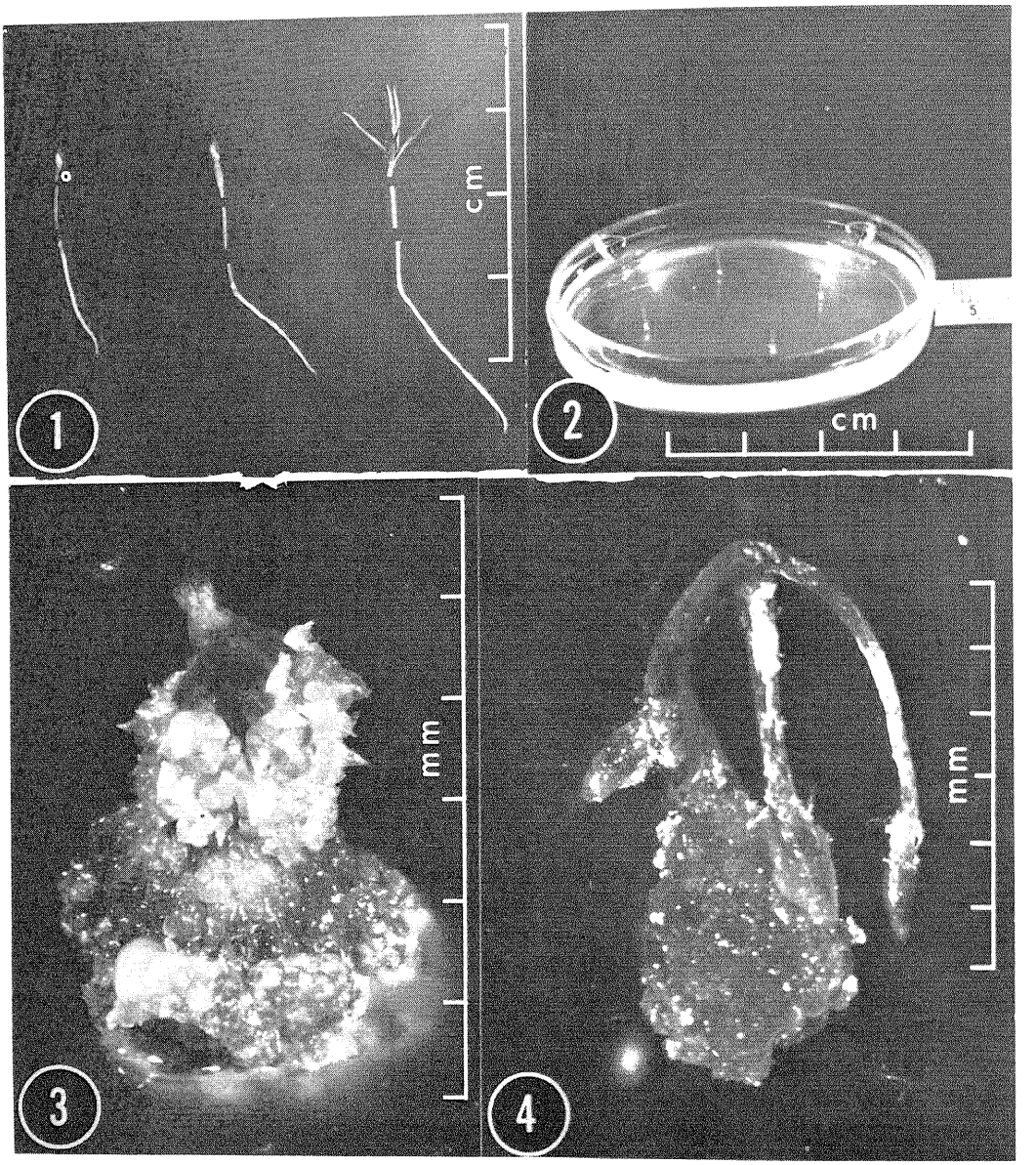

Figure 1.--Six to 12-day-old white spruce seedlings. A 5 to 7 mm segment of hypocotyl has been excised from each seedling taking care to exclude the apical meristem.

Figure 2.--Hypocotyl segments with one end embedded in agar medium in culture dish.

Figure 3.--Hypocotyl segment after incubation on a medium containing a high level of BAP and low NAA. There is a basal callused region in contact with the agar medium, a swollen intermediate region covered with scalelike organs, and an unswollen tip. There has been little longitudinal growth.

Figure 4.--Hypocotyl segment after incubation on a medium containing a low level of BAP and a high level of NAA. There is a basal callused region and above this a portion of unchanged hypocotyl bearing three roots. There has been considerable elongation of the explant (cf figure 3).

METHODS AND RESULTS

Detailed methods and media have been reported elsewhere (Campbell and Durzan 1975). Hypocotyl segments used were 5 to 7 mm long and were cut carefully to exclude the apical meristem (fig. 1). The segments were cultured in an agar medium in 50-mm petri dishes in a vertical position with the end formerly attached to the radicle embedded in the medium (fig. 2). The segments differentiated by expanding laterally but very little vertically (fig. 3). There is some callusing at the base, i.e., the portion in contact with the medium. Above this there is a portion that is swollen and has scalelike outgrowths and at the top there is an unswollen portion. The fact that the scalelike outgrowths are below the tip, together with the cutting procedure, demonstrates clearly that the outgrowths did not arise from a pre-existing apical meristem.

The key factor controlling this differentiation is the cytokinin-auxin balance (table 1). In this experiment, segments were cultured on media containing different amounts of the plant growth regulators 1-naphthaleneacetic acid (NAA) and 6-benzylaminopurine (BAP). The frequency of induction increased with increasing BAP and decreasing NAA concentration.

Table 1.--Explants that had produced at least one scalelike organ after 20 weeks in culture under varying conditions,[1] as a function of 1-naphthaleneacetic acid (NAA) and 6-benzylaminopurine (BAP) concentration in the medium[2]

(In percent)

BAP (M)	NAA (M) 10^{-5}	10^{-7}	0
10^{-5}	14	64	69
10^{-7}	0	0	0
0	0	0	0

1 13.5 hours light at 20°C and 10.5 hours of dark at 7°C.

2 The number of segments per treatment ranged from 11 to 17 (differences due to contamination losses).

The active range and different environmental conditions were examined in more detail in a further experiment (table 2). Induction occurred better and faster under constant than under varying conditions but by

98 days the number of explants that had produced organs had reached a maximum in both environmental conditions. The explants cultured under varying conditions were inhibited by the highest level of BAP whereas those under constant conditions could not be induced to develop further with subsequent treatments.

Table 2.--Explants that had produced at least one scalelike organ after 14 weeks in culture, as a function of NAA and BAP concentration in the medium[1]

(In percent)

	NAA (M)			
BAP (M)	10^{-5}	10^{-6}	10^{-7}	0
10^{-4}	25(13)[2]	78(38)	82(28)	89(19)
10^{-5}		75(50)	84(56)	86(58)
10^{-6}		45(22)	59(50)	53(38)

[1] The number of segments per treatment ranged from 20 to 36

[2] The figures in parenthesis were obtained under varying conditions (13.5 hours light at 20C and 10.5 hours dark at 7C). The figures not in parenthesis were obtained under constant light and temperature at 20C.

Those induced by lower levels of BAP did develop. Thus, the optimum induction occurs under constant environmental conditions with 10^{-5} M BAP and 10^{-7} M or 0M NAA.

In the previous experiments, the explants were placed right side up. When they were inverted (i.e., the end formerly attached to the apical meristem was placed in the medium) organs were again induced at about the same frequency with the same BAP-NAA optima. The difference was that roots could also be induced (fig. 4, table 3). The requirement for root induction was high NAA and low BAP. This is opposite to that for induction of the scalelike organs and thus follows the pattern of differentiation control reported by Skoog and Miller (1957).

Having induced roots and scalelike organs, attempts were made to control further development. The roots did not develop much beyond that shown in figure 4 and eventually died, although a number of different media were tried. The scales did develop if the explants were left on the same media for a period or were transferred to a medium containing neither BAP nor NAA (this was probably the same treatment because the

growth regulators likely got depleted in the first instance). The scales became elongated and more needlelike (fig. 5). When explants at this stage were broken up and cultured for a further period on medium lacking NAA and BAP, numerous elongated shoots bearing normal-looking needles developed (fig. 6).

Table 3.--Explants that had produced at least one root after 12 weeks in culture, as a function of NAA and BAP concentration in the medium[1]

(In percent)

	NAA (M)		
BAP (M)	10^{-5}	10^{-7}	0
10^{-5}	15(19)[2]	0(0)	0(0)
10^{-7}	28(28)	0(0)	0(0)
0	47(25)	0(0)	0(0)

[1] The number of segments per treatment ranged from 25 to 36.

[2] The figures in parenthesis were obtained under varying conditions (13.5 hours light at 20C and 10.5 hours dark at 7C). The figures not in parenthesis were obtained under constant light and temperature at 20C.

All of the previous steps have been repeated and occur with a high frequency. The final step, the rooting of induced shoots to regenerate a whole plant, has only occurred recently so its frequency, control, and repeatability have not been fully determined. The shoot in figure 7 produced roots following shoot elongation on the medium lacking BAP and NAA. It has been transferred to a sand-sphagnum mixture in a pot and is thriving.

DISCUSSION

We have demonstrated that it is possible to regenerate whole white spruce plants from a small piece of tissue. Similar results have also been recently reported for longleaf pine (Sommer *et al.* 1975), *Cryptomeria japonica* (Isikawa 1974), and Douglas fir (Rediske 1975). Once the process has been optimized, the next step would be to attempt to repeat it with tissue from a mature tree. Because the shoots in the present work apparently arose from epidermal tissue (Campbell and Durzan 1975), a

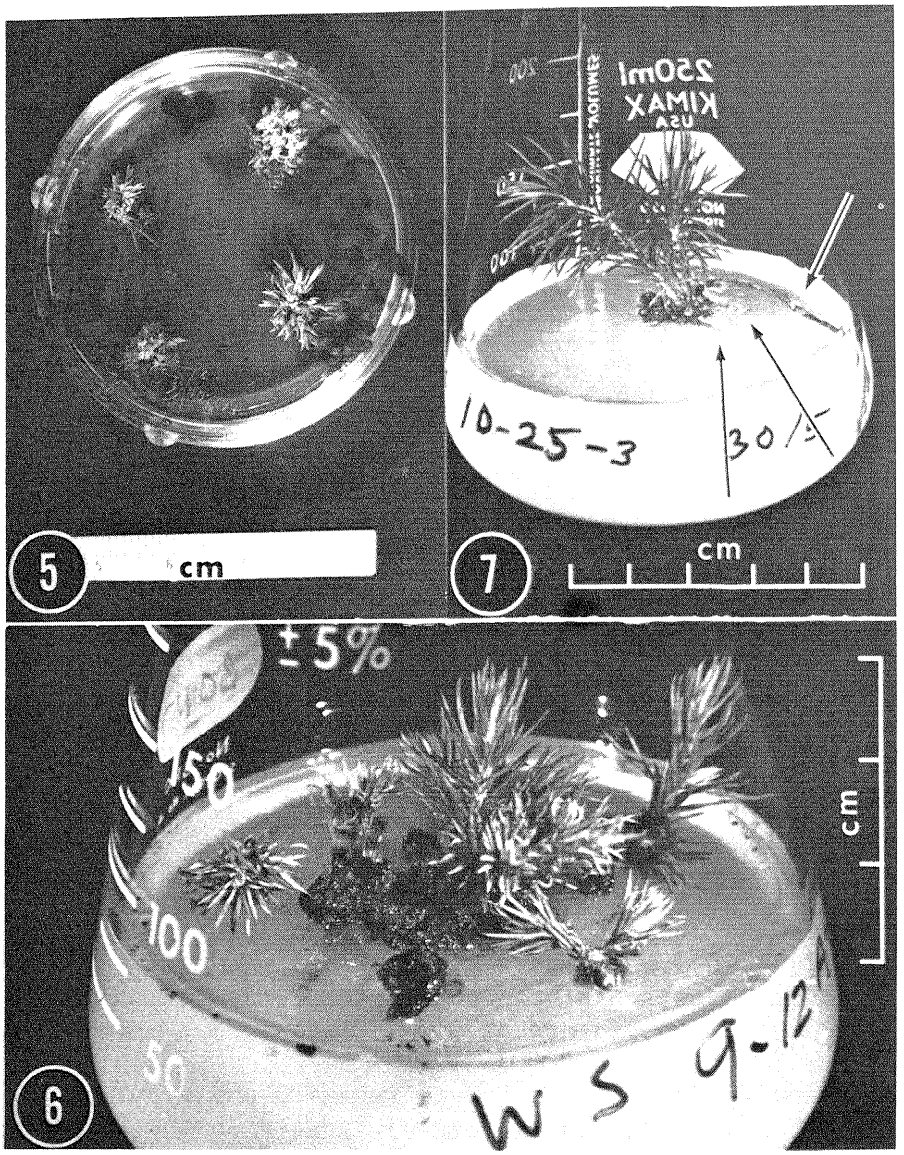

Figure 5.--Hypocotyl segments after a prolonged incubation on a medium containing high BAP and low NAA. Depletion of the growth regulators in the medium has allowed the scalelike outgrowths to elongate.

Figure 6.--A number of shoots obtained by dividing up an explant, such as those in figure 5, and incubating the pieces on a medium lacking BAP and NAA.

Figure 7.--An explant bearing two induced shoots that have rooted (arrows).

logical tissue to try would be needles. Further justification for this choice comes from the fact that leaf epidermal tissue from mature herbaceous plants has been readily induced to form shoots (Tran Than Van and Drira 1971, Chlyah and Tran Than Van 1971). If each needle could produce 10 shoots and a single bud has 100 to 200 needles, the number of potential propagules from a single tree would be enormous. It should also be noted that because the plantlets induced from tissue cultures do not arise from pre-existing apical meristems, their apical meristems may be rejuvenated. Slow growth, plagiotropic growth, and poor rooting may not be a problem with such vegetative propagules.

A slightly different way of using tissue culture in propagation would be through suspension cultures. Tissue from many plant species can be grown in an agitated liquid medium as single cells and small clumps--much like bacteria. We have done this in our laboratory with white spruce, jack pine, and American elm. The doubling time of such cultures is less than a week, so a tremendous number of cells and clumps can be generated in a short time. For example, starting with 1 gram of tissue, 1 kilogram could be generated in 10 weeks and 1,000 kilograms in 20 weeks. With a number of plant species it has been possible to induce the free cells and clumps to form embryos that develop into normal plants capable of producing viable seed (Steward et al. 1964). The best studied example is carrot. If white spruce embryos could be induced in suspensions at the same rate as carrot embryos (Halperin 1967), then in only 100 liters of medium, 73 million plantlets could be produced and raised in containers for field planting. This is more than enough to plant 100,000 acres (the predicted rate of white spruce planting in Canada to 1985 at 8 by 8 spacing (Carlisle and Teich 1971).

Is this possible? We can grow spruce tissue as suspensions. A number of herbaceous species have been induced to form embryos in suspensions (Halperin 1967, Thomas and Street 1970). White spruce tissue cultures have been induced to regenerate whole plants. Cambia and vascular tissue have been induced in white spruce suspensions (Durzan et al. 1973). Thus, the probability is high that it is only a matter of time and effort to achieve effective vegetative propagation of spruce by tissue culture.

The predicted world wood shortage by the year 2000 makes it seem mandatory that all methods of increasing forest productivity be fully explored immediately. Through tree improvement and genetics, Canada will be able to increase the productivity of its forests and thus fulfill its responsibility to help minimize the predicted world wood shortage.

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THE CLONAL TEST: AN AID TO PROGENY TESTING AND A WAY TO SPEED UP GENETIC GAINS

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ABSTRACT.--Phenotypic measurements of loblolly pine (*Pinus taeda* L.) and Virginia pine (*Pinus virginiana* Mill.) were obtained from parent trees in wild stands, from ramets in clonal orchards, and from seedlings in control-pollinated progeny tests. Total tree height, stem diameter, crown form, bole straightness, and wood density were the characteristics assessed. The degree of resemblance between ortets and ramets, ramets and progeny, and parent trees and progeny was determined by estimating variances and heritabilities. Broad- and narrow-sense heritabilities were estimated from clonal and progeny populations. Ortet-clone, ortet-seedling, and clone-seedling regression and correlation analyses were used to measure the likeness between parent and progeny. These analyses revealed greater similarities between progeny and ramets than between progeny and mature parent trees in the forest indicating the possibility of improving the efficiency of selection through clonal testing. Calculations of expected genetic gains confirmed the importance of roguing inferior clones from the seed orchards as a step toward maximizing gain in a single generation. Clonal tests that can be converted into seed production orchards are recommended as aids to the more standard and expensive progeny tests and to speed up genetic gains both for Virginia and loblolly pines.

In Canada 300 million seedlings are produced annually in forest nurseries. With such a large program, even small genetic improvements in the planting stock will result in appreciable returns at time of harvest.

Selection of elite trees is the basis of a forest tree breeding program and much effort has been expended on this activity during the

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past 15 years. Tree breeders now recognize a large amount of variation in growth, form, and wood characteristics within most tree species. How much of this variation is caused by the genetic make-up of the tree and how much represents a response to the environment is a function of the degree of inheritance; this is usually expressed through the concept of heritability.

The demand for genetically improved trees is great, but the time and costs involved in setting up a tree improvement program to produce large quantities of genetically improved seed are important. The task involves tree selection, seed orchard establishment and maintenance, progeny testing, plus many more activities such as disease and insect control. In order to progeny test selected clones to determine their breeding value, a "tester" system is often adopted. However, despite the fact that clonal orchards produce seed early, it is difficult to obtain sufficient seeds of the crosses needed. The result is that after 5 or more years of effort, seeds of only a portion of the planned crosses may be available for the test. Because any delay in an applied program costs money, evaluations of the general combining ability of the clones must be based on an insufficient number of crosses, or tests must be delayed until all crosses are available. Therefore, a method that could reduce the costly procedure of progeny testing would be of great value to tree improvement programs.

Faster testing may be possible through the use of the resemblance between ortets[2/] and ramets,[3/] between ortets and seedling progeny or between ramets and progeny. A greater similarity between clones[4/] and seedling progeny than between ortets and seedling progeny would suggest the possibility of selection, using a clonal test in a clonal seed orchard or a clone bank; this would be more effective than selection based upon the parent tree in the forest. Clonal testing would be of value for preselection of suitable material for use in the breeding program and could reduce the cost of the very expensive progeny tests.

This paper reports the results of an investigation of the resemblances between ortets and grafts, grafts and seedling progeny, and between ortets and progeny of loblolly pine (*Pinus taeda* L.) and Virginia pine (*Pinus virginiana* Mill.) and suggests a technique to speed up genetic gains.

2/ The original plant from which a clone has been derived.
3/ An individual member of a clone.
4/ A group of genetically identical plants derived asexually from a single individual.

MATERIAL AND METHODS

Plant Material

Three distinct groups of genetically related material were used in the study: parent trees, grafts of the parents, and control-pollinated progeny. The loblolly pine population consisted of 20 mature trees growing in wild forest stands, grafted clones of first and second propagation, and 57 control-pollinated families of the clones. Loblolly pine ortet ages were between 29 and 75 years and averaged 52 years.

The Virginia pine population was composed of 21 parents, their 10-year-old grafts, and 105 progeny families obtained from the diallel mating[5/] of the clones. The complete failure of certain crosses or an insufficient number of seeds produced resulted in the reduced number of families available. In addition, the selfs were excluded from the study. The Virginia pine ortets averaged 23 years old, their age varied between 17 and 36 years. For both species progenies were 4 years old at the time of measurement.

Vigorous scions were collected in the lower portion of the ortet crowns and pot grafted on 2-year-old rootstocks. The first propagation clones were 9-years old at the time of the second propagation.

Measurements

Measurements of stem diameter were taken to the nearest 0.1 inch at breast height on the ortets, at 2.5 feet above the scion-rootstock union on grafts, and at 2.5 feet above the root collar on the seedlings.

Total heights of both parents and grafts were determined to the nearest foot using a Blumes-Leiss altimeter. The progeny heights were measured with a graded height pole.

Crown form and stem straightness were subjectively evaluated using a scoring system based on crown radius, crown density, uniformity, dominance of the main leader, and branch characteristics such as branch diameter and length, branch angle, and sinuosity. Trees with the best crown scored 6 and trees with the poorest scored 1. Subjective stem grading was similar using a 1 to 6 scale depending on the trees' stem straightness. In all instances measurements were taken on 10 ramets per clone and on a maximum of 60 seedlings per full-sib family.

Unextracted wood specific gravity was determined for wood samples obtained at breast height from each ortet using a 3/8-inch increment borer. In the orchards, three grafts per clone were chosen at random

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5/ Mating design resulting from the crossing of the parents in all possible combinations.

and a wood sample obtained at 2.5 feet above the graft union, also using the increment borer. In the progeny test, 2 trees per plot, for a maximum of 20 per full-sib family, were cut and a disk 2 inches thick was taken at 2.5 feet above the ground level.

Statistical Procedures

A general least squares analysis for a diallel experiment was done on the progeny population (Schaffer and Usanis 1969). A nested analysis of variance assuming a random model was used to separate the genetic and environmental sources of variation from the clonal population.

Narrow-sense heritabilities were estimated using the formula appropriate to the diallel mating:

$$h^2 = \frac{4\sigma^2_{gca}}{\sigma^2_{gca} + \sigma^2_{sca} + \sigma^2_b + \sigma^2_\omega}$$

where h^2 = narrow sense heritability,

σ^2_{gca} = variance due to difference in general combining ability,

σ^2_{sca} = variance due to difference in specific combining ability,

σ^2_b = plot to plot variance, and

σ^2_ω = within plot variance.

Broad-sense heritability was calculated as the ratio of the among clone variance to the total variance and may be presented as follows:

$$H = \frac{\sigma^2_c}{\sigma^2_c + \sigma^2_r}$$

where: H = broad sense heritability,

σ^2_c = among clone variance, and

σ^2_r = within clone variance.

Heritability estimates have also been calculated from the regression of progeny on ortet and on the grafted clone. The appropriate formula is:

$$b = \frac{\text{Cov (O-P)}}{\sigma_p^2} = \tfrac{1}{2} h^2$$

where: b = regression coefficient of progeny on parent,

Cov (O-P) = covariance of progeny mean on one parent,

σ_p^2 = phenotypic variance of parent, and

h^2 = narrow-sense heritability.

To assess the reliability of the heritability estimates, standard errors of the variance components were calculated using the equation given by Anderson and Bancroft (1952) and by Falconer (1960) (Corriveau 1974).

RESULTS

Nested analyses of variance of clonal populations revealed highly significant genetic differences among clones for all characteristics studied. Results obtained from first and second propagation grafts of loblolly pine indicated that cloning effects[6/] (topophytic and cyclophytic effects) biased estimates of total genetic variance upward for growth but had little effect on variances in the cases of stem straightness and crown form (table 1). Differences among ramets of the same clones are less than among clones because they share the same genotype as well as a common nongenetic component caused by physiological and morphological properties of the mother tree. When scions or cuttings are taken from ramets adapted to different micro-environments, this common nongenetic component may be partially or totally lost. Two-stage cloning has been proposed by Libby and Jund (1962) to reduce cloning effects. Virginia pine clone height and diameter were negatively correlated with ortet age ($r = -0.62^{**}$ and -0.56^{**}, respectively), reflecting strong cyclophytic effects. Environmental components were found to be less affected and broad-sense heritability values were less biased due to the presence of the confounded cloning component in both the numerator and denominator of the variance ratios.

Mean clone repeatabilities were moderate to strong. Due to large environmental variances, broad-sense heritabilities were smaller than were the repeatabilities (table 2).

6/ Combined effects of the scion position within the crown and of ortet age on the vegetative and reproductive development of the ramet.

Table 1.--Genotypic and environmental variance estimates of loblolly and Virginia pine height, diameter, stem straightness, and crown form from grafted clones 1/

Characteristic	Among clones (σ_c^2) 2/			Within clones (σ_r^2) 3/		
	Loblolly		Virginia	Loblolly		Virginia
	First propagation	Second propagation	First propagation	First propagation	Second propagation	First propagation
Height	4.631±1.772	1.108±.653	4.782±2.263	9.638	9.333	26.705
Diameter	.795± .324	.494±.486	.091± .470	2.263	10.348	.649
Crown form	.426± .173	.496±.190	.376± .147	1.209	1.040	1.022
Straightness	.135± .038	.225±.102	.446± .165	.660	.954	1.010

1/ Variances are based on 10 ramets per clone.

2/ σ_c^2 = Among clone variance = total genetic variance plus a component due to cloning effects.

3/ σ_r^2 = Within clone variance = environmental variance.

Table 2.--Comparison of heritability estimates by variance components, regression, and correlation analysis

Characteristic	Clonal population 1/		Progeny population 2/		Regression 3/		Correlation 4/	
	R_r	R_c	Family	Individual	Progeny-ortet	Progeny-clone	Ortet-progeny	Clone-progeny
LOBLOLLY PINE								
Height	0.32	0.83	0.15	0.05	0.01	0.08	0.01	0.04
Diameter	.26	.78	--	--	--	--	--	--
Crown form	.26	.78	.23	.07	.02	.16	.01	.14
Straightness	.17	.67	.37	.13	.08	.25	.04	.04
VIRGINIA PINE								
M.A.H.B. 5/	0.15	0.64	0.33	0.13	0.12	0.22	0.14	0.22
M.A.D.G.	.12	.58	.15	.06	.09	.12	.09	.08
Height	.15	.64	.33	.13	.04	.12	.01	.22
Diameter	.12	.58	.15	.06	.01	.04	.01	.15
Crown form	.27	.79	.43	.10	.04	.08	.09	.16
Straightness	.31	.82	.37	.10	.08	.06	.01	.12
Specific gravity	.77	.91	.47	.38	.53	.25	.24	.27

1/ R_r = repeatability of grafts of a clone or broad-sense heritability.
R_c = repeatability of a clone mean

2/ Family = family mean heritability, individual = individual heritability or narrow-sense heritability.

3/ Heritability estimates for loblolly pine are from the regression of seedling progeny on the female parent.

4/ Heritabilities obtained by the correlation of single parent and progeny and adjusted for the number of siblings per family (Franklin 1973).

5/ M.A.H.G. = mean annual height growth, M.A.D.G. = mean annual diameter growth.

In most instances, variance component analyses done on progeny data indicated statistically significant differences in general combining ability. Specific combining ability was an important source of genetic variation for growth characteristics while additive genetic variance was of prime importance in explaining genetic differences in crown form, straightness, and wood density. The same analyses also indicated that heritability values and efficiency of selection for advanced generations could be increased by the reduction of the environmental variance through careful planning of the field design.

Before progeny tests are available, clonal material is sometimes used to obtain estimates of heritability and to calculate expected genetic gains from selection and breeding. Results from analyses of clones and seedling progeny indicate that broad-sense heritabilities may be used with confidence as the upper limit for narrow-sense heritabilities. However, due to the variable importance of the first and second order genetic interaction from one characteristic to another, it would be unsafe to use a constant portion of the broad-sense heritabilities for any trait when calculating expected genetic gain. Too large or too small gains could be predicted leading to the acceptance or rejection of specific breeding programs. Narrow-sense heritabilities were 30 to 85 percent of the broad-sense heritabilities depending on the character involved (table 2).

Narrow-sense heritabilities calculated for growth and form characteristics from the regression of progeny on mature parent trees (table 2) were generally low and unreliable, reflecting the difficulty of predicting offspring juvenile performance on the basis of parental phenotypic measurement alone and vice versa. When growth of the parent trees are significantly correlated with age differences, the genotypic differences are partially or totally masked. Adjustments made to a common parental age were useful in revealing the presence of additive gene effects on the expression of the characters. The estimates obtained from the regression of seedling progeny on clonal parents were more precise and compared favorably to estimates obtained by variance analyses (table 2).

Correlations were also used to measure the degree of resemblance between ortet and graft, ortet and progeny, and between graft and progeny. Significant relations were found between mean annual growth of Virginia pine ortets and grafted clones ($r = 0.63^{**}$ for height and 0.52^{**} for diameter) and between clonal parents and seedling progeny ($r = 0.43^{**}$ for height and $r = 0.48^{*}$ for wood density). A significant correlation was also found between specific gravity of parent trees and their seedlings ($r = 0.45^{*}$). The correlation coefficient between parent and progeny is often preferred to the regression coefficient as an estimate of the heritability when large scale measurement differences exist between the relatives due to important age or environmental differences. Uncorrected heritabilities obtained by correlation

were larger than estimates obtained by regression and variance analyses. When adjustments were made for the number and degree of relation among members of a single family (Falconer 1960), the heritabilities were of the same magnitude as those calculated by regression analysis (table 2). However, correlation and regression methods can give exactly the same estimates only when the parent and progeny variances are equal.

DISCUSSION

Progeny testing is costly in time and effort. Even with a simple testing system, and despite the fact that clonal material reproduces early, complications still occur. Sometimes, after 5 or more years of effort, only a portion of the crosses necessary to obtain the required genetic information are available. An additional 5-year period is also required before reliable data can be obtained from the progeny tests for roguing. During the waiting period, seeds produced are improved by only one selection cycle. The longer the waiting period, the greater the proportional loss.

Greater similarities between progeny and clonal parent than between progeny and mature parent tree make possible the utilization of clonal testing as a tool to increase and speed up genetic gains through the roguing of seed orchards. Calculations have shown roguing[7] of the inferior clones in a seed orchard to be an important step toward maximizing gains in a single generation. Predicted genetic gains following two cycles of selection in loblolly and Virginia pine populations are presented in table 3. A 1 percent selection intensity was assumed for wild forest stands during the first cycle of selection. The second cycle represents the roguing of 50 or 70 percent of the clones in the seed orchard on the basis of either progeny testing or clonal testing. Results indicate that roguing of the loblolly pine orchard on the basis of the clones' own performance would result in substantially higher gains than roguing on the basis of the clones' 4-year-old progeny performance. More important gains are also predicted for Virginia pine height and diameter following roguing on the basis of clonal performance than on the basis of progeny performance.

Clonal testing has the advantage of not requiring time beyond the 5 or 6 years after clonal establishment. Clonal testing is a method of testing where the co-ancestry coefficient between parent and progeny (as well as among progeny of the same parent) is equal to one, and thus offers more reliable genetic information than any other type of relatives. However, clone performance includes effects due to cyclophysis and topophysis, which may give false information. To obtain

7/ Systematic removal of undesirable clones.

Table 3.--Expected genetic gains for growth and form characteristics and wood specific gravity of Virginia and loblolly pines from selection in wild stands followed by roguing of the clonal seed orchards on the basis of progeny testing and clonal testing

	Expected gain from selection in natural forest stands				Expected gain from roguing on the basis of 4-year progeny tests				Expected gain from roguing on the basis of clonal tests				Unselected population mean	Total expected gain from two cycles of selection (percent)	
	i_1 1/	h_1^2	σ_p	G_1	i_2 2/	h_2^2	σ_f	G_2	i_3 2/	h_3^2	σ_c	G_3	$\bar{X}$	(G_1+G_2)	(G_1+G_3)
LOBLOLLY PINE															
Height	2.66	0.01	6.12	1.9	0.80	0.15	0.726	2.0	0.80	0.08	3.74	3.5	8.73	3.9	5.4
					1.16	.15	.726	2.9	1.16	.08	3.74	8.0	8.73	4.8	9.9
M.A.H.G.	2.66	.07	0.31	2.6	.80	.15	.182	2.0	.80	.31	0.37	8.4	2.18	4.6	11.0
					1.16	.15	.182	2.9	1.16	.31	0.37	12.2	2.18	5.5	14.8
Straightness	2.66	.08	1.12	6.3	.80	.37	.276	4.3	.80	.25	1.26	13.3	3.78	10.6	19.6
					1.16	.37	.276	6.3	1.16	.25	1.26	19.3	3.78	12.6	25.6
Crown form	2.66	.02	1.03	1.6	.80	.23	.199	2.0	.80	.16	1.07	7.8	3.53	3.6	9.4
					1.16	.23	.199	3.0	1.16	.16	1.07	11.3	3.53	4.6	12.9
VIRGINIA PINE															
Height	2.66	0.04	5.24	8.1	0.80	0.33	0.426	3.3	0.80	0.12	2.30	6.5	6.85	11.4	14.6
					1.16	.33	.426	4.8	1.16	.12	2.30	9.4	6.85	12.9	17.5
Diameter	2.66	.01	1.14	2.4	.80	.15	.100	1.9	.80	.04	0.56	2.8	1.28	4.3	5.2
					1.16	.15	.100	2.7	1.16	.04	0.56	4.1	1.28	5.1	6.5
Straightness	2.66	.08	1.03	6.0	.80	.37	.224	3.6	.80	.06	1.09	2.9	3.66	9.6	8.9
					1.16	.37	.224	5.3	1.16	.06	1.09	4.1	3.66	11.3	10.1
Crown form	2.66	.04	1.02	2.6	.80	.43	.202	3.3	.80	.09	1.06	3.2	4.23	5.9	5.8
					1.16	.43	.202	4.8	1.16	.09	1.06	4.6	4.23	7.4	7.2
Wood specific gravity	2.66	.53	0.30	11.1	.80	.47	.015	3.0	.80	.25	0.30	3.1	.382	14.1	14.2
					1.16	.47	.015	4.3	1.16	.25	0.30	4.6	.382	15.4	15.6

Table 3 continued on next pag

Table 3 (continued)

1/ i_1 = coefficient of selection intensity corresponding to a 1 percent intensity of selection in wild stands, i.e., $i_1 = 2.66$

2/ i_2 and i_3 = coefficients of selection intensity corresponding to the selection of 50 percent ($i_{(i)} = 0.80$) and 30 percent ($i_{(i)} = 1.16$) of the clones in the seed orchards.

h_1^2 = narrow-sense heritability estimated by regression of seedling progeny on parent trees,

h_2^2 = family mean heritability,

h_3^2 = narrow-sense heritability estimated by regression of seedling progeny on clonal parent,

σ_p = phenotypic variation of unselected natural loblolly pine population (Porterfield 1973) and phenotypic variation of 104 crop Virginia pine trees,
σ_c = phenotypic variation of clone means,

σ_f = phenotypic variation of half-sib family means,

$$G_1 \text{ percent} = (i_1 h_1^2 \sigma_p) \frac{100}{\bar{X}}, \quad G_2 \text{ percent} = 2(i_2 h_2^2 \sigma_f) \frac{100}{\bar{X}}, \quad G_3 \text{ percent} = 2(i_3 h_3^2 \sigma_c) \frac{100}{\bar{X}},$$

$\bar{X}$ = means of the 4-year-old commercial check seedlings included in the progeny tests,

M.A.H.G. = mean annual height growth.

maximum gain and reliability, only trees of the same age class and growing in similar environmental conditions should be selected. In addition, scions should be collected at the same position within the crown of each tree.

On a clonal basis, a seed orchard could be rogued 5 years after establishment depending on its growth rate and precocity of seed production. From that time on, the seeds produced would be improved by two cycles of selection. In addition, the gain resulting from roguing the orchard can be increased by initially using a larger number of clones and closer spacing of the ramets. At 20-foot spacing, using the same number of ramets per clone, 100 clones require less area than 50 clones with 30-foot spacing between ramets. The difficulty of progeny testing large numbers of clones limits the number of parent trees that can be initially included in the seed orchard; this problem can be overcome if clonal tests are used for initial roguing.

A breeding program using clonal testing could be begun as follows. First, select 100 phenotypically superior trees in wild forest stands at a maximum intensity of selection. Second, establish the 100 clones in the orchard. If the final spacing required for maximum seed production is 30 feet, the initial spacing should be 16 feet between the ramets. Third, after 5 years, if the growth is rapid and the fructification is early, rogue 50 percent of the clones on the basis of their vegetative performance. As shown in table 3, the expected gain is twice as high as from roguing 50 percent of the clones on the basis of 4-year-old progeny performance for height in loblolly and Virginia pines and for stem straightness and crown form in loblolly pine. After the orchard is in production, control pollinate the clones left to provide seedlings for advanced generation selection. A modified diallel type of mating design would provide reliable information about the mode of gene action and would produce the maximum number of unrelated families. On the basis of progeny performance, inferior clones that escaped the initial roguing could be liminated.

SUMMARY

An investigation of the inheritance of growth, form characteristics, and wood density of Virginia and loblolly pine was undertaken with the objective of determining the possibility of using vegetative clonal performance of parent trees selected from wild stands as a measure of their breeding value. Measurements were taken from three distinct but genetically related tree groups: parent trees, grafts of the parents, and control-pollinated progeny.

Analyses were made of total tree height, diameter, bole straightness, and crown form. Virginia pine wood density was also investigated.

Analyses of variance were performed on clones and seedling progenies, while regression and correlation analyses were used as measures of the degree of resemblance between related groups and to estimate heritabilities. Nested analyses of variance of clonal populations revealed highly significant genetic differences among clones for all characters. Variance component analyses done on progeny data indicated statistically significant differences in general combining ability. Narrow-sense heritability on an individual tree basis was estimated to be 0.30 to 0.05 for height, 0.07 for crown form and 0.09 to 0.13 for stem straightness of loblolly pine. Virginia pine narrow-sense heritabilities were as follows: height = 0.13, diameter = 0.06, straightness = 0.10, crown form = 0.20, and wood density = 0.38.

Narrow-sense heritabilities estimated by regression of seedling progeny on the mature parent trees were generally low and unreliable, reflecting the difficulty of predicting offspring juvenile performance on the basis of parental phenotypic measurement alone. Higher and more accurate estimates were obtained by regression of progeny on the clonal parent than by regression on the ortet. Heritabilities equal to 0.22, 0.12, 0.06, 0.08, and 0.25 were found for mean annual height growth, diameter, straightness, crown form, and specific gravity of Virginia pine.

Greater similarities between progeny and clonal parent than between progeny and mature parent tree make possible the use of clonal testing as a tool to increase and speed up genetic gains. Clonal testing of loblolly and Virginia pine is proposed as a supplement or substitute to progeny testing, especially for early roguing. A gain of 11 percent in mean annual height growth, 20 percent in straightness, and 9 percent in crown form are expected for loblolly pine following a selection intensity of 1 in 100 applied to wild forest stands plus roguing of 50 percent of the clones in a specially designed clonal test. Gains of 15 percent in height, 9 percent in straightness, 6 percent in crown form, and 14 percent in wood specific gravity are expected for Virginia pine after initial selection and roguing.

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PRESCRIPTION FOR THE AERIAL ENVIRONMENT OF A PLASTIC GREENHOUSE NURSERY

D. F. W. Pollard and K. T. Logan[1]

ABSTRACT.-- Investigations into the aerial environment favoring rapid growth of tree seedlings in plastic greenhouses are described. Controllable factors studied were day and night temperatures, and high and low intensity extension of photoperiod; a confounding influence of carbon dioxide enrichment and high humidity was also examined. Experiments were designed within the limits of applicability of results to the greenhouse control system, and were made on three commercially important species: jack pine, black spruce, and white spruce. Recommendations are given for each species, and also a single prescription is given for greenhouses containing all three species. The merits of high and low intensity photoperiod supplements are discussed.

The idea of using controlled environments for rapid production of tree seedlings is not new, but only recently has the principle been applied to forest nurseries in Canada.

Development of controlled-environment nurseries in North America has been stimulated by a trend toward container-grown seedlings and increasing interest in mechanized production systems. Typically, controlled environment nurseries comprise plastic greenhouses with various systems of temperature and photoperiod control. Automatic watering is usually included and may incorporate a nutrient delivery system. More elaborate units include means for carbon dioxide enrichment of the atmosphere, humidity control, and high intensity light supplement. Forest tree seedling production is approaching the sophistication that has characterized commercial horticulture for many years.

Three important factors of environmental control systems are engineering feasibility, cost, and the plant's requirements. The first two are intimately related and readily assessed so usually dominate design of a unit. The plant's requirements, however, are often poorly understood. Despite several decades of research into tree growth, the nurseryman equipped with controlled

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environments does not have adequate information on the requirements of tree species, especially northern conifers.

This was the position of the Ontario Ministry of Natural Resources (OMNR) when they ventured into production of seedlings in controlled environments at Swastika, Ontario. Their objective was to produce two, and eventually three, batches of seedlings each year by extending the effective growing season and by accelerating growth during the first year. This paper is an account of a series of experiments that were conducted at the Canadian Forestry Service laboratories at Petawawa to prescribe the environments best suited to meet this objective.

The controlled environment facilities at the Swastika nursery consist of two plastic greenhouses (40 m long, 10 m wide, and 4 m high at center) fitted with raised platforms for pallets of containerized seedling trees. Main environmental control is for temperature, through heat exchangers in a plenum at the end of each greenhouse, and for photoperiod, through a series of overhead incandescent lamps providing night illumination of about 400 lux. Ventilators are used in hot weather. Atmospheric carbon dioxide can be increased by a propane burner, although the benefit is lost when ventilators are open. A central trough carries a motorized boom for spraying water and nutrient solutions. Each house holds about a million 5/8 inch tubelings.

The effects of day and night temperature, carbon dioxide enrichment, and photoperiod were tested on the three main species raised at Swastika--jack pine (*Pinus banksiana*), black spruce (*Picea mariana*), and white spruce (*Picea glauca*) to determine the optimum aerial environment for the species.

METHODS

As far as possible, materials and cultural methods used in the Swastika nursery were duplicated in the controlled environment experiments. Seed of the three species was sown in 5/8-inch plastic tubes. In the first three experiments a fine-screened 3- to-1 mixture of peat and vermiculite was used; in subsequent experiments tubes were filled with peat muck as used by the nursery. A nutrient solution (Ingestad 1967) was applied daily (except in Experiment 1, where solution was applied three times a week).

Unless stated otherwise, experiments were conducted in small growth cabinets with illumination of 22,000 lux provided throughout a 16 h photoperiod. The duration of each experiment was 8 to 12 weeks from sowing. Treatment effects were assessed from ovendry weight (95°C) and heights attained over this period.

Experiment 1--Effect of Daytime Temperature

After germination, about 100 tubelings of each of the 3 species were placed in each of 5 cabinets. Temperatures in the cabinets were 15, 20, 25, 30, and 35°C, respectively, during the day and 15°C at night. The range of temperatures tested was restricted to the practical limits of greenhouse operation. The tubelings and treatments were rotated weekly through the five cabinets to eliminate the cabinet effects as a source of experimental error.

Twelve weeks after sowing, 15 seedlings were taken from each species for individual analysis of roots and shoots and height measurements (table 1). For both spruce species a 25° day temperature yielded tallest and heaviest seedlings. Jack pine growth did not peak in a single temperature regime but remained high over a daytime range of 25 to 35°C. Evidently daytime temperature is less critical in jack pine than in spruces.

Experiment 2--Effect of Nightime Temperature

Experimental design was similar to Experiment 1. Four night temperatures were tested, 15, 20, 25, and 30°C with a daytime temperature of 30°C. Again, the test temperatures were based on practical limitations of greenhouse control at Swastika (table 2).

As in Experiment 1, temperature was most critical in spruce with both species achieving maximum height and weight with a night temperature of 20°C. Jack pine weight was unaffected by changes between 15 and 25°C, although height decreased slightly over this range.

The seedlings in Experiment 2 weighed approximately three times as much as the seedlings in Experiment 1. This is probably a result of differences in watering and feeding because the seedlings in Experiment 2 were given a daily top watering with nutrient solution that the Experiment 1 seedlings were not.

Experiment 3.--Effect of Carbon Dioxide Enrichment

Seedlings were raised as in Experiment 1 and subjected to either ambient or enriched CO_2 concentration. For enrichment CO_2 was bled into the growth cabinet from tanks of compressed gas and maintained at a concentration of between 1,010 and 1,150 ppm. The cabinet atmosphere was monitored continuously with an infra-red gas analyzer. Cabinets were maintained under a day/night regime of 30/15°C and 16 h photoperiod. The enriched cabinet was sealed to prevent excessive leakage of CO_2.

Results with jack pine in this experiment did not correspond to those obtained in previous work (table 3) (Yeatman 1970). Carbon dioxide enrichment apparently depressed shoot growth by about 11 percent although this was partly offset by a 40 percent increase in root growth. Root growth

Table 1.--Height and dry weight[1] of jack pine, white spruce, and black spruce grown for 12 weeks in day temperatures of 15 - 35°C and night temperatures of 15°C

Day temperature (C°)	Height	Dry weight: Shoot	Dry weight: Root	Dry weight: Seedling
	[2] cm	g	g	g
JACK PINE				
15	7.2a	0.096a	0.038a	0.134a
20	10.0b	.172b	.059c	.231b
25	12.0c	.215xc	.053bc	.268bc
30	12.5cd	.219c	.045ab	.255bc
35	12.8d	.238c	.050bc	.288c
WHITE SPRUCE				
15	4.3a	0.030a	0.007ab	0.037a
20	4.6a	.051b	.008bc	.059b
25	5.6b	.068c	.010c	.078c
30	4.5a	.048b	.007ab	.055ab
35	4.2a	.034ab	.005a	.039a
BLACK SPRUCE				
15	4.6a	0.036a	0.010a	0.046a
20	5.8b	.071c	.015b	.086c
25	7.4d	.094d	.017c	.111d
30	6.4c	.079c	.013b	.092c
35	5.5b	.050b	.010a	.060b

[1] Mean of 15 seedlings

[2] Means followed by common letters are not significantly different at P = 0.05 by Duncan's Multiple Range Test.

Table 2.--Height and dry weight[1] of jack pine, white spruce, and black spruce grown for 12 weeks in day temperature of 30°C and night temperature 15 - 30°C

Night temperature (C°)	Height	Dry weight		
		Shoot	Root	Seedling
	cm[2]	g	g	g
JACK PINE				
15	18.1a	0.600a	0.096a	0.696a
20	17.8a	.579a	.108a	.687a
25	16.4b	.591a	.105a	.696a
30	14.2c	.482a	.094a	.576a
WHITE SPRUCE				
15	8.0b	0.197b	0.042a	0.239ab
20	10.5a	.264a	.042a	.306a
25	9.1b	.215ab	.030a	.245ab
30	8.1b	.196b	.036a	.232b
BLACK SPRUCE				
15	12.4a	0.247b	0.036b	0.283b
20	13.7a	.357a	.051a	.408a
25	13.5a	.296ab	.041ab	.337ab
30	12.4a	.258b	.039ab	.297b

1 Mean of 15 seedlings.

2 Means followed by common letters are not significantly different at P = 0.05 by Duncan's Multiple Range Test.

was also strongly stimulated in the spruces and significant increases in shoot growth occurred in these species.

Table 3.--Mean weight and height of 15 conifer seedlings grown for 12 weeks in ambient and enriched CO_2 concentrations (day/night temperature: 30/15°C)

CO_2	Shoot weight	Root weight	Height
	g	g	cm
JACK PINE			
Ambient	0.60	0.10	18.1
Enriched	.53	.14	16.4
WHITE SPRUCE			
Ambient	0.20	0.04	8.0
Enriched	.24	.06	7.4
BLACK SPRUCE			
Ambient	0.25	0.04	12.4
Enriched	.34	.07	12.7

The results with jack pine are believed to be the result of higher humidity in the sealed cabinet (rh = 80 to 85 percent in the CO_2-enriched cabinet compared to rh = 65 to 70 percent in the control cabinet). High humidity reduced evapotranspiration directly, both from the plants and from the soil surface. Seedlings were weighed every 2 or 3 days before watering; considerably less water was lost from seedlings in the enriched environment. Carbon dioxide enrichment induces stomatal closure which also reduces transpiration. The combined effect would be to promote water-logging in the soil and consequently to affect redox potential and nutrient exchange. Treatment seedlings of all three species were slightly chlorotic. Subsequent analysis revealed a total nitrogen content of between 11 and 33 mg/g in CO_2-enriched plants compared to 20 to 25 mg/g for controls.

The fact that better results with CO_2 enrichment were obtained with species of wetter habitats, and particularly with black spruce, supports the view that poor results with jack pine are more likely to be associated with soil moisture problems than with CO_2 enrichment itself.

A second experiment was conducted with jack pine in which drying loops were linked to atmospheres in the control and CO_2-enrichment cabinets. Jack pine will respond positively to CO_2 enrichment but humidity problems may arise (table 4). The need for more thorough experiments was obviated when OMNR nurserymen frequently found it necessary to augment the cooling system of greenhouses by ventilation which made enrichment impracticable.

Table 4.--Mean height and weight of 25 jack pine seedlings grown for 12 weeks in ambient and enriched CO_2 concentrations at different relative humidities (day/night temperatures: 30/15°C)

CO_2	r.h.[1]	Shoot weight	Root weight	Height
		g	g	cm
Ambient	low	0.48a[2]	0.15a	19.2a
Ambient	high	.54ab	.18ab	21.3a
Enriched	low	.68b	.30c	20.9a
Enriched	high	.54ab	.23bc	18.4a

1 low = 65 to 70 percent r.h.; high = 80 to 85 percent r.h.
2 Means followed by common letters are not significantly different at p = 0.05 by Duncan's Multiple Range Test.

Experiment 4--Effect of Photoperiod

Extended photoperiods affect growth in a variety of ways depending on the physiological state of the plant and on the intensity of light provided. Low intensity (less than 1,000 lux) extension of photoperiod will usually prevent the onset of dormancy in young seedlings and appears to stimulate growth by effects on plant metabolism. At higher intensities (above 10,000 lux) both of these effects will be present, but in addition is stimulated by the significant increase in energy available for photosynthesis. Natural photoperiods can be supplemented by low intensity light at reasonable cost, but the cost of installing and operating entirely artificial high intensity lighting is usually prohibitive for production nurseries.

This investigation was conducted in two parts. First, as a demonstration, seedlings were grown in four high intensity photoperiods. The experiment was then repeated with low intensity extensions of a fixed high intensity photoperiod.

High intensity photoperiod

Thirty-six seedlings of each species were reared in tubes at day/night temperatures of 25/20°C in each of four growth cabinets. The cabinets provided illumination of 22,000 lux for 15, 18, 21, and 24 h photoperiods,

respectively, from a mixed source of fluorescent and incandescent lamps. As a precaution against unknown cabinet effects, the seedlings and treatments were rotated through the four cabinets at weekly intervals. The seedlings were harvested for growth measurement 8 weeks after sowing (table 5). All species grew faster under long photoperiods, with continuous light consistently yielding the heavier seedlings. Seedlings grown under continuous light were at least twice the weight of those grown under only 15 hours of light. The response of height growth was slight in all species. The shoot weight increase was principally in foliage.

Table 5.--Height and dry weight[1] of jack pine, white spruce, and black spruce seedlings grown for 8 weeks in photoperiods of 15, 18, 21, and 24 hours of high intensity light (day/night temperature: 25/20°C)

Photoperiod (h)	Height	Dry weight: Shoot	Dry weight: Root	Dry weight: Seedling
	cm	g	g	g
JACK PINE				
15	10.4a[2]	0.189a	0.062a	0.251a
18	10.8ab	.260b	.088b	.348b
21	11.4c	.331c	.127c	.458c
24	10.9b	.371d	.131c	.502d
WHITE SPRUCE				
15	4.8a	0.062a	0.011a	0.073a
18	5.0a	.080b	.015a	.095a
21	5.1a	.104c	.021ab	.125b
24	4.8a	.122d	.028b	.150c
BLACK SPRUCE				
15	6.5a	0.065a	0.014a	0.079a
18	6.7a	.090b	.021b	.111b
21	7.0a	.126c	.030c	.156c
24	6.7a	.145d	.033c	.178d

1 Mean of 36 seedlings
2 Means followed by common letters are not significantly different at p = 0.05 by Duncan's Multiple Range Test.

The cost of maintaining high intensity lighting is directly proportional to the duration of the photoperiod. But the response of seedling growth exceeds a simple linear relation: increasing photoperiod by 60 percent (from 15 to 24 hr) increased growth at least 100 percent. Thus in systems relying entirely on artificial lighting for high intensity illumination, long photoperiods are more efficient and more effective than short photoperiods.

Low intensity photoperiod

Eighteen seedlings per species were reared in each of four treatments for 10 weeks as for the high intensity experiment. Photoperiods were based on 14 h of high light intensity with 0, 2, 6, and 10 h of supplementary incandescent light of about 400 lux. The treatments and seedlings were rotated at weekly intervals (table 6).

Table 6.--Height and dry weight[1] of jack pine, white spruce, and black spruce seedlings grown for 10 weeks in photoperiods extended by low-intensity light (day/night temperature: 25°/20°C)

Photoperiod (h)	Height	Shoot	Root	Seedling
	cm	g	g	g
JACK PINE				
14 + 0	[2]12.3a	0.54a	0.20a	0.74a
14 + 2	13.1a	.43a	.19a	.62a
14 + 6	15.3b	.58a	.21a	.79a
14 + 10	15.1b	.57a	.19a	.76a
WHITE SPRUCE				
14 + 0	5.4a	0.063a	0.021a	0.084a
14 + 2	6.0ab	.075ab	.018a	.093a
14 + 6	6.0ab	.075ab	.018a	.093a
14 + 10	6.8b	.087b	.019a	.096a
BLACK SPRUCE				
14 + 0	8.3a	0.091a	0.018a	0.109a
14 + 2	8.1a	.098a	.021a	.119a
14 + 6	8.5a	.098a	.020a	.118a
14 + 10	8.2a	.105a	.018a	.123a

1 Mean of 18 seedlings.
2 Means associated by common letters are not significantly different at $p = 0.05$ by Duncan's Multiple Range Test.

Jack pine results were somewhat inconsistent, but there appeared to be some additional height growth under the long photoperiods. White spruce showed a steady, though moderate, increase in shoot weight and height growth while black spruce showed neither. In no species was root growth enhanced.

The additional growth of white spruce might justify photoperiod control. But results with other species do not support the use of low intensity photoperiod extension in the range tested. However, the base illumination of 14 h is comparable to photoperiods in mid-April and mid-August at the Swastika nursery. Attempts to rear seedlings earlier in the spring or later in summer would almost certainly require some low intensity extension of photoperiod to prevent early cessation of growth.

CONCLUSIONS AND RECOMMENDATIONS

The three northern conifers investigated--jack pine, black spruce, and white spruce--appeared to have similar requirements in the aerial environment. This simplifies the prescription for environmental control in nurseries rearing these species in plastic greenhouses.

The most suitable temperature for all three species is a 25°/20°C day/night regime. This is specific for spruces, yet lies within the broader range of 25-35°/15-25°C suitable for jack pine.

Photoperiodic effects can be divided into two broad categories, those related to development and those related to dry matter production. Even at low light intensities, photoperiod exercises great control over the development of a seedling. A short photoperiod will usually result in cessation of growth and the onset of dormancy; thus when natural day-lengths are critically short, photoperiods can be effectively and inexpensively extended through the use of low intensity incandescent lamps. But our results indicate that there is little gain in using very long photoperiods (more than 16 h) of low intensity supplements once the continuous growth of seedlings has been ensured.

The effectiveness of very long photoperiods would be different in systems employing high light intensity supplements. Additional light in the form of longer, high intensity photoperiods means more energy for photosynthesis and results in more and more dry matter growth; an increase in light of 60 percent (from 15 to 24 h) doubled dry matter growth in all three species in our experiment.

Daylength exceeds 14 hours between mid-April and mid-August at latitude 48°N and should not need supplementing. Photoperiod extensions earlier in spring and later in summer should maintain 14 hours of light. A preferable regime would be 16 hours of light, in view of the lesser vigour of greenhouse-grown seedlings.

The advantages of carbon dioxide enrichment depend largely on whether a greenhouse requires ventilation during hot weather. Concentrations up to 1,000 ppm or more are undoubtedly beneficial, especially when light intensities are high. But it is usually these conditions that call for ventilation to prevent excessively high temperatures. Carbon dioxide enrichment appears to be impracticable in summer in view of the limitations of cooling systems employed in many plastic greenhouses.

The prescription suggested for the aerial environment of a plastic greenhouse nursery is inexpensive to implement because both temperature and photoperiod optima are close to natural conditions. Implementation is simplified by the similar requirements of the three northern species, so that mixed or successive batches can be reared under the same conditions.

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MANAGEMENT OF TREE GROWTH AND RESEARCH PLANTATIONS

Donald A. Fraser[1]

ABSTRACT.--Reports vegetative growth and reproductive responses in white and black spruce resulting from: control of soil moisture by means of overhead sprinklers, photoperiod by means of incandescent lamps, and thermoperiod by means of heated plastic shelters. Increased lateral branch and diameter growth resulted in white spruce from irrigation(a). Continuous light (b), increased apical growth but no diameter. Raised temperature (c) forced early bud growth as well as early apical growth cessation. (b) plus (c) caused early bud growth and late apical growth cessation. (a) as well as (b) plus (c) resulted in early, more abundant male and female conelet production in white spruce. Root-pruning had an immediate, additional stimulatory effect, but subsequently reduced vigor had deletorious effects on conelet production. The responses in black spruce were similar. Photoperiod and gibberellic acid responses in growth chambers are briefly mentioned.

Understanding the basic ecology, physiology, and genetics of forest trees is essential for manipulating and interpreting the physical and biological factors of the environment. Moisture, nutrients, photoperiod, thermoperiod, and growth-influencing substances are physical factors that may be manipulated. Biological elements include the beneficial presence of nitrogen-fixing organisms in the soil, and the deleterious effect of insects and pathogens.

We conducted experiments to determine what effect these factors had on tree growth and on production of reproductive buds. The experimental work was conducted in special outdoor testing areas and in growth chambers. Although other species were also investigated, chief emphasis was on white (*Picea glauca* (Moench) Voss) and black spruce (*P. mariana* (Mill.) BSP.).

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FIELD EXPERIMENTS

Methods

In 1956 the Corry Lake Tree Physiology Area was established in Chalk River, Ontario, on a 3-acre site of loamy sand with adequate fertilization. It was designed for progressive plantings of white and black spruce and selected hardwood species for managing environmental factors. Special emphasis was placed on the following factors that might accelerate the production of flower primordia and consequent seed production in commercial plantations.

(1) Soil moisture was regulated with overhead irrigation sprinklers drawing untreated water from Corry Lake. During early stages of plantation establishment, white clover was sown around the young saplings to improve the soil and to limit competition from weeds present in the original field.

(2) Photoperiod was extended with incandescent lamps (Fraser 1966).

(3) Thermoperiod was altered with heated plastic enclosures that extended the frost-free period.

The control was the cleared 6-acre Loon Lake Physiology Area on a loam sand represented by moisture regimes from dry to wet (Fraser 1954). This control area was planted concurrently with the Corry Lake Physiology Area, so that growth under a natural environment could be compared with growth of trees treated to accelerate vegetative and reproductive growth.

Apical as well as diameter growth of leaders and lateral branches were measured periodically from March 21 to August 29, 1969 on 9-year-old white spruce. In addition, the development of reproductive buds was tallied annually for 9 years in four plantations of white spruce and one of black spruce, established in 1956 with 3-year-old seedlings of local provenances as follows:

1. White spruce under natural environment.

2. White spruce under natural light, but irrigated and fertilized.

3. White spruce under continuous light, irrigated, and fertilized.

4. Same as "3" but root-pruned twice in a circle 18 inches from the trunk to an 8-inch depth, when the saplings were 7 and 8 years old.

5. Black spruce under the same conditions as "3" and "4".

RESULTS AND CONCLUSIONS

Vegetative Growth Response

Soil Moisture--Apical and diameter growth of white spruce control trees were greatest on the dry site (fig. 1). However, greater lateral branch and diameter growth were found on the irrigated area where adequate moisture and nutrients were provided throughout the season. The influence of the natural range of moisture on radial growth of mature trees was reported earlier (Fraser 1956).

Photoperiod--Continuous light increased apical growth, yet more important was the longer period over which the apical extension took place, for this represented a longer time available for the formation of reproductive buds (fig. 1).

Thermoperiod--Raised temperatures altered the time of the period of apical growth, but did not affect diameter growth (fig. 1). When temperatures were raised in mid-April, when frost is still frequent under natural conditions, growth began in April rather than in late May. Therefore, temperature controls the time growth begins in the spring (Fraser 1972). However, growth also stopped earlier in the warmer environment.

The combination of continuous light and raised temperature caused apical growth to begin earlier and stop later (fig. 1). The first time the saplings were exposed to such an extended photoperiod a 30 percent increase in leader growth was obtained. This response gradually diminished when the extended photoperiod was continued over a period of years on the same saplings. Therefore, lengthened photoperiod will accelerate growth of nursery material but the next use should be when accelerated production of reproductive buds is desirable.

Reproductive Growth Response

The white spruce control trees developed reproductive buds when 13 years old: 8 percent male and 11 percent female buds which was less than one male and two females per plantation tree (fig. 2). A peak of 32 percent of the saplings produced male and 19 percent female buds with an average of 15 male and 9 female buds the next year. No reproductive buds were formed the following year.

When white spruce was grown under natural light, irrigated, and fertilized, reproductive buds were produced earlier and in larger quantities. Reproductive buds were first developed when these trees were 10 years old--5 percent of the trees produced male and 8 percent female buds; both male and female buds averaged one per tree. When the saplings were 14 years old, 40 percent of the trees produced male and 33 percent female buds

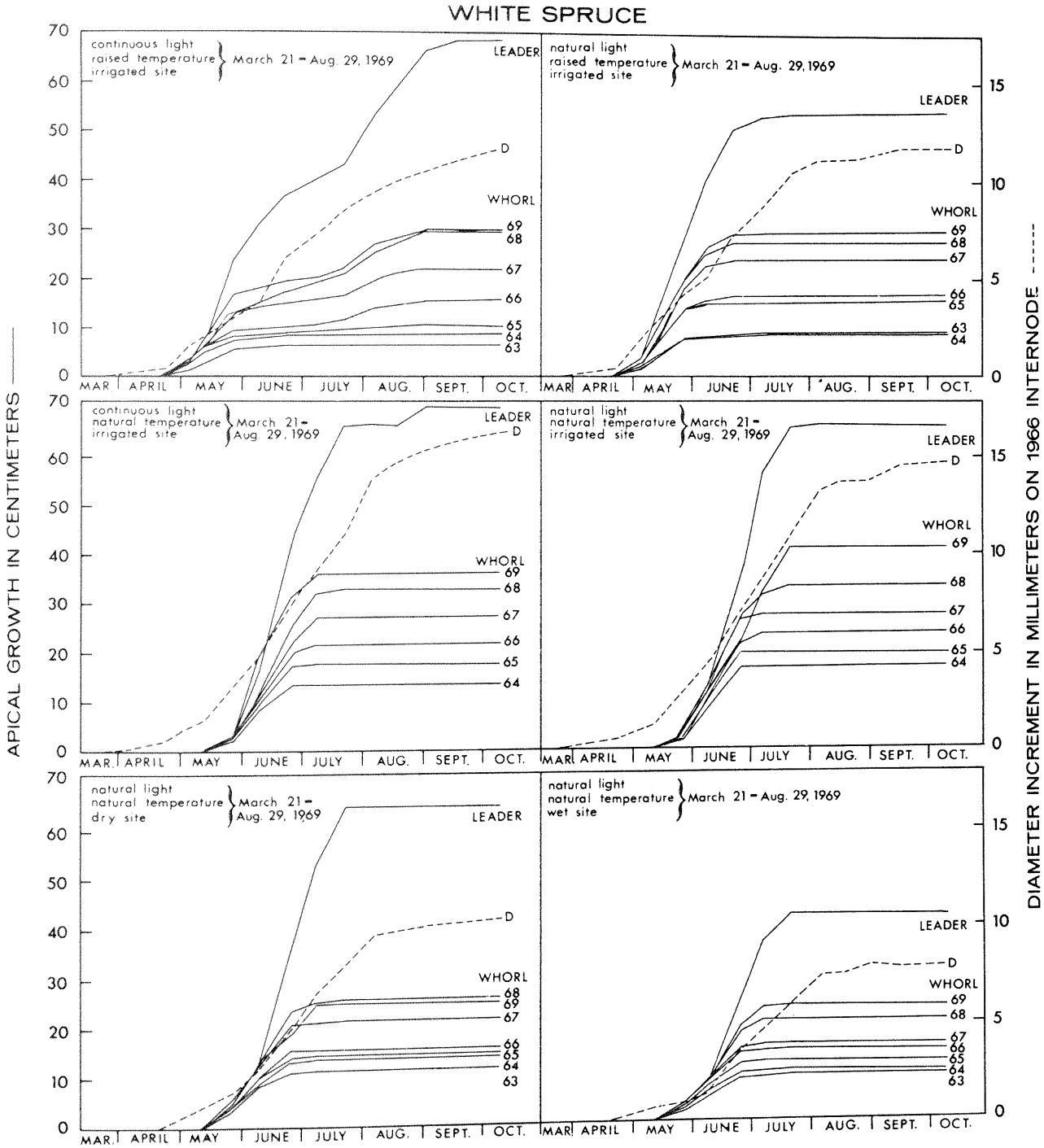


Figure 1.--Average leader (four trees per sample), lateral shoots (four branches per whorl), and diameter growth of 9-year-old white spruce grown under various combinations of photoperiod, thermoperiod, and soil moisture.

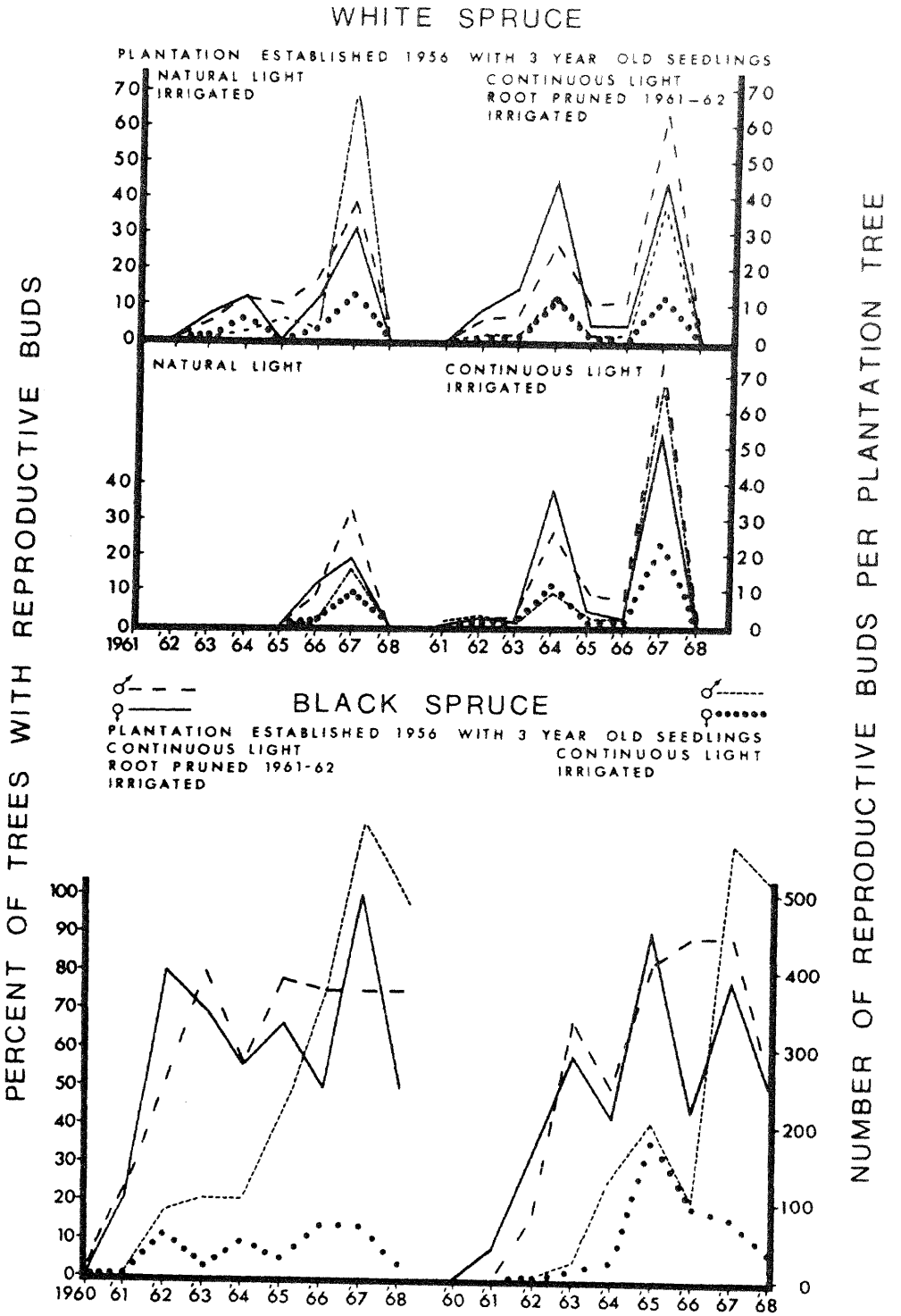


Figure 2.--White and black spruce trees with reproductive buds and reproductive buds per tree during the 1961-1968 period.

with an average of 70 male and 13 female buds per tree, compared with the 32 percent and 19 percent peak in the control trees.

White spruce saplings under continuous light in an irrigated and fertilized soil also produced reproductive buds earlier than those grown in a natural environment; 26 percent produced male and 40 percent female buds with an average of 10 male and 13 female buds per tree at an age of 11 years. In 1967 when all plantations had trees producing reproductive buds, this continuous light plantation had male buds on 74 percent of the trees and females on 53 percent with an average of 67 male and 23 female buds per tree.

Root-pruned trees produced reproductive buds more abundantly at a slightly earlier age--30 percent male and 45 percent female buds from an 11-year-old sapling, but 3 years later only 55 percent male and 45 percent female buds were produced compared with the 74 percent male and 53 percent female buds in the unpruned continuous light saplings.

Black spruce was also studied in the irrigated and fertilized Corry Lake Physiology Area plantation under continuous light in a preliminary experiment (fig. 2). Reproductive buds appeared when the saplings were 7 years old. Two years later, 80 percent of the root-pruned saplings were producing male buds under continuous light compared with 65 percent of the unpruned saplings; 100 percent of the root-pruned saplings produced female buds at an age of 14 years. Yet, this gave an average of only 50 female buds per tree, while the unpruned trees, although they were not developing uniformly, had a maximum of 200 female buds per tree 2 years earlier, when 90 percent of them produced female buds. Thus, root-pruned black spruce saplings produced more reproductive buds in the first year after root-pruning, but the number decreased later when the effect of reduced tree vigor due to root-pruning became evident.

It would appear that genetical response might be favored by the supply of nutrients alone, as attested in the white spruce by reproductive bud production at the earliest data. However, the two peaks in reproductive bud development indicate an overall effect of light, especially on the production of female buds. Both black and white spruce responded to root-pruning by immediate production of reproductive buds, but these trees did not remain as productive over several years as did the undisturbed trees. The method might, therefore, be suitable when only one year of high reproductive bud development is required.

GROWTH CHAMBER EXPERIMENTS

Borthwick *et al*. (1956) established that alternations of the light and dark period in combinations other than that of two uninterrupted periods within 24 hours will often stimulate additional growth. Thus, three different light-dark combinations were selected: (1) 12 hrs light and

Figure 3.--White spruce seedlings sprayed weekly with 100 ppm GA_3 and grown for 8 months under three different photoperiods as indicated under each seedling.

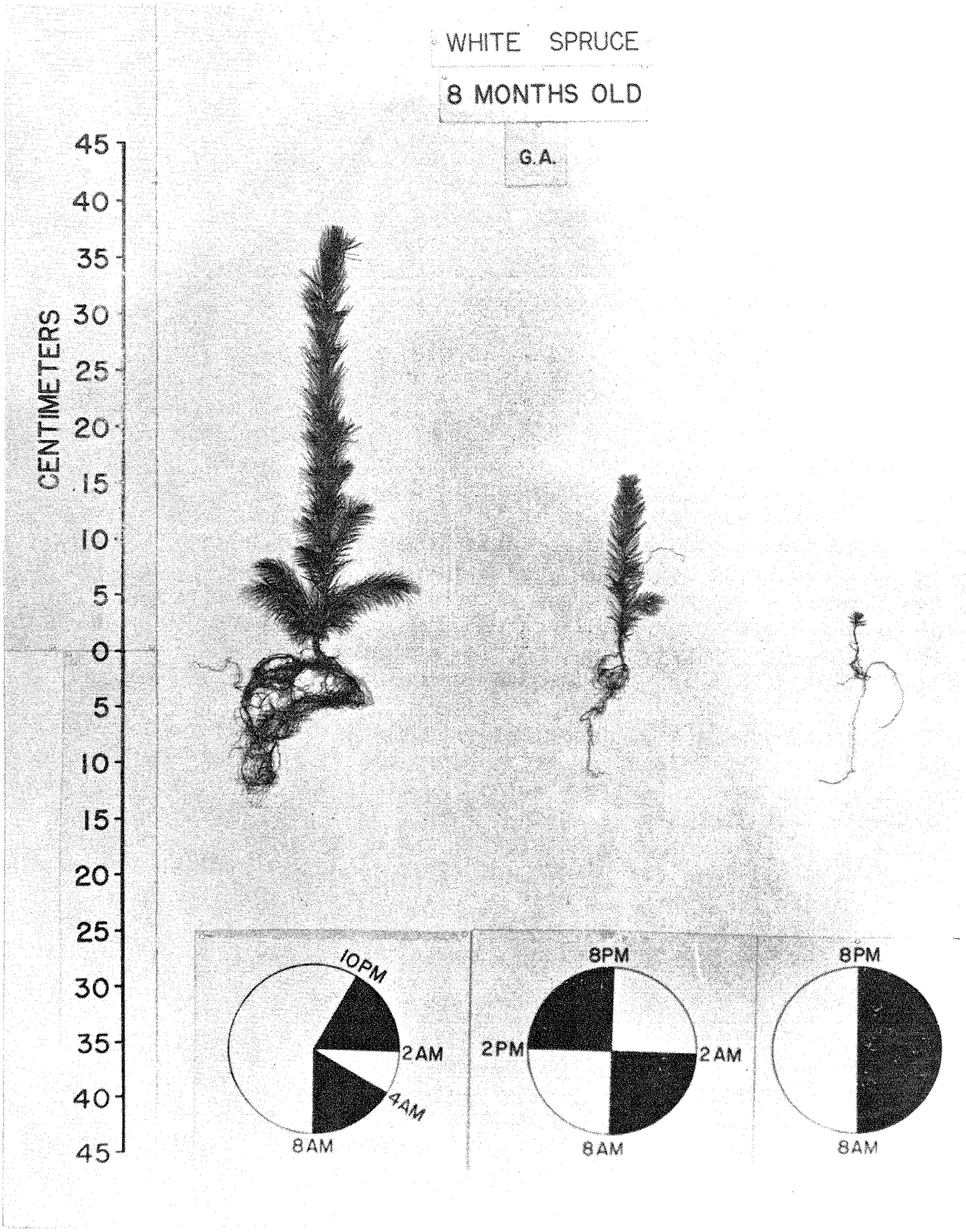
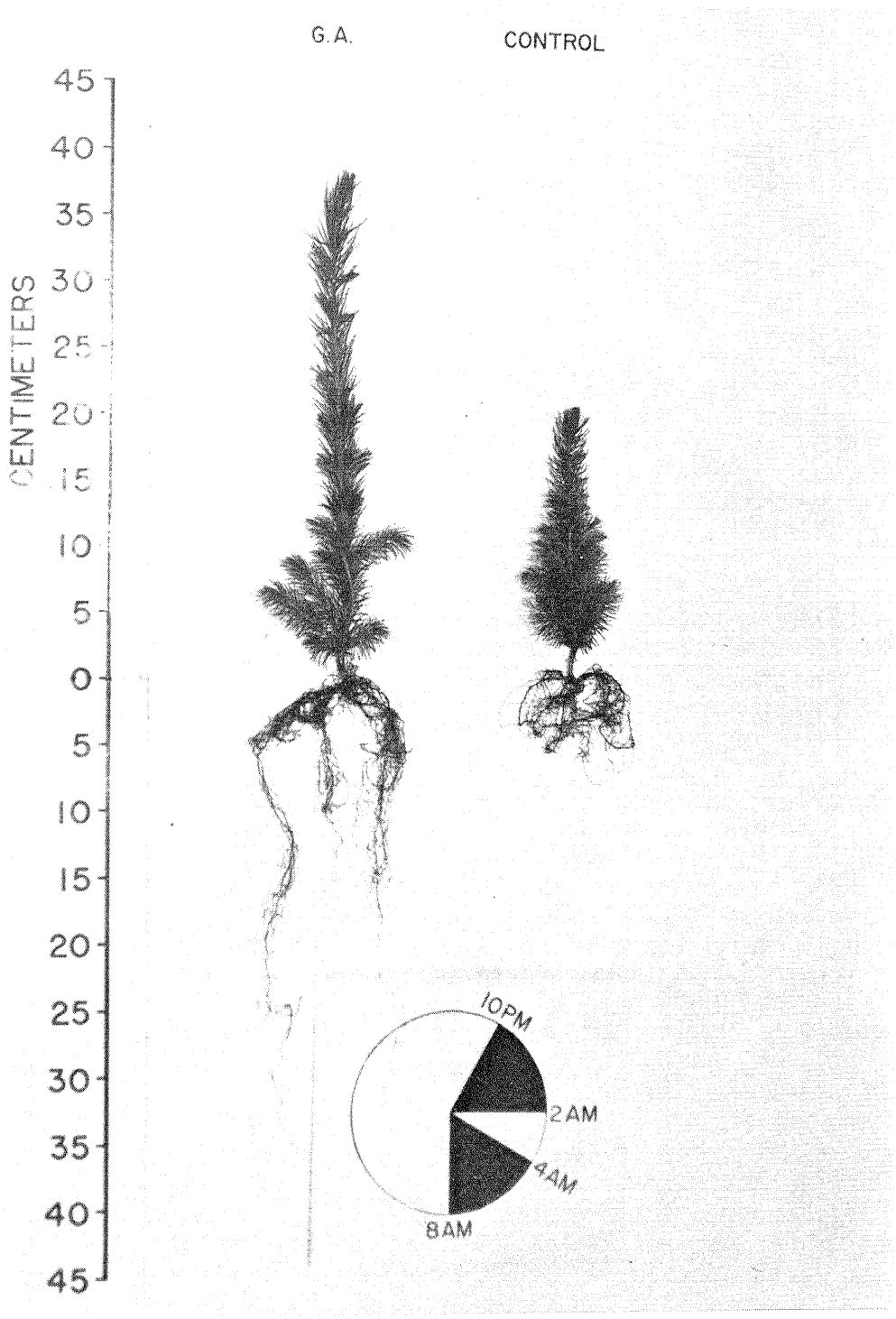


Figure 4.--White spruce seedlings sprayed weekly with 100 ppm GA_3 and grown for 8 months under the photoperiod as indicated below. Control seedling shown on the right grown under the same conditions but without gibberellic acid treatment.



12 hrs dark; (2) 6 hrs light and 6 hrs dark alternating; and (3) 14 hrs light, 4 hrs dark, 2 hrs light, and 4 hrs dark. The effect of the dark and light combinations on the growth of spruce seedlings follows that for herbaceous plants. The 6 hrs light and 6 hrs dark periods produced more growth than the 12 hrs light and 12 hrs dark periods. Greater growth was obtained with the 14 hr light period followed by 4 hrs dark, 2 hrs light, and 4 hrs dark (fig. 3).

Gibberellic acid (GA_3) was applied weekly as a foliar spray after germination at a concentration of 100 ppm. Its effect was additive to that of the lengthened photoperiod in that it accelerated leader growth (fig. 4). This response is opposite to that reported for white-cedar (*Thuja occidentalis* L.) where GA_3 application reduced apical growth and started flower primordia development within a few weeks of the first application (Fraser 1970).

SUMMARY

Management of tree growth in research plantations decreases the time required for seed production and hence assists in tree breeding experiments. Production of larger seedlings within a shorter time, if root systems are not reduced at the expense of shoot elongation, should provide material better suited for plant competition when out-planted.

ACKNOWLEDGMENTS

I wish to express my appreciation to members of the former Tree Physiology Section of the Petawawa Forest Experiment Station, Chalk River, Ontario, who assisted in the conduct of these experiments. Dr. E. E. Gaertner provided help both with the field work and in the compilation of results.

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BREEDING STRATEGY

M. A. K. Khalil[1/]

ABSTRACT.--The development of breeding strategy is of paramount importance before breeding is begun in any given species. The purpose of improvement, degree and pattern of variation, and species biology are primary factors to be considered. The choice of the breeding method is another aspect to be taken into account, and selection, hybridization and mutation breeding are discussed here. Selection is well adapted to the characteristics of many species and is most commonly applied. The potential of hybridization has not been fully realized but must usually be combined with some form of selection. The use of special research environments, such as growth chambers, greenhouses and nurseries can give useful partial answers and direction to tree improvement in species where little basic information is available.

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ADAPTIVE VARIATION -- MANIFESTATIONS IN TREE SPECIES AND USES IN FOREST MANAGEMENT AND TREE IMPROVEMENT

Hans Nienstaedt[1/]

ABSTRACT.--Adaptive variation and the genetic system that maintains adaptive fitness are described. It is emphasized that optimum fitness and genetic flexibility are opposing demands on the plant populations and have led to a compromise between fitness to existing environments and the capacity for further change. Using as examples phenological and edaphic adaptation, patterns of variation are discussed. Clinal variation is described and the importance of adequate sampling stressed in establishing discontinuous, ecotypic variation patterns. Variation patterns are character specific and may be highly complex depending on the pattern of the environmental variation -- clines within clines, clines within ecotypes and ecotypes within clines must exist within north temperate tree populations. The breeding systems and the factors determining the size of breeding groups are important aspects of the genetic system and must be considered in planning by foresters and tree breeders alike.

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PLUS TREE SELECTION IN BRITISH COLUMBIA

Gyula K. Kiss[1/]

ABSTRACT.--Two plus tree selection programs developed by the Research Division of the British Columbia Forest Service are discussed. The first of the programs developed for coastal Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) is a strict phenotypic selection program. Untested ramets of plus trees thus selected are established in seed orchards for seed production. The second program developed for interior spruces (white spruce, *Picea glauca* (Moench) Voss and Engelmann spruce, *P. engelmannii* Parry) employs a much more relaxed selected standard followed by open pollinated progeny trials. Advantages and disadvantages of both programs are discussed. Evaluation and recommendations are given based on experience gained during execution of the program.

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PROGENY TESTING IN PRACTICAL TREE IMPROVEMENT

J. W. Wright[1/]

ABSTRACT.--Good quality planting stock and good plantation care during the early years are very important. A well executed progeny test of moderately good design usually gives much more information than a poorly executed experiment of the most refined design. Half-sib progeny tests are less expensive, and give less gain and information than full-sib progeny tests. The cost, gain and information ratios between the two types vary considerably, depending on several factors. Often, with northern conifers, half-sib tests are preferable for first-generation work and full-sib tests in more advanced breeding programs. Progeny tests often need to contain a few hundred families. With tests that large it is desirable to compute optimum family size (often smaller than has been used in the past) and to consider carefully the effects of plot size and number of replications on efficiency; otherwise the tests may become unmanageable. A variation of the randomized complete block design is often regarded as the most practicable for large tests. A few hints are included as to desirable measurement and analysis procedures.

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THE ECONOMICS OF TREE IMPROVEMENT

A. Carlisle and A. H. Teich[1/]

ABSTRACT.--The report discussed the sources of costs and benefits of tree improvement in the context of plantation economics, and considers the ways a forester can control the profitability of establishing and managing forests. The response of Canadian tree species to selection and breeding, the ways tree improvement can help the forester, the costs and benefits of different tree improvement strategies, and quantification of costs and benefits are described. The sources of cost in tree improvement research, development and the plantation operation are compared. Future research needs are suggested.

1/ Canadian Forestry Service, Petawawa Forest Experiment Station, Chalk River, Ontario.

TREE SEED PROGRAM IN ONTARIO

C. H. Lane[1/]

ABSTRACT.--The regeneration program in the province of Ontario requires large quantities of tree seed annually. It is estimated that 1 billion viable seeds will be required to meet the 1975 needs of the program. Forest tree improvement has been developed to effectively improve the availability of tree seed and to improve seed quality on a scale sufficiently massive to meet the requirements of the reforestation program.

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SEED ORCHARDS

J. P. van Buijtenen and E. M. Long[1/]

ABSTRACT.--The objective of a seed orchard is to mass produce improved seed of the desired quality as economically as possible, and usually also as quickly as possible. The major steps involved are: (1) mass selection of desirable trees; (2) establishing the seed orchard; (3) progeny testing the seed orchards, and (4) roguing the seed orchards on the basis of results of the progeny tests. Seed orchard establishment includes the following major steps: site selection, site preparation, seed orchard design and graft establishment. Site fertility, drainage and location are all important considerations. Site preparation should be done thoroughly but usually presents no serious problems. Spacing should be such that the orchard will not require roguing before information from progeny tests is available, but close enough to give reasonable cone production at an early age. The design should consider such factors as providing a sufficient number of clones to form an adequate genetic base, optimizing cross pollination among clones, providing an adequate supply of improved pollen for the orchard, minimizing the proportion of contaminating pollen, and limiting the amount of inbreeding in the orchard. Three systems of handling grafts are in common use: pot grafting, bed grafting, and field grafting. Each of them is an acceptable method but has its own advantage and disadvantages. Seed orchard management practices are designed directly or indirectly to keep seed orchard trees healthy and to produce the maximum amount of seed. Increased flower production is secured by a combination of subsoiling, irrigation and fertilization. The seed orchard is protected and kept in a healthy condition by mulching, fire protection, protection from diseases and insects, and proper care of the orchard during harvesting.

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FOREST TREE SEED QUALITY

B. S. P. Wang[1/]

ABSTRACT.--With the increase in reforestation in Canada, seed is becoming a valuable commodity because of increasing demand. Seed production is governed by many factors, but this paper only discusses seed quality as affected by the post-harvest handling of cones and processing, testing, storing and shipping of seeds. To improve seed quality and the economic use of collected seeds, a close control is required from time of cone collection through cone handling, and processing, testing and storage to shipping of the seeds for field sowing. Until all these factors are adequately controlled, the success of reforestation programs is unpredictable.

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