INSTITUTE OF
PAPER CHEMISTRY
Appleton · Wisconsin

PRODUCTION AND INTENSIVE MANAGEMENT OF GENETICALLY IMPROVED ASPEN

Project 3537

Report Two
A Progress Report
to
MEMBER OF GROUP PROJECT 3537

February 27, 1987

THE INSTITUTE OF PAPER CHEMISTRY

Appleton, Wisconsin

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Blandin Paper Co.

Consolidated Papers, Inc.

Michigan Department of Natural Resources

Owens-Illinois, Inc.

TABLE OF CONTENTS

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PRODUCTION AND INTENSIVE MANAGEMENT OF GENETICALLY IMPROVED ASPEN

SUMMARY

The 1986 crossing program took advantage of a good flowering year and uced over 950,000 hybrid aspen seeds for both cooperator and IPC seedling uction. An additional 100,000 full-sib *P. tremuloides* seeds were produced use in the hypoxylon canker bioassay work.

IPC seedbeds produced 25,000 plantable seedlings for a 40-acre nstration planting with Consolidated Papers, Inc., and for two smaller plant- with Blandin Paper Co. and the Michigan DNR. The State of Wisconsin will hipping 44,000 hybrid aspen seedlings this spring produced from Project 3537 l. Clonally propagated planting stock was also produced by IPC to begin the al testing of selected individuals.

A number of test trials were measured for growth and disease incidence past fall, the oldest of which was 30 years and the youngest was one year. addition, several operational trials were measured in Minnesota and Michigan. particular interest was an 8-year-old triploid hybrid aspen sucker stand on ndin Paper Co. lands that is growing at the rate of 230 ft^3/acre/year. tly, a 23-year-old triploid hybrid aspen sucker stand that produced 63 cords m 1-1 1/2 acres was harvested this fall.

Five clonal selections with exceptional growth rate were collected from al X on the Ripco test area and will be propagated this spring. They raged 71 feet and 10.6 inches in diameter at 23 years.

Hypoxylon research continued this past year utilizing actual levels of infection data recorded from field trials and plantations. Full-sib seedlots representing parental combinations with 20 to 25 year field data were subjected to the leaf bioassay with toxin produced by Hypoxylon mammatum (mammatoxin). Multiple correlations were used to determine if there is a relationship between the bioassay results and field incidence. The standard error of estimate was so large that the predicted value was of limited use. It appears that more crosses need to be evaluated, and changes made in handling the bioassay data to improve the calculations.

Plans for 1987/88 include: (1) seed and seedling production, (2) establishment of a 40-acre demonstration planting, (3) continued selection of hybrid aspen clones, (4) propagation of hybrid aspen clones, (5) establishment of a triploid hybrid aspen seed orchard, (6) evaluation of hybrid aspen suckering in Trial X, (7) measurement of an 18-year-old native aspen sucker stand that had been irrigated and fertilized, (8) reexamination of earlier project work with seed production from boxed grafts, (9) if time and funds permit, the development of a tissue culture propagation method for hybrid aspen selections, and (10) continuation of hypoxylon bioassay and field evaluations for canker incidence.

INTRODUCTION

The aspen tree improvement project has been evaluating native aspen and aspen hybrids for over 30 years. During that time, Lake States aspen utilization has increased dramatically. From 1968 to 1984, aspen roundwood harvests increased from 1,753,000 cords to 3,557,000 cords. Although recent Lake States wood supply inventories indicate aspen acreage is adequate to meet current demand, there appear to be imbalances in age classes. Older age classes are available to offset these imbalances, but recent surveys taken in Wisconsin indicate these older stands are deteriorating and converting to other types. They also tend to be marginal in wood quality and stocking. As fuel and harvesting costs increase, an inevitable situation, these older, marginal quality stands will become less attractive as a wood source.

IPC breeding work has been directed at producing a hybrid suitable for establishment on the types of sites most widely available. Those areas tend to be upland and sandy with site indexes ranging from 50-70 feet. The growth rate of hybrid aspen has been measured on a range of those types of sites. On all but the poorest sites, hybrid growth has been double that of native aspen, and rotation age shortened to 20 years. Prolific and vigorous suckering after harvest assures that fully-stocked stands will be reproduced.

Our breeding work has also emphasized the improvement of wood properties affecting pulp and paper quality. A significant increase in wood density has increased the amount of fiber available from an acre of land. A concurrent improvement in fiber length contributes to increased tearing strength, a factor in hardwood pulps often improved by the addition of expensive conifer fiber.

Hybrid aspen can help meet future wood requirements. Site selection guidelines have been worked out, establishment requirements have been determined, and seedbed and vegetative propagation methods are available. The direction of future work is toward increased availability of planting stock. Future research must address disease resistance and continued wood quality improvement.

1986 CROSSING PROGRAM

The 1986 breeding work again emphasized the production of hybrid seed for planting stock and the production of *P. tremuloides* seed for hypoxylon bioassay work (Table 1). The availability of pollen from the tetraploid clone Ta-10, and large numbers of female flowers from the diploid *P. tremuloides* clones T-50-60 and Clone 5 allowed the production of two large seedlots. Both clones were collected from the former Packaging Corporation of America seed orchard near Free Soil, Michigan. Although the land on which the orchard is located was recently sold, an arrangement with the new owner through which the orchard is leased on an annual basis has been agreed to. Large collections of branches bearing flowerbuds will be made from this orchard allowing several clones in the IPC Greenville arboretum to recover from the repeated annual collection of branches.

Thirteen hybrid crosses were made for seedling production by cooperators, IPC, and the State of Wisconsin. An additional eleven small *P. tremuloides* crosses were made for seedling production for use with the hypoxylon bioassay. Six of the bioassay crosses had Clone 5 as the female parent. Progeny tests with Clone 5 have shown a low incidence of hypoxylon infection. It has been several years since this clone flowered, and full advantage was taken of last year's flowering to produce crosses with corresponding hypoxylon canker field data. A discussion of the bioassay results from tests of two of these crosses is given in the Hypoxylon Screening section of this report.

Seed production was sufficient to meet cooperator requests last year, but it is apparent that work is needed to both improve our seed production capabilities, and to get future seed production areas (orchards) established with cooperators. Long-term progeny tests have identified the best parent clones. Those same progeny tests are now providing diploid second generation selections.

Table 1. Summary of 1985 crosses and location of parent trees.

Cross Number[a]	Parents (Female x Male)		
XT-1-86	Clone 5 (Wausau, WI)	x	T-44-60 (Ralph, MI)
XT-2-86	Clone 5 (Wausau, WI)	x	T-46-60 (Ralph, MI)
XT-3-86	Clone 5 (Wausau, WI)	x	T-10-60 (Ontonagon, MI)
XT-4-86	Clone 5 (Wausau, WI)	x	T-20-60 (Alston, MI)
XT-Ta-5-86	Clone 5 (Wausau, WI)	x	Ta-10 (Ekebo, Sweden)
XT-6-86	T-24-60 (Wausau, WI)	x	T-6-61 (Fern, WI)
XT-7-86	T-24-60 (Wausau, WI)	x	T-10-60 (Ontonagon, MI)
XT-8-86	T-50-60 (Ralph, MI)	x	T-10-60 (Ontonagon, MI)
XT-Ta-9-86	T-50-60 (Ralph, MI)	x	Ta-10 (Ekebo, Sweden)
XT-10-86	T-53-60 (Fern, WI)	x	T-44-60 (Ralph, MI)
XT-Ta-11-86	T-53-60 (Fern, WI)	x	Ta-10 (Ekebo, Sweden)
XT-12-86	T-5-61 (Ontonagon, MI)	x	T-10-60 (Ontonagon, MI)
XT-Ta-13-86	T-5-61 (Ontonagon, MI)	x	Ta-10 (Ekebo, Sweden)
XTa-T-14-86	Ta-6-68 (West Germany)	x	XT-33-68, S-201 (Greenville, WI)

1 (Contd.) Summary of 1985 crosses and location of parent trees.

ross Number[a]	Parents (Female x Male)		
T-Ta-15-86	T-12-58 (Clintonville, WI)	x	Ta-10 (Ekebo, Sweden)
T-Ta-16-86	T-1-58 (Ontonagon, MI)	x	Ta-1-68 (West Germany)
T-Ta-17-86	T-1-58 (Ontonagon, MI)	x	XTa-3-68, S-1 (Greenville, WI)
T-18-86	Clone 5 (Wausau, WI)	x	Clone 7 (Wausau, WI)
T-19-86	Clone 5 (Wausau, WI)	x	T-6-68 (Ontonagon, MI)
T-Ta-20-86	T-20-56 (Watersmeet, MI)	x	Ta-10 (Ekebo, Sweden)
Ta-T-21-86	Ta-7-68 (West Germany)	x	XT-33-68, S-201 (Greenville, WI)
Ta-T-22-86	Ta-7-68 (West Germany)	x	T-44-60 (Ralph, MI)
Ta-T-23-86	Ta-6-68 (West Germany	x	T-44-60 (Ralph, MI)
Ca-T-24-86	Ca-2-66 (Czechoslovakia)	x	T-44-60 (Ralph, MI)
Ta-0-25-86	Ta-6-68 (West Germany)	x	Wind
Ta-0-26-86	Ta-7-68 (West Germany)	x	Wind

Appendix for description of crossing code.

The availability of flowerbuds for use this year is less than last , but cooperator needs should be met. Although not a project member, the : of Wisconsin has sought access to hybrid aspen seed, and an agreement was during the phasedown of the aspen project that allowed the state to purchase seed, and provided for aspen cooperator access to a portion of the seedlings grown by the state. Cooperator seed requirements are met first, and remaining seed is made available for state use. There apparently is some confusion regarding seedling access from the State of Wisconsin and hopefully it will be resolved.

HYPOXYLON SCREENING CROSSES

Eleven crosses were made in 1986 (Table 2) for use in research utilizing a toxin screening method. Five additional crosses were made using four female selections and three male selections. Three of those crosses have field performance ratings with low hypoxylon incidence, and the remaining three are part of a crossing matrix that pairs widely used males with widely used females. Those crosses will be bioassayed, and the results used to determine parental reactions to mammatoxin.

Information regarding the bioassay can be found in the Hypoxylon Screening section of this report. A summary and discussion of results is also included in that section.

TRIPLOID HYBRID SEED PRODUCTION

The 1986 crossing program again emphasized the production of triploid hybrid aspen seed. The availability of pollen from the tetraploid *P. tremula* (Ta-10) ramets in the IPC Greenville arboretum, and good flowering of three female clones in the former PCA orchard near Free Soil, Michigan, along with several female clones in the IPC arboretum, allowed the production of 918,000 triploid hybrid seeds. All cooperator requests for triploid seed were met.

Table 2. Summary of 1985 seed production.

Cross[a]	Number of Catkins Pollinated	Number of Catkins Collected	Number of Seeds	Seeds/Catkins Pollinated	Germination, %	Purpose
XT-1-86	12	12	10,251	854	90	Hypoxylon screening
XT-2-86	12	12	6,652	554	90	Hypoxylon screening
XT-3-86	14	13	9,389	671	89	Hypoxylon screening
XT-4-86	15	15	17,464	1,164	95	Hypoxylon screening
XT-Ta-5-86	1,055	1,006	374,811	355	70	3N seedling production
XT-6-86	15	13	16,008	1,067	97	Hypoxylon screening
XT-7-86	14	13	13,264	947	99	Hypoxylon screening
XT-8-86	16	16	14,184	886	99	Hypoxylon screening
XT-Ta-9-86	835	802	305,657	366	84	3N seedling production
XT-10-86	14	14	6,945	496	88	Hypoxylon screening
XT-Ta-11-86	381	377	49,392	130	48	3N seedling production
XT-12-86	11	11	10,418	947	83	Hypoxylon screening
XT-Ta-13-86	274	247	122,790	448	23	3N seedling production
XTa-T-14-86	318	--	6,005	19	65	3N seedling production
XT-Ta-15-86	392	384	36,373	93	4	3N seedling production
XT-Ta-16-86	196	190	2,488	12	95	Seedling production
XT-Ta-17-86	459	392	43,122	94	98	Seedling production
XT-18-86	11	10	711	65	81	Hypoxylon screening
XT-19-86	11	11	1,925	175	92	Hypoxylon screening
XT-Ta-20-86	579	552	29,162	50	31	3N seedling production
XTa-T-21-86	313	306	2,364	8	92	3N seedling production
XTa-T-22-86	244	238	54,212	222	85	Seedling production
XTa-T-23-86	76	--	3,088	41	92	Seedling production
XCa-T-24-86	170	163	4,710	27	92	Seedling production
XTa-O-25-86	--	--	22,375	--	100	Seedling production
XTa-0-26-86	--	--	98,458	--	99	Seedling production

[a]See Appendix for description of crossing code.

Pollen production from Ta-10 was greater than needed and a large quantity was stored for the 1987 crossing program. Stored pollen was used in the 1986 crossing work as an extender for fresh pollen, and will also be used as an extender this year.

The germinative ability of the triploid hybrid seed, on the average, is less than that of _P. tremuloides_ x _P. tremuloides_ seed but varies by clone. For example, the female _P. tremuloides_ clone T-5-61, has high numbers of seed per catkin, but the germination of hybrid seed from that clone is always less than 40%. This year's seedlot (XT-Ta-13-86, Table 2) averages 23%. Other triploid hybrid crosses, most notably XT-Ta-15-86 and XT-Ta-20-86, also have low germination rates (Table 2). The female clones used in those crosses do not have the history of low germination. Poor germination may have been due to the low vigor of one of the female clones, and pollinating at a nonreceptive stage.

In addition to the triploid crosses made with Clone Ta-10, triploid crosses were made using pollen from one of the two tetraploid _P. tremuloides_ clones produced at the Institute. Neither of these clones flowered in 1985, and therefore stored pollen from 201-68 (XT-33-68, S-201) was used in two crosses (XTa-T-14-86 and XTa-T-22-86). Seed set was low, but germination was 65 and 92%. The two _P. tremuloides_ tetraploid clones are still relatively young, and flowering has been sporadic and light. When pollen is available, crosses will be made and performance evaluated. Seedlings from one cross have been established in a replicated trial by the U.S. Forest Service on their Harshaw Farm near Rhinelander, Wisconsin. As reported last year, growth and survival have been very good, and this new triploid hybrid is expected to perform as well as the triploids from Ta-10. Additional replicated trials are planned to evaluate performance and wood quality as soon as seed and seedling production allow.

1986 SEEDBEDS

The production of hybrid aspen seed and seedlings is both a major objective and a bottleneck to expanding the use of hybrid aspen for operational plantings. The technique for seedbed production of hybrid aspen seedlings is to the point where reliable production can be achieved from both mechanical and hand sowing.

Container-grown seedlings are also being produced for direct field planting, and as an intermediate step for "plug plus one" seedlings where the growing season is not long enough for a plantable 1-0 seedling. Container-grown hybrid aspen seedlings for direct field planting present problems different from those encountered in seedbed production. As the seedlings grow, large leaves prevent even distribution of water, and with the large leaf surface area and small volume of growing medium, considerable water is required. Seedling caliper and height are frequently marginal because of competition for light in close-spaced containers. The use of containers as an intermediate growing step before transfer to a nursery lineout area has been shown to be a feasible method for seedling production. Growing container seedlings for direct planting may require using containers with large volumes of growing medium and relatively few cells per square foot. These constraints on hybrid aspen container growth may make production uneconomical. However, an advantage to container production is the efficient use of scarce, high value seed. Results to date suggest that further work on growing regimes is warranted.

IPC SEEDBEDS

For several years, the IPC Greenville Nursery has not been used to produce significant quantities of hybrid aspen seedlings. Seed from annual crossing programs was directed to cooperators to meet their requirements. Seedling production was therefore being transferred to cooperators.

A possibility for additional funding developed in 1985 when the U.S. Department of Energy (DOE) sent out proposal requests for development of short rotation plantations for energy production. Although not an advocate of short rotation ("biomass") energy plantations, it was believed that a suitable proposal could be developed utilizing hybrid aspen as the woody species for volume production evaluation. A proposal was developed and submitted in cooperation with Consolidated Papers, Inc. that attempted to address the objectives outlined by the DOE request, but which would also allow other evaluations of plantation development more suitable to project goals. To be prepared to meet the DOE timetable, if the proposal was accepted, required a commitment to seed production in the 1986 crossing program, and a commitment to seedling production in the 1986 seedbeds. Unfortunately, the hybrid aspen proposal was not accepted.

The plus side to this expenditure of resources and materials was the production of over 25,000 hybrid aspen seedlings, part of which will be used to establish a 40 acre planting with Consolidated Papers on the site selected for the joint DOE study. The Michigan Department of Natural Resources and Blandin Paper Company will also utilize some of the production for field plantings.

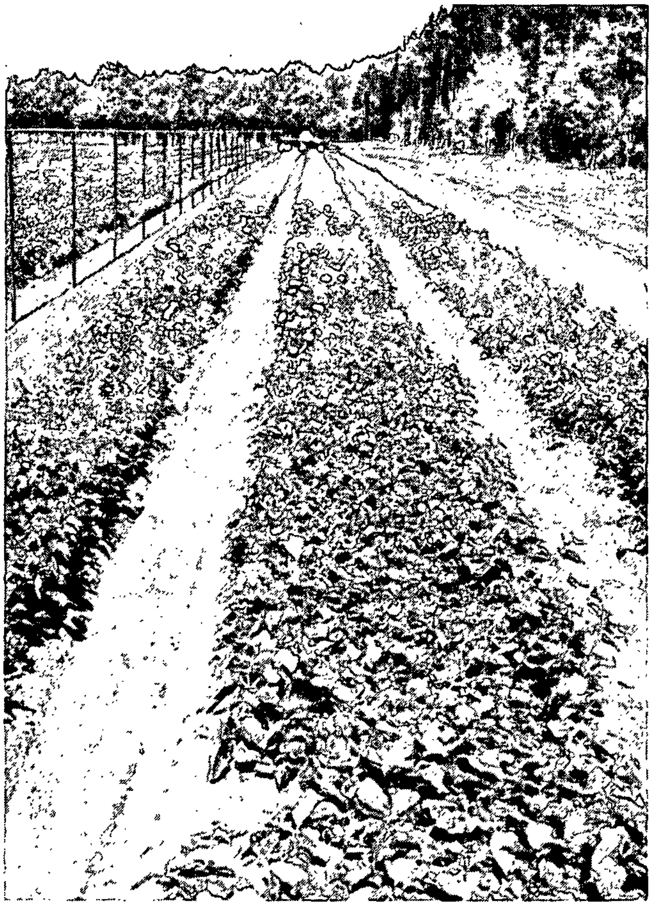
Seedbed production in 1986 is given in Table 3. The beds were sown at the rate of 25 viable seeds per square foot with a target density of five plantable seedlings per square foot. A total of 5620 square feet were sown, and 4.5 plantable seedlings per square foot were lifted. An additional 1.8 undersize seedlings per square foot were also produced and will be lined-out and grown for another year. Figure 1 illustrates the appearance of the seedbeds just prior to leaf drop.

Table 3. 1986 IPC seedling and clonal production.

Material	Total No. Plantable	No. Undersized
XT-Ta-5-86[a]	14,200	5,900
XT-Ta-5-86 and XT-Ta-20-86 mix	1,000	175
XT-Ta-11-86	5,250	2,200
XT-Ta-13-86	4,350	1,750
XT-14-82	320	--
Raverdeau	550	--
XT-Ta-10-58, S-5	267	--
XT-Ta-14-58, S-23	17	--
XT-Ta-14-58, S-24	65	--
XT-Ta-6-64, S-1	241	--
XT-Ta-10-69, S-1	234	--
XT-Ta-10-69, S-2	88	--

[a]See Appendix for description of crossing code.

As in past years, the seedlings were lifted in late fall after leaf drop, sorted, bundled, then heeled-in in sand, watered, and allowed to freeze. With



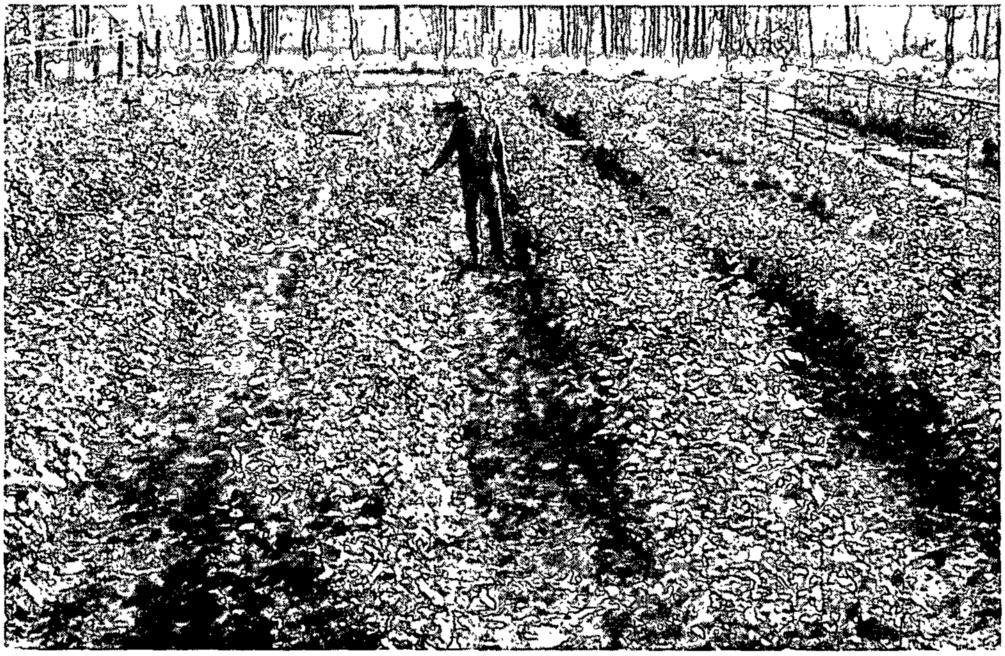


Figure 1. The Wisconsin DNR Hayward Nursery seedbeds shown in the upper photograph contain triploid hybrid aspen seedlings as they appeared in June. The beds were sown in midsummer the previous year and will be lifted as 1 1/2-0 stock. The beds shown in the lower photograph are 1-0 triploid hybrid aspen in the IPC Greenville Nursery in late fall.

the removal of the barn in which the seedlings had been stored, alternative methods for winter storage were considered. The most practical method, and one that had been used to a limited extent, was to place the graded and counted seedlings directly into wax coated corrugated boxes with plastic liners. Sphagnum moss was used as the packing, and root systems were well covered. In the previous year, the boxed seedlings were allowed to freeze, and were subjected to wide variations in temperature in an unheated pole barn. The wide range of temperature was not acceptable so freezer storage space was rented at a surprisingly low cost. The storage regime is held at a constant 30°F, and the seedlings are examined every six weeks to monitor their condition.

The clonal material shown in Table 3 (the cross codes with an S-number designation) were propagated from roots taken from superior hybrid individuals. The relatively small number of individuals produced from each clone represents the initial buildup of material for large-scale propagation. Five of these clones will be planted this spring in a small clonal trial. As additional clonal selections are propagated in sufficient numbers for field testing, larger trials will be planted.

WISCONSIN DEPARTMENT OF NATURAL RESOURCES' HAYWARD NURSERY SEEDBEDS

The method of mechanical sowing of hybrid aspen seed being developed by the Wisconsin DNR and described in last year's annual report was used to sow beds again this past summer. Germination and seedling development has been very good and bed densities are near the target of 10 seedlings per square foot (Fig. 1). John Borkenhagen, WDNR Hayward Nursery Manager, was disappointed with previous years' bed densities when sown for 5 seedlings per square foot, and felt that his nursery conditions required sowing for twice that amount. The Northern location of this nursery made it difficult to produce an acceptable number of plantable 1-0 seedlings. By sowing at the end of June, and growing the seedlings through the following year (1 1/2-0), an acceptable seedling was grown. However, under this growing regime, the number of seedlings per square foot had to be increased or the seedlings grew excessively and were too large for conventional field planting.

The inventory indicates that 44,000 hybrid aspen seedlings will be shipped this spring. An additional 20,000 seedlings are in beds and will be available the spring of 1988. The State of Wisconsin has asked for triploid hybrid seed for sowing this summer. Although not a member of the cooperative project, it was deemed worthwhile to sell seed to the state. An agreement was made whereas a portion of the stock would be made available for purchase by cooperative members. The implementation of that agreement has not been forthcoming and further discussions are necessary before additional seed is made available.

FIELD TRIAL EVALUATION

The success of selection and breeding to improve growth rate, wood quality, and disease resistance can only be evaluated through well designed field trials and established block plantings. The aspen tree improvement project is in a unique position to evaluate growth rate and the inheritance of wood properties for several types of aspen hybrids because of the foresight of the IPC tree improvement workers that began the breeding work with aspen. A series of replicated field trials have been planted since 1956 and continue to add to the evaluation of project parent trees. They are also providing second generation selections which will lead to further gains.

There is a need to test progeny from these second generation selections. The aspen project originally had test areas on eight different sites. The majority of these test areas are either occupied by past field trials or have been lost through transfer of company ownership. The need for additional space and a commitment to provide long-term access to that space is critical for continued development of the improved materials now available.

EXPERIMENTAL TRIAL VII

A series of Populus tremuloides crosses were made from 1956-1959 to study the heritability of selected properties. The trial was originally planted at a 6 x 3 foot spacing. At age five every other tree was cut and used for wood quality study, the results of which have been given in early Project 1800 progress reports. Trial VII was not replicated but is a series of 55 tree blocks that were thinned to 30 trees. The growth and disease incidence of these 30 tree blocks are summarized in Table 4.

Trial VII tests several of the first crosses made in the aspen project and is contributing to the understanding of hypoxylon canker frequency in progeny from a number of parent trees currently being used in the project. The growth rates given in Table 4 are similar to or greater than native aspen. The incidence of hypoxylon is also similar to what occurs in natural stands although there are crosses with high incidence and one cross, XT-5-58, with a very low level of hypoxylon infection.

Selections being made within several of the "best" families for growth, form, or low incidence of hypoxylon will be used in this year's crossing program to produce both hybrid and intraspecific seed for possible progeny testing. Figure 2 illustrates the form and size of one of the family selections.

EXPERIMENTAL TRIAL X

Trial X is located on the Ripco Test Area in Oneida County, Wisconsin, south of Eagle River. It compared the growth and suckering ability of two triploid hybrid crosses, two native triploid clones, and two diploid progeny groups. The trial had a four replicate, randomized-block design with three replications cut at age five to form a suckering study, and the fourth replication was left intact.

Growth measurements were taken at several ages for 15 years on the suckers and for 20 years on the intact replication. The data acquired from this trial have contributed significantly to our evaluation of triploid hybrids on medium quality hardwood sites.

Cross	Year Planted	20 Years Av. Ht., feet	Av. dbh, inches	Survival, %	Hypoxylon, %	29 Years Av. Ht., feet	Av. dbh, inches	Survival, %	Hypoxylon, %
XT-1-56	1957	35	4.5	23	91	58[a]	7.6	10	91
XT-5-56	1958	45	4.4	77	32	57	5.9	50	54
XT-6-56	1958	46	4.4	50	48	55	6.1	43	62
XT-12-56	1957	38	4.2	25	48	48[a]	5.7	40	48
XT-22-56	1958	50	5.3	57	47	62	6.8	50	63
XT-32-56	1958	51	5.4	50	46	59	7.4	40	62
XT-36-56	1958	51	5.2	57	39	64	7.1	43	48
XT-10-57	1958	53	5.6	57	44	61	7.4	43	52
XT-11-57	1958	49	5.9	43	67	59	7.6	33	70
XT-12-57	1958	42	5.3	80	21	52	6.6	77	45
						28 Years			
XT-4-58	1959	50	5.5	47	60	58	6.7	27	75
XT-5-58	1959	51	4.9	80	15	58	6.3	70	21
XT-7-58	1959	48	5.2	40	64	62	6.7	27	70
XT-8-58	1959	42	4.6	53	48	46	5.9	40	65
XT-9-58	1959	45	4.9	73	30	54	5.9	50	44
XT-12-58	1959	50	5.7	67	48	61	7.2	40	67
						27 Years			
XT-6-59	1960	42	5.7	53	48	49	7.4	40	71
XT-7-59	1960	43	4.9	50	48	51	6.1	47	63
XT-8-59	1960	47	5.6	63	38	55	6.5	53	55
XT-10-59	1960	46	5.4	57	43	56	7.1	43	54
XT-11-59	1960	49	5.3	43	59	54	7.1	37	66
XT-12-59	1960	44	4.6	73	24	54	5.9	60	46
XT-13-59	1960	46	5.1	60	31	55	6.2	50	58
XT-14-59	1960	44	5.0	40	48	58	6.9	30	57
XT-15-59	1960	45	5.1	50	46	51	6.2	43	59

[a]30 Year data.

Figure 2. Second generation P. tremuloides selections from the best families are now being made for use in the aspen project's breeding program. The individual shown is from cross XT-5-58, and was planted in Greenville Trial VII in 1959. The selection is from the family with the lowest hypoxylon canker incidence of the 25 families in the trial; growth rate and form are also above average.

An opportunity arose this past year to cut Trial X for the second time. The sucker stand portion of the trial was 23 years old and the intact portion was 28 years old. Figure 3 depicts the size of trees harvested. Several superior hybrid individuals were selected for propagation and use in the clonal forestry aspect of the aspen project. Table 5 presents the growth information for five of the selected individuals and for 22 dominant and codominant stems randomly measured during harvest. All heights and diameters shown were taken after the trees were felled. Individual tree volumes were calculated from form factor volume tables.[1]

Figure 3. This 23-year-old sucker stand was harvested this past fall in Trial X on the Ripco Test Area near Eagle River, Wisconsin. Fifty-seven cords with a top diameter of 5 inches, and 6 cords with a 3-inch top diameter were removed from approximately 1-1 1/2 acres.

Table 5. Comparison of selected tree size to dominant and codominant trees in Trial X.

Material	Age	Total Ht., feet	Ht. to 4-inch top, ob	Dbh, inches	Form Factor	Volume, ft^3
Average of 6 clones	23	72	51	10.9	80	17.6
Average of 22 dominant and codominant stems	23	62	36	7.0	81	6.8

The superiority of the selections is clearly illustrated by the size and volume differences given in Table 5. Height growth for the selections averaged over 3 feet per year as compared to 2.7 feet per year for the average dominant stems. Diameter growth averaged 0.46 inch per year for the selection and 0.30 inch for the dominants. The difference in diameter growth accounted for the significant differences in individual tree volumes.

Although growth rate has been greatly increased, an attendant loss in wood density has not occurred. To the contrary, the average specific gravity of the triploid hybrid wood removed from Trial X was 0.40. Native P. tremuloides has an average wood specific gravity of 0.35. The gain in density is significant in that a cubic foot of ovendry wood from native P. tremuloides would weigh 21.8 lb and from the triploid hybrids would weigh 25 lb. The increase in wood density means more fiber per unit of volume, and when considered at a pulp yield of 50%, the difference between an 80 ft^3 cord of native aspen (1744 oven dry lb) and hybrid aspen (2000 o.d. lb) is considerable. Previous fiber length analyses have also shown an increase of 25%.

A total of 57 cords with a minimum top diameter of five inches, and 6 cords to a three inch top were removed from approximately one and a half acres. It should be noted that only two of the seedling sources established in Trial X were hybrid. Although these hybrids suckered prolifically, and were the major component of the stand at harvest, there was considerable area occupied by lower quality stems.

A condition known as mineral stain was present in the majority of the stems cut. It has a reddish appearance attributable to the iron component frequently associated with this type of stain. It is not a normal occurrence and is associated with injury to boles or root systems. It is speculated that soil compaction from early attempts to keep lanes between plots free of suckers by annual mowings may have inadvertently damaged root systems.

Part of the wood harvested was used in a stone groundwood pulping trial and in a flakeboard trial. The mineral stain condition contributed to a lower brightness compared to native aspen (62.2 vs. 64.2) in the groundwood trial, but the effect on brightness was surprisingly minimal. The mineral stain components are primarily water soluble, and the IPC Pulping and Bleaching Group indicate that bleach chemical requirements to achieve brightness levels of 80+ would most likely be equivalent to that required by mill run native aspen. Mineral stain is not expected to occur at such levels under operational conditions.

The land on which Trial X is located belongs to Wausau Paper Mills but an agreement has been reached that will allow the project continued access to the site. Measurements will be taken this fall to evaluate suckering, and frequency of hybrid suckers.

EXPERIMENTAL TRIAL LI

Trial LI is located on the Cunard Test area in Menominee County, Michigan. The site is usually wet, and prohibited the use of machinery for grass control. Of the seven trials on the area, Trial LI had the best vegetation control during establishment.

The trial compares triploid progeny from interspecific crosses of *P. tremuloides* and *P. tremula* made in 1970. The trial design is a four replicate, randomized block, 16 tree plots with borders. Table 6 summarizes 10 and 17 year growth, survival, and hypoxylon data. The drop in survival between year 10 and year 17 was in part due to *Hypoxylon mammatum*, but there were other factors contributing to mortality that could not be determined. The incidence of hypoxylon infection was greater than average for four crosses and average for four others. Height and diameter growth was also average, although there were a number of individuals over 50 feet and 7.0 inches dbh in both the diploid and triploid TxTa crosses. The individual illustrated in Fig. 4 is 55 feet and 7.6 inches dbh and will be propagated for clonal testing.

Figure 4. The 17-year-old triploid hybrid aspen shown is from cross XT-Ta-7-69 in Trial LI on the Cunard Test Area near Norway, Michigan. The tree is 55 feet in height, 7.6 inches dbh, and has excellent form. It will be propagated and tested for use in the clonal forestry aspect of the aspen project.

Table 6. Ten and seventeen year growth in Trial LI.

Material	10-Year Av. Ht., feet	10-Year Av. dbh, inches	10-Year Survival, %	17-Year Av. Ht., feet	17-Year Av. dbh, inches	17-Year Survival, %	17-Year Hypoxylon,** %
*XTa-T-9-68	30.2	3.2	91	40.7	4.7	75	31
*XT-17-68	22.2	2.4	52	35.0	5.3	30	28
XCa-Ta-6-69	23.8	2.9	66	38.8	5.1	54	15
XT-Ta-7-69	28.1	3.2	75	39.6	5.0	58	37
XT-Ta-8-69	27.8	3.1	83	38.0	4.8	52	40
XT-Ta-9-69	29.6	3.2	82	41.2	5.6	41	26
XT-Ta-10-69	30.0	3.4	86	31.3	4.4	12	22
XTa-11-69	23.0	2.4	56	24.7	3.2	48	22

*Diploid crosses; all others are triploid.
**Total hypoxylon canker incidence. Values given reflect all trees that presently have hypoxylon or have died from hypoxylon.

EXPERIMENTAL TRIALS LXV AND LXVI

Two replicated trials were established on IPC's Greenville Test Area II in 1985. These trials will evaluate the material produced by Dr. Steven Wann in his Ph.D. thesis work with a Hypoxylon mammatum bioassay.

Trial LXV compares Populus tremuloides seedlings with tissue culture produced clones from those seedlings. In addition to testing the bioassay, the trial will evaluate the performance of tissue culture plants (Fig. 5) produced from one of the cotyledons of a seedling that was grown to field plantable size with the remaining cotyledon. Individuals selected for the trial were subjected to a mammatoxin leaf puncture bioassay. Trees exhibiting a sensitive reaction to the mammatoxin (large leaf lesion), and those showing no reaction were planted as part of a long-term evaluation of the bioassay procedure. A seedling and four tissue cultured derived ramets from that seedling were planted side by side. Growth and form will be monitored to evaluate tissue culture as a means of clonally propagating superior hybrid selections.

Trial LXVI also evaluates tissue culture derived plants and full-sib seedlings that tested reactive and nonreactive in the mammatoxin bioassay. The trial differs from LXV in that the donor plant is not contrasted with tissue culture ramets. The trial is designed for a long-term evaluation of Hypoxylon mammatum canker incidence and correlation with the bioassay information. Seedling material will be added annually to form a large population from which data can be acquired.

ure 5. The tissue culture propagated P. tremuloides individual shown is in Trial LXV on the Greenville Test Area. The trial compares the original seedling (ortet) with the tissue culture plants (ramets) derived from it. The trial also tests individuals showing a reaction or nonreaction to mammatoxin. Growth of the tissue culture plants at the end of one year is no different than that of the seedlings.

DEMONSTRATION PLANTING EVALUATION

BLANDIN PAPER COMPANY - AUDETTE FARM

In 1965, a small planting comparing a triploid hybrid aspen source with a native Minnesota P. tremuloides source was established on Blandin Paper Company's Audette farm, south of Grand Rapids, Minnesota. All seedlings were grown in Blandin's nursery and planted as 1-0 stock. The soil around each tree was treated with simazine immediately following planting, and some injury and mortality as a result of the herbicide treatment occurred.

The planting was measured in 1972 at age eight. The hybrid aspen averaged 33.1 feet in height and 4.1 inches dbh. The native aspen averaged 29.3 feet in height and 3.4 inches in diameter (Table 7). The triploid hybrid portion of the planting was measured at age 15 for diameter only, just prior to harvesting. Volume estimates at the time of harvesting indicated growth was at 249 cubic feet per acre per year, which was greater than the 205 ft^3/acre/year from a triploid cross at age 15 in Ripco Trial X.

The sucker stand that developed after harvesting was measured this past fall at age 8. Eight 1/100 acre plots were taken within the stand. Stems per acre ranged from 4800 to 7600 with an average of 5988. No trees less than 12 feet were present. The dominant and codominant stems were 36 to 44 feet in height with diameters from 2.0-3.9 inches bh and numbered, on the average, 14 per plot (Fig. 6). Volumes were calculated from Bella's formula[2] which had been used extensively in earlier IPC project work with sucker stand measurements, and was found to agree closely with regression equations developed through IPC weight table work. Cubic foot volumes were calculated to a half inch top minus branches. Stem weights were determined by using an average specific gravity of 0.367. The specific gravity will increase with age and should average 0.40 at the time of harvest.

Table 7. Blandin Paper Co. triploid hybrid growth information.

Material	8-Year Plantation[a]			15-Year Plantation[a]		
	Av. Ht., feet	Av. dbh, inches	Survival, %	Av. dbh, inches	Survival, %	Volume, ft^3/acre/yr
Triploid hybrid	33.1	4.1	88	7.2	64	249
Native *P. tremuloides*	29.3	3.4	92	Not Measured		

	8-Year Suckers[b]				
	Stems/ acre	Av. Ht., feet	Av. dbh, inches	Volume,[c] ft^3/acre/year	Stem o.d., wt./acre/year
Triploid hybrid	5988	27.5	1.7	230	4814

[a]Data provided by Blandin Paper Co.
[b]Data from IPC measurements.
[c]Wood plus bark to 1/2-inch top minus branches.

Although it is a small sucker stand, it is a further demonstration of the potential of hybrid aspen. Suckering was prolific and growth is as rapid as predicted. The stand will continue to be measured and compared to previous growth data.

Figure 6. The 8-year-old triploid hybrid aspen sucker stand on Blandin Paper Co. land averaged 33 feet in height and 4.1 inches in diameter. Volume growth averaged 230 cubic feet per acre per year, the same growth rate the stand had at age 15 when it was cut back.

BLANDIN PAPER COMPANY - CARLSON CUT AREA PLANTING

A 13 acre planting of triploid hybrid aspen was established the spring of 1985 on a northern hardwood site (S.I. 55-65) that had been sheared and raked in 1983. The site was further prepared for planting by patch scarifying and banding with Oust (4-5 oz/acre) in 1984.

A total of 7195 container grown seedlings were hand planted at an approximate spacing of 10 feet within rows and eight feet between rows.

Measurements on 10 randomly chosen rows indicated survival was 67%, ranging from a low of 50% to a high of 89%. There was considerable variation in height with an average of 3 feet and the better stems over 5 feet. The best individual in the planting is shown in Fig. 7. It was 12 feet in height after two years in the field. Roots will be collected from this individual, and root sprouts produced for testing and possible inclusion for use in clonal forestry.

Figure 7. An exceptional 2-year-old container grown triploid hybrid aspen on Blandin Paper Co. land in Northern Minnesota. The tree was 12 feet in height and had grown 10 feet this past year. It is the best seedling in the planting and will be propagated and evaluated for possible use in clonal forestry.

The returning herbaceous vegetation is producing considerable competition. Bull thistle in particular is extremely dense as a result of the Oust treatment. The effect of the competition appears to be shading rather than moisture availability. The smaller stems (less than two feet) are spindly with elongated internodes. Larger stems are vigorous and appear capable of surpassing the competition this coming growing season.

MICHIGAN DEPARTMENT OF NATURAL RESOURCES - MELSTRAND PLANTING

A poor quality hardwood site in Upper Michigan's Alger County was selected in 1976 for a hybrid aspen conversion trial. The area consisted of poor quality hardwood saplings and sparsely vegetated openings. The hardwoods were pushed out and windrowed with the outside row serving as a deer barrier.

The soil is classified in the Seney series which is between Kalkaska and Rubicon in quality. The area was under management for sharptailed grouse during the 1930's and was burned several times. The ground cover in the opening was orange hawkweed, lichens, and sparse grass.

Three different aspen hybrids (*P. canescens* x *P. grandidentata*, *P. canescens* x *P. tremuloides*, and *P. tremuloides* x *P. tremula*) were hand planted by an inmate crew from the Cusino Corrections Camp. First year survival varied from total failure on the open portion of the site to greater than 90% on the formerly wooded area. The open area was replanted the following year and failed once again. A number of factors were considered and examined, but no specific cause of mortality could be determined. Growth on the formerly wooded portion of the site was good. At about age 5, the leaf bronzing we reported on *P. grandidentata* hybrids in other plantations began to appear on the *P. canescens* x *P. grandidentata* hybrid in this planting and to a lesser extent on the *P. canescens* x *P. tremuloides* hybrid. The *P. tremuloides* x *P. tremula* hybrid was not affected.

Over the past several years the leaf disease has gotten progressively worse, and the *P. canescens* x *P. grandidentata* hybrid suffered almost complete mortality. The *P. canescens* x *P. tremuloides* hybrid also had mortality but to a lesser extent. The *P. tremuloides* x *P. tremula* hybrid remains unaffected.

The *P. tremuloides* x *P. tremula* portion of the planting was measured this past fall at age 11. Growth and survival was very good with heights averaging 31.7 feet and diameters averaging 4.2 inches. The dominant individuals were over 40 feet and 5.5 inches (Fig. 8). The tallest tree measured was 45 feet and 5.7 inches dbh. *Hypoxylon mammatum* canker incidence was 11.5%, a low average rate. The *P. tremuloides* x *P. tremula* hybrid material is suckering profusely and occupying open areas.

Despite the mortality that occurred for two of the hybrids, the planting is considered successful because of the information being gained from the one established hybrid. The planting will continue to be observed and measured as warranted. Cutting back the planting was considered as a means to more fully occupy the site but long term plantation growth observations may be more important. Several individuals are suitable for selection, propagation, and testing for possible use in clonal forestry.

Figure 8. The best hybrid aspen source on a poor quality hardwood site on Michigan DNR land near Munising, Michigan was a diploid P. tremuloides x P. tremula cross. At age 11, height growth averaged 31.7 feet and diameters averaged 4.2 inches. The hybrid is beginning to sucker in open areas adjacent to established trees.

CLONAL SELECTIONS

Exceptional hybrid aspen individuals are being selected for use in the lonal aspect of Project 3537. Clonal forestry has numerous advantages over onventional seedling-based forestry, most of which have been well described in n article by Libbey and Rauter.[3] The aspen project's selections now available or testing meet all of the criteria put forth in that paper, and include an ıdditional criterion that was not specifically mentioned, that being exceptional ;ood quality. With increased harvesting and transportation costs, it is not :nough to improve growth rates; a concurrent improvement in wood quality is also ıeeded.

For the large-scale use of selected hybrid aspen clones to be feasible, it is necessary to develop a method of rapid, cost-effective mass propagation. Current propagation methods are restricted to the rooting of root sprouts or greenwood cuttings from young, hedged plants. Conventional propagation methods can produce large numbers of rooted individuals at a competitive cost with seedlings when factors such as growth rate, adaptability, wood quality, disease resistance, etc., are included in the cost analysis. Micropropagation is another tool that may become available for use.

Vegetative propagation methods to exploit exceptional clonal material are available. What is required is a large-scale demonstration of the advantages of clonal plantings. To this end, the aspen project is considering the production of material for testing for site adaptability, compatibility with other clones, and for demonstration block plantings.

1986 SELECTIONS

Six triploid hybrid aspen selections were made in Trial X on the Ripco Test Area. One of those selections was made in 1985 and is described in last year's report. Five additional selections were made this past year prior to the cutting of Trial X. Potential triploid hybrid selections were noted in several other plantings and will be evaluated this coming year. Table 8 lists the five selections made. Wood quality information is also being gathered and will be presented in a future report.

Table 8. 1986 clonal selections.

Clone Identification[a]	Location	Age	Total Height, feet	Height to 4-Inch Top, ob	Dbh, inches
XT-Ta-14-58, S-25	Trial X	23	68	47	11.1
XT-Ta-14-58, S-26	Trial X	23	69	50	9.9
XT-Ta-14-58, S-27	Trial X	23	70	52	10.5
XT-Ta-14-58, S-28	Trial X	23	75	53	12.0
XT-Ta-14-58, S-29	Trial X	23	73	51	9.6

[a]See Appendix for description of identification code.

Although the majority of the selections made to date have been triploid hybrids, individuals from other hybrid crosses have been located and will be propagated this coming year. They include diploid P. tremuloides x P. tremula, P. tremuloides x P. davidiana, P. canescens x P. tremuloides, and possibly P. grandidentata. Several selections within these types of hybrids may be adaptable to lower quality sites and should be tested for this use. A potential use clones capable of occupying conifer sites is that of a firebreak. Although growth rate as a firebreak on poor quality sites would be considerably less n that on more appropriate sites, the primary function would be to break up rge blocks of conifers.

HYPOXYLON RESEARCH

INTRODUCTION

Last year in Project 3537 Progress Report One, we discussed the importance of hypoxylon canker (Hypoxylon mammatum Wahl. Miller) as the most serious forest management problem influencing the future use of aspen and aspen hybrids. All aspects of the "hypoxylon problem" have been the subject of research at some time during the past 30 years. Results from the Institute's crossing program, the aspen tissue culture research, and our recent work on mammatoxin bioassay procedures suggest the hypoxylon canker problem can be solved using a combination of tree breeding, plant pathology, and tissue culture techniques. We feel the Institute is in the unique position of having: (1) several hundred 15 to 25-year-old full-sib individuals in field plantings that have been evaluated periodically for hypoxylon since establishment, (2) hypoxylon evaluated parent trees, and (3) tissue culture and toxin bioassay capabilities. Most of the research conducted this past year and presently underway involves determining the validity and reliability of the toxin leaf bioassay procedure used in evaluating seedlings and rooted root sprouts.

The approaches we are presently using include:

1. Evaluating parent trees by examining the incidence of hypoxylon canker in full-sib progeny groups.

2. Determining the reliability and validity of the leaf bioassay method by: (a) making repeated measurements of the same clone, and (b) measuring

the bioassay response of rooted root sprouts from trees (seedling origin) that are disease free after 25 years, and comparing the results with the bioassay response of trees from the same cross that are still alive but have hypoxylon.

3. Using the leaf bioassay procedure to (a) evaluate highly valued parent trees, and (b) determine the relative toxin resistance of seedling populations, primarily crosses where long-term field records on hypoxylon incidence exist.

4. Using the leaf bioassay procedure to evaluate the toxin resistance of quaking aspen and hybrid aspen clones selected for outstanding growth.

Although the work on these studies has not been completed this past year, progress has been good and a summary of progress follows.

FIELD DATA

Three additional field trials were evaluated, bringing to ten the number of replicated field trials and cooperator plantings evaluated over the past three years. As a result, information is available on 40 full-sib crosses. Hypoxylon incidence (occurrence and mortality) information has allowed us to rate experimental crosses after 20 to 25 years with regard to their relative susceptibility to hypoxylon canker. The rating system used provides hypoxylon losses for each cross after 10, 15, 20, and 25 years in the field. When, for example, a cross is listed as having 20% hypoxylon, 20% of the trees either have died or presently have hypoxylon canker. Many of our full-sib crosses have an infection rate of 2% a year. This is a rate similar to that occurring in native quaking aspen stands.[4,5] A table summarizing those crosses for which long-term field evaluation data are available is being prepared and will be available for use in next year's evaluations.

Although we have reevaluated little over half of the total number of crosses we plan to consider, there are a number of patterns that seem to be developing. One interesting result is the greatly differing patterns of hypoxylon infection within a cross over time, suggesting more than one mechanism of resistance exists. Some crosses quickly develop a high level of infection (many trees are infected), and then very few trees in the cross become infected after year 15. Most crosses, however, have relatively few trees infected prior to year 10, and then the percentage of trees infected increases gradually until only a few trees remain alive at age 25. We also have crosses with low levels of infection until age 20 and then some condition (hail, insects, temperature, etc.) apparently triggers a major jump in the level of infection. The most encouraging information emerging from the field data from full-sib evaluations is the specific "female x male" combinations that produce crosses with lower than average hypoxylon incidence. The hypoxylon mortality information on full-sib crosses is expected to be valuable in rating the parent trees, and in determining the validity of the leaf bioassay method for predicting hypoxylon resistance in untested crosses.

HYPOXYLON SELECTION AND CROSSING PROGRAM

The selection and crossing research was designed to determine the validity and reproducibility of the leaf bioassay procedure, and at the same time generate hypoxylon resistant individuals that can be used in future breeding and clonal forestry research. The crosses used in the full-sib evaluation work include those made in 1984, 1985, and 1986, along with stored seed from crosses made earlier. Most are repeats of earlier crosses for which we have field evaluation data for 20 to 25 years. The crosses vary in the incidence of hypoxylon canker (based on field data). The objective is to evaluate crosses using 25-50 seedlings, and determine the degree of correlation that exists between the bioassay information (from leaves from 16 to 18 week-old seedlings) and the field incidence data. To date, more than 28 full-sib crosses have been made, and the leaf bioassay work is underway. Included are 11 crosses made in 1986.

The hypoxylon work is an attempt to test the validity of the leaf bioassay method for screening clones that have cellular-level resistance to the toxin that causes injury to aspen. During 1986, seven selections were made, bringing the total to 47. The selections included (1) disease free hybrids of exceptional form and growth rate, (2) disease free quaking aspen of good form and growth, and (3) hypoxylon infected aspen from the same full-sib crosses used in 2 above.

BIOASSAY RESULTS

Progeny Group Bioassay Evaluations

The hypoxylon bioassay work this past year concentrated on the evaluation of full-sib seedling populations. The bioassay procedure used was the leaf puncture method described in some detail in Progress Report One (page 25).

Mature leaves from 16- to 18-week-old greenhouse-grown seedlings and/or rooted root sprouts were excised at the point of attachment to the stem and placed in vials of water. Small holes were made in the leaf blade with a minutin insect pin and a 3 μL drop of mammatoxin placed over the hole. Following incubation in a humidified chamber at 28°C for 48 hours, response to the toxin was measured as lesion diameter. Replication was obtained by using six tests (lesion measurements) per leaf and three leaves per plant.

Approximately 320 seedlings from 13 full-sib crosses were evaluated last year using the leaf bioassay procedure. The results obtained in 1986 are summarized in Table 9, along with the progeny group evaluations completed in 1985. Although the work is not complete, there are a number of trends developing. First, as was discussed in Progress Report One, there are a relatively large number of seedlings within progeny groups considered highly resistant to the toxin at a cellular level (lesions < 2 mm in diameter). Second is the large variation that exists between crosses in the number of highly and moderately resistant individuals. This variation is not unexpected and may be useful in developing procedures for predicting hypoxylon losses.

Preliminary statistical comparisons were made combining the leaf bioassay data with hypoxylon incidence field data. Using information on 12 full-sib crosses, an attempt was made to predict hypoxylon losses at year 20. The variables used in attempting to predict hypoxylon losses at year 20 (L-20) for specific full-sib crosses included % highly reactive progeny (lesion diameter > 5 mm), % nonreactive progeny (lesion diameter < 2 mm), hypoxylon response rating (mean lesion diameter for cross), selection index (a numerical

Table 9. Results of leaf bioassay evaluations of progeny groups.

Cross No.[a]	Parent female	x male	No. Seedlings Evaluated	No. Seedlings by Lesion Class <2 mm	2-5 mm	>5 mm	Mammatoxin Response Rating[b]
XT-6-80	T-20-56	T-46-60	25	5	5	15	4.5
XT-8-80	T-25-56	T-46-60	21	4	7	10	4.2
XT-4-82	T-53-60	T-44-60	25	8	12	5	3.2
XT-3-83	XT-22-56 S-2	T-44-60	25	7	13	5	3.3
XT-2-84	T-24-60	T-13-58	25	5	18	2	3.2
XT-16-84	T-5-61	T-44-60	25	3	9	13	4.5
XT-17-84	T-50-60	T-44-60	25	4	7	14	4.5
XT-18-84	T-5-61	T-20-60	25	3	7	15	4.7
XT-19-84	T-50-60	T-20-60	25	1	0	24	5.8
XT-3-85	T-5-61	T-46-60	33	0	14	19	4.9
XTA-T-4-85	Ta-6-68	T-46-60	29	15	11	3	2.5
XTa-T-6-85	Ta-7-68	T-46-60	27	6	11	10	3.9
XT-18-85	T-50-60	T-46-60	28	2	7	19	5.0
XT-19-85	T-50-60	T-6-61	18	1	5	12	5.3
XT-23-85	T-53-60	T-46-60	26	8	9	9	3.6
XTa-T-11-82	Ta-6-68	T-22-72	31	4	20	7	3.7
XT-13-84	T-1-58	T-6-61	25	2	16	7	4.0
XTa-T-14-84	Ta-7-68	T-44-60	17	3	12	2	3.4
XT-20-84	T-1-58	T-20-60	25	11	5	9	3.3
XT-15-85	T-1-58	T-44-60	20	7	9	4	3.0
XT-16-85	T-1-58	T-46-60	9	3	2	4	3.8
XT-23-85	T-23-85	T-46-60	26	8	9	8	3.6
XT-1-86	Clone 5	T-44-60	49	24	24	1	2.3
XT-2-86	Clone 5	T-46-60	18	9	8	1	2.4
XT-3-86	Clone 5	T-10-60	50	13	35	2	3.0
XT-4-86	Clone 5	T-20-60	50	17	27	6	3.0
XT-18-86	Clone 5	Clone 7	50	28	22	0	2.1

[a]See Appendix for description of crossing code.
[b]Equivalent to the weighted average lesion diameter.

rating based on a compilation of morphology, growth, site, and wood quality factors) of the female parent, selection index of the male parent, and actual field losses at age 10. Table 10 summarizes five of about 40 multiple correlation calculations that were tried. Only 12 crosses for which we have both bioassay data and adequate 10 and 20 year field data were used in the calculations. The experimental error of both the bioassay and field evaluation data are fairly large. This resulted in our being unable to predict year 20 hypoxylon losses with any degree of accuracy using just bioassay data. The best prediction equations were obtained when age 10 losses (L-10) were included in the calculations. However, even in equations three, four, and five (Table 10), where the prediction value was based on both information on field losses at age 10 and bioassay data, the standard error of estimate was so large that the predicted value was of only limited practical use. By increasing the number of crosses used in preparing the basic prediction equations, and making very modest changes in how the bioassay data are handled, the predictive ability for estimating age 20 hypoxylon losses may be improved.

Evaluation of Selected Clones

The reliability of the bioassay method was evaluated during 1985 using a number of clones of potential commercial interest. Rooted root sprouts were grown for 16 to 18 weeks. Leaves were then collected and evaluated using the leaf bioassay procedure. The results demonstrated that the method is very reproducible, and a closer examination of the data suggested an evaluation could be made using as few as 8 to 10 ramets per clone. Of particular interest was the high, cellular level toxin resistance demonstrated by hybrid clones selected for their exceptional form and growth rate from Northern Wisconsin field trials.

tional clones were selected in 1986 but time did not permit their evaluation. Bioassay evaluation is scheduled for the spring and summer of 1987.

le 10. Preliminary multiple correlation calculations[a] for predicting losses at 20 years (L-20).

ation o.	Dependent Variable	Independent Variables	Equation Constants	Multiple Correlation Coefficient[b]	Standard Error of Estimate
1	L-20	% Nonreactive	0.59	0.43[NS]	19
		% Highly reactive	0.16		
			12.5[c]		
2	L-20	% Nonreactive	-0.20	0.53[NS]	19
		% Highly reactive	0.97		
		Response rating	-30.4		
			119.8[c]		
3	L-20	L-10	2.69	0.76*	14
		Response rating	-3.52		
			31.5[c]		
4	L-20	Response rating	-9.34	0.77*	14
		% Highly reactive	0.24		
		L-10	2.48		
			46.1[c]		
5	L-20	Selection Index F	-1.14	0.79*	14
		Response rating	0.56		
		L-10	3.02		
			45.4[c]		

Equation of form $y = a + bx_1 + CX_2 + \ldots$
Mult. correlation coefficient levels of significance.
NS = nonsignificant, * = significant at 0.05 level.
General equation constant.

omparisons Between Diseased and Disease Free Clones

Root collections were made the fall of 1985 in 20- to 25-year-old full-ib field plantings. These collections consisted of six trees from five crosses with three trees free of hypoxylon and three trees from the same cross with hypoxylon. Rooted root sprouts were produced, the leaf bioassays run, and comparisons made using the toxin resistance information. Inconsistent results were obtained, apparently due to the poor physiological condition of some of the plants. Roots from these original clones will be recollected along with additional disease-free clones, and the bioassays completed in the coming year. This work is preliminary in nature and may be expanded. The number of comparisons to be completed in the coming year will depend on the level of funding.

PLANS FOR 1987/88

The development of a method to aid in the prediciton of *Hypoxylon mammatum* canker incidence in progeny groups will again be emphasized. Correlations between actual canker incidence in field trials and plantings and laboratory bioassays will be attempted as new data are added to existing information.

The production of hybrid seed and seedlings will proceed to the extent that flowerbuds from selected parent trees are available. The exceptional flowering that occurred last year is being followed by considerably less this year. The selection of outstanding hybrid clones will continue, and the production of planting stock for clonal testing from previous selections will be part of our efforts to make exceptional plant materials available. If time and funds permit we will also look at the feasibility of micropropagating selected hybrid clones for field planting.

Because of the bottleneck in producing large amounts of hybrid aspen seed it is being proposed that further work be done with boxed grafts as a nondestructive means of utilizing flower bearing branches. Grafts will also be made for the establishment of a hybrid aspen seed orchard with the State of Michigan and for the expansion of Blandin Paper Company's existing seed orchard.

A 40-acre planting of triploid hybrids, which was originally part of a DOE funding attempt in cooperation with Consolidated Papers, Inc., will be established in Northern Wisconsin on a site owned by Consolidated Papers. The planting represents an acceleration of our efforts to make genetically improved planting stock operational.

An aspen sucker stand on Owens-Illinois land near Tomahawk, Wisconsin (Willow Flowage, Silvicultural Trial V) that was part of Project 2774 work with fertilization and irrigation to maximize growth of native and improved aspen, will be measured this coming fall. The long-term effects of fertilization and irrigation will be determined. The stand from which the 10-acre study developed was 18 years of age at harvest. The sucker stand will be 18 years of age and the surrounding stand will be 36 years. A number of growth comparisons can be made, and the opportunity would represent the completion of a long-term study.

The sucker stand that develops from the harvesting of Trial X will also be evaluated.

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APPENDIX

A code system was devised early in the aspen program to handle the numbering of individual parent trees and crosses. It was necessary to incorporate into the system the ability to identify the species of selected parent trees, the type of cross (parentage) when used for crosses, and the year the trees were selected or the cross was made. The following list alphabetically gives the symbols encountered in the selected tree and crossing system.

To illustrate, T-2-56 = the second *P. tremuloides* selected in 1956. XT-Ta-14-58 is the 14th cross made in 1958 and the cross involves a *P. tremuloides* female and a *P. tremula* male. XCa-G-18-70, S-1 = the first selected individual from the 18th cross in 1970 involving a *P. canescens* female and a *P. grandidentata* male.

A = *P. alba*

An = *P. angustifolia*

B = *P. alba* var. bolleana

Ca = *P. canescens*

D = *P. deltoides*

Da = *P. davidiana*

E = *P. euphratica*

G = *P. grandidentata*

Gla = *P. glandulosa*

H = hybrid

I = *P. ilicifolia*

M = *P. maximowiczii*

N = *P. nigra*

O = open pollinated

S = *P. sieboldii*

S-1, S-2, S-3, ... = selected individuals

T = *P. tremuloides*

Ta = *P. tremula*

Tc = *P. balsamifera*

Tr = *P. trichocarpa*

Ts = *P. tristis*

X = cross

GLOSSARY

Aspen. Refers to P. davidiana, grandidentata, sieboldii, tremula, and tremuloides. As used in this report, refers to species in the Populus section Leuce, which includes the aspens (subsection Trepidae Dode) and the true white poplars (subsection Albidae Dode) which includes P. alba and P. canescens. Hybrids of species within this section are also covered by this term. (See poplar.)

Auxins. A class of growth hormones causing cell enlargement.

Bh. Breast height (4.5 feet).

Bioassay. Determination of the relative effective strength of a substance by evaluating its effect on a test organism.

Bisexual. Having both functional male and female reproductive organs in the same flower, or in the case of Populus, a tree having both male and female flowers.

Canker. A necrotic area caused by fungi or bacteria. Hypoxylon cankers have a flat, sunken appearance that may or may not have callus ridges along the margin of the necrotic area.

Catkin. A scaly spike of usually unisexual flowers, as in Betula and Populus.

Chromosome. A microscopic, usually thread- or rodlike body carrying the units of inheritance (genes). The chromosomes are the primary constituents of the cell nucleus but are individually distinguishable only during nuclear division.

Chromosome number. The number or complement of chromosomes characteristic of a species. The number of sets must also be specified; thus, in Pinus the chromosome number may be expressed as "n equals 12" or as "$2n$ equals 24," depending on whether sex cells or vegetative cells are observed.

Chromosome set. The chromosomes inherited as a unit from one parent. Most eggs or sperm carry only one set. A set usually includes one of each kind of chromosome characteristic of the species.

Clone. A group of plants derived from a single individual (ortet) by asexual reproduction. All members (ramets) of a clone have the same genotype and consequently tend to be uniform.

Cross. As used in the Aspen Genetics Program the term applies to progeny produced by mating trees of the same species (intraspecific) or of different species (interspecific).

Dbh. Diameter of the tree stem at breast height (4.5 feet).

Diploid. Having two sets of chromosomes in the nucleus - indicated by "2n." One-half of the chromosomes are contributed by the female parent, one-half by the male parent. Many higher organisms are diploid except for their sex cells and associated tissue.

Fertilization. Union of a haploid male sex cell with a haploid female egg cell to form a diploid zygote which develops into the normal tree.

Gene. The smallest unit that can be shown to be consistently associated with the occurrence of a specific genetic effect. The genes are ultramicroscopic and act as if linearly arranged at fixed places (loci) on the chromosomes. Each gene interacts with other genes and the environment to produce within the cell certain physiological effects that control the development of one or more characters of an individual.

Genotype. An individual's hereditary constitution, expressed or hidden, underlying one or more characters; the gene classification of this constitution expressed in a formula. The genotype is determined chiefly from breeding behavior and ancestry.

Haploid. Having the reduced chromosome number (n), i.e., having one set of chromosomes in the nucleus. This is normal in sex cells, which have only half of the number of sets occurring in diploid (2n) vegetative cells.

Heritability. A measure of the relative degree to which a character is influenced by heredity as compared to environment. The heritability (in the narrow sense) of a character in a population is the fraction of the total variation that is contributed by transmissible (additive) genetic differences, i.e., it is the ratio of genotypic variance to phenotypic variance. High heritability indicates that an individual's phenotype is indicative of its genotype and that differences in environment will cause little modification, i.e., that genetic control is high.

Heterosis. Hybrid vigor; the increased vigor of a hybrid as compared to the better parent. Heterosis is at a maximum in the F1 generation.

Heterozygosity. Presence in an organism of different members of the same allelic set, i.e., both the dominant and the recessive gene. For example, an Aa plant is heterozygous, whereas AA and aa plants are homozygous. A heterozygous individual characteristically does not breed true and is known as a hybrid with respect to the genes in question.

Homozygosity. Presence of identical alleles, either both dominant or both recessive, as for example AA or aa. A homozygous individual breeds true when mated with the same genotype for the character(s) in question.

Hybrid. As used in the Aspen Genetics Program the term applies to progeny produced as the result of mating trees of different species (interspecific).

Hybrid vigor. Same as heterosis.

Inbreeding. Intercrossing or selfing related organisms. This procedure, especially if carried out for a number of generations, exposes undesirable recessive characters and "fixes" desirable ones, i.e., renders them true-breeding.

Interspecific. Between species; e.g., interspecific hybridization is the production of offspring by cross-pollinating one species with another.

Intraspecific. Within a species; e.g., intraspecific hybridization is the production of offspring by cross-pollinating one individual of a species with pollen from another individual of the same species.

Mammatoxin. A term describing a poisonous substance produced by the fungus *Hypoxylon mammatum*.

Mutation. A sudden variation from the ancestral phenotype, due to gene or chromosome changes. If the cause can be demonstrated as a chromosome change, the mutation is preferably referred to by the specific phenomenon involved, e.g., a change in structure (aberration) or number (ploidy). Although mutations are infrequent, and usually recessive and harmful, they are the raw material of evolution and plant breeding.

Nucleus. The cell part made up chiefly of the chromosomes.

Ortet. The one plant from which members of a clone were originally derived.

Pathotoxin. A poisonous substance that is a product of metabolic activities.

Phenotype. (1) The demonstrable characteristic(s) of an organism; the product of the interaction of the genes of an organism with the environment. (2) Individual(s) described on the basis of demonstrable characteristics. Similar phenotypes do not necessarily breed alike.

Plantlet. A complete plant derived from a tissue culture system.

Ploidy. The chromosome situation with respect to number of sets, e.g., two sets (diploid), or variation from full sets (aneuploid).

Pollination. When pollen reaches the receptive catkin.

Polyploid. Having three or more times the haploid number of chromosome sets in its cells. A cell or organism having three sets ($3n$) is called triploid; four sets ($4n$) tetraploid.

Poplars. Refers to trees in the genus *Populus*. As used in this report, refers to species outside the section Leuce (see aspen).

Popple. A colloquialism which refers to native aspen, *P. tremuloides* and *P. grandidentata*.

Progeny test. Evaluation of the breeding value of parents by suitable comparisons among their offspring.

ıtative. Suspected.

amet. An individual member of a clone.

eciprocal cross. The repetition of a cross where the sexual function of the parents is reversed, i.e., female A x male B is the reciprocal of female B x male A.

election. Artificial selection is the propagation by man of organisms possessing desired characteristics. The aim generally is to improve the population or gain knowledge of its hereditary potentials. Natural selection is part of the evolutionary process resulting in the survival of the "fittest," i.e., of the best adapted individuals.

Sibs (siblings). Offspring, irrespective of sex, from the same parents but from separate fertilizations. Full sibs have both parents in common, half-sibs, only one in common.

Sprout. Vegetative shoot arising from the stump or roots. Root sprouts may also be designated as suckers.

Suckers. Vegetative shoots arising from ~~subterranean~~ roots or stems.

Tetraploid. See polyploid.

Tissue culture. A general term for organs, callus, or cells growing _in vitro_ on an artificial medium. Cultures can be started from a variety of plant parts which have cells capable of dividing.

Triploid. See polyploid.

Vegetative propagation. Propagation of a plant by asexual parts, as in tissue culture, budding, dividing, grafting, rooting, and air layering. Hereditary characteristics of the resulting clone (ramets) are identical with those of the original plant (ortet).