MASS PROPAGATION TECHNIQUES FOR
ASPEN CLONES

Project 2987

Report Two

A Progress Report

to

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

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MASS PROPAGATION TECHNIQUES FOR ASPEN CLONES

SUMMARY

Described is the second phase of a two-phase project investigating mass propagation techniques for producing aspen clones. The first phase dealt with root storage and late winter-early spring rooted root sprout production. The second phase investigates possibilities of rooting excised sprouts in late fall and early winter, growing them to 4 to 6 inches, forcing them to dormancy and transplanting them to the nursery in the spring.

The same two materials were used in both phases. One material was AG-1-60, a hybrid between Populus alba and P. grandidentata. The second was XT-Ta-14-58, S-3, a selected progeny from a triploid family of a cross between a diploid P. tremuloides and a tetraploid P. tremula. It was determined that, regardless of material or growth container used, 4 to 6-inch plants could be forced into dormancy with short days of high light intensity and cool temperatures, stored over winter and grown in the nursery the next growing season with 80 to 100% success. The biggest deterrent to successful propagation with this system was found to be reduced success in rooting excised sprouts in the late fall and early winter.
INTRODUCTION

This is a report of the second phase of a two-phase project investigating the possibilities of large scale propagation of improved aspen clones. In the first phase, a system utilizing stored aspen roots and stored rooted sprouts of aspen was tested. The results, confounded by atypical nursery conditions present that year, indicated: that fall and winter storage of aspen roots did not affect their ability to sucker, that rooted sprouts may be kept on minimum nutrition from 5 to 6 weeks with no ill effects, and that the date (May to mid-June) of transplanting the rooted suckers into the nursery had no immediate consequence other than loss of growing time that particular year.

The first phase investigated root sprout production from approximately February through June. Several advantages in both total root sprout production and efficient use of nursery personnel can be obtained by year around production of improved aspen clones. For this reason the second phase of the project investigates a fall and winter root sprout production and storage technique which forces small (4-6 inch) rooted sprouts into dormancy for storage through the remainder of the winter.

This is a report of the second-phase results and some additional information about the first-phase results.
MATERIALS AND METHODS

The same two clones used in the first phase of the project were used for the second phase. One is a "alba x bigtooth" clone, AG-1-60. The other is a selected individual from a triploid interspecific hybrid progeny of *P. tremuloides* diploid and *P. tremula* tetraploid, XT-Ta-14-58-S-3.

The objective of this phase of the project was to investigate means by which rooted root sprouts could be grown in the greenhouse late in the year, forced into dormancy and stored over winter. Short days and low temperatures bring on dormancy while high light intensity and fertilization encourage a buildup of nutrients. It was the plan to use high light intensity, short days, low temperatures, and fertilization to force rooted root sprouts into dormancy with as high a reserve of carbohydrates as possible to encourage vigorous flushing in the spring*. It was not known whether fertilization would stimulate further height growth in spite of short days and low temperatures. A preliminary study was begun in September to determine whether fertilization would delay dormancy and result in excessive height growth. The initial result showed that height growth and time to dormancy was prolonged but not enough to hinder use of the procedure. Additionally, the fertilized plants were much healthier in appearance and the gains were felt to be worth the small inconvenience in extra time needed.

Experience has shown that the ability to consolidate greenhouse space is one of the important considerations for the successful development of a mass vegetative propagation system. For this reason the size of the plants to be

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*For convenience this procedure has been referred to as the "dormancy treatment."
forced into dormancy must be limited. A stem height of 4 to 6 inches was arbitrarily established as the maximum allowable size. It was felt that smaller size stems would be too difficult to over-winter successfully.

Two types of growing containers were used. The first was a clear plastic shoe box approximately 6-1/2 inches wide by 12 inches by 3-1/2 inches tall. The dormant rooted sprouts were held in this container in an unheated building until planted in the nursery. The container had many advantages in greenhouse use, as described in Report One, and allowed the materials to be over-wintered in a small space without disturbing the sprouts. Two disadvantages were the difficulty of extracting individual sprouts for nursery planting because of intermingling roots and the unknown buffering potential of the rooting media to harsh winter. The second type of container is known as a Styroblock and is manufactured by Beaver Plastics, Limited of Edmonton, Alberta, Canada. The container (Fig. 1) essentially is a styrofoam block with about 96, 15/16-inch diameter by 4-1/4-inch length tapered holes per square foot. The holes were filled to within one inch of the top with a sandy loam potting soil and the last 1/2 to 3/4 inch with a 1:1 sand-vermiculite mixture. Excised root sprouts were placed in the sand-vermiculite mixture and the block covered with a pane of glass to give a cold frame effect. When the sprouts had rooted, the glass was removed and they were grown under the same procedures and conditions as the sprouts in the shoe box.

Due to the multiple compartments, the styrofoam block has a disadvantage of uneven watering and unused growing space resulting from mortalities. The advantages include better containment of propagules due to the compartmentalization,
a sturdier container with adequate buffering and over-wintering potential and
the plant plugs, if plant size is restricted, are easily extracted and planted.

Figure 1. Pictured Above is a Subsection of One of the Styrofoam Blocks Illustrating the Plant Plugs that Developed

The original plan called for a monthly production of 100-200 suckers of each material. Half of this number would be put into styroblocks and the other half in the shoe boxes. They were to be grown in the greenhouse with 16 hours of 400-foot candle supplemental light (Gro-lux fluorescent bulbs) and fertilized
every two weeks with a 20-20-20 liquid greenhouse fertilizer. The plants reached an average size of 4 inches in 2 to 3 weeks and were then moved into a chamber with 10 hour 2000 (approximate) foot candle days and a temperature of 60°F. The plants took 4 to 5 weeks to set buds and were moved to the greenhouse and held on 10-hour days of available light and minimum temperatures of 30 to 40°F. When the buds turned hard, the plants were moved to a third chamber, kept on short days and at between 26 and 40°F., where the leaves then changed color. The dormant plants were then moved to an unheated shelter and kept for the remainder of the winter.

On June 1, 1972 all the plants were outplanted in the IPC nursery at Greenville, Wisconsin. The February plants were not yet dormant and had green leaves and soft buds. Survival and flushing were observed several times during the growing season. Figure 2 illustrates the size the triploid hybrid aspen normally attain after one growing season in the nursery.
RESULTS

SURVIVAL AND GROWTH

Sprout survival and growth during the fall and early winter months has always been erratic in the IPC greenhouse. In spite of using supplemental light and closer temperature control, the sprouting started slowly and rooting success was reduced, particularly for the triploid hybrids. In addition, the sucker production decreased earlier than usual, probably due to the use of small roots which were a reflection of the previous year's nursery problems. As a result, the hoped for 100 to 200 sprouts were not available for each treatment and no tests were made in the shoe boxes for the month of December. Table I gives by material, the number of sprouts started, number of sprouts rooted successfully, number of sprouts transplanted on June 1, number surviving on June 7, and the percentage of survivors at June 7th in terms of sprouts rooted, i.e., unrooted sprouts were ignored.

The rooted sprouts in which "dormancy treatments" were initiated in December and January developed well-formed terminal buds, dropped their leaves and were stored and outplanted in the spring without difficulty. Dormancy treatments started on rooted sprouts in February were only partially successful, perhaps due to the influence of warming daytime greenhouse temperatures in April and May which prevented keeping temperatures at 30-40°F. The 10-hour day length was maintained by covering the plants for part of the day. The rooted sprouts for which dormancy treatments were initiated in February set up soft buds, showed only minor change in leaf color and were transplanted in the nursery on June 1 in that condition. Observations made on July 7 indicated these sprouts had flushed and
shoot elongation was under way. Ignoring the variation in dormancy of the three different starts, the results in Table I indicate the most serious problem in using the technique of fall and early winter production of sprouts using "dormancy treatments" lies in the sprout production and success in rooting the excised sprouts.

TABLE I
DATA SUMMARY
ROOTING, TRANSPLANTING AND SURVIVAL

<table>
<thead>
<tr>
<th>Material</th>
<th>Month</th>
<th>Dormancy Treatment Initiated</th>
<th>Number of Sprouts Started</th>
<th>Rooted</th>
<th>Transplanted</th>
<th>Sprout Survival No. %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shoe Box Sprouts</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AG-1-60</td>
<td>Jan.</td>
<td></td>
<td>125</td>
<td>71</td>
<td>68</td>
<td>59 83</td>
</tr>
<tr>
<td>XT-Ta-14-58, S-3</td>
<td>Jan.</td>
<td></td>
<td>44</td>
<td>43</td>
<td>39</td>
<td>39 89</td>
</tr>
<tr>
<td>AG-1-60</td>
<td>Feb.</td>
<td></td>
<td>75</td>
<td>43</td>
<td>39</td>
<td>39 91</td>
</tr>
<tr>
<td>XT-Ta-14-58, S-3</td>
<td>Feb.</td>
<td></td>
<td>34</td>
<td>32</td>
<td>32</td>
<td>32 100</td>
</tr>
<tr>
<td>Styroblock Sprouts</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>AG-1-60</td>
<td>Dec.</td>
<td></td>
<td>213</td>
<td>165</td>
<td>157</td>
<td>128 78</td>
</tr>
<tr>
<td>XT-Ta-14-58, S-3</td>
<td>Dec.</td>
<td></td>
<td>183</td>
<td>165</td>
<td>155</td>
<td>145 88</td>
</tr>
<tr>
<td>AG-1-60</td>
<td>Jan.</td>
<td></td>
<td>120</td>
<td>89</td>
<td>87</td>
<td>74 85</td>
</tr>
<tr>
<td>XT-Ta-14-58, S-3</td>
<td>Jan.</td>
<td></td>
<td>24</td>
<td>15</td>
<td>15</td>
<td>15 100</td>
</tr>
<tr>
<td>AG-1-60</td>
<td>Feb.</td>
<td></td>
<td>72</td>
<td>29</td>
<td>29</td>
<td>29 100</td>
</tr>
<tr>
<td>XT-Ta-14-58, S-3</td>
<td>Feb.</td>
<td></td>
<td>24</td>
<td>5</td>
<td>5</td>
<td>5 100</td>
</tr>
</tbody>
</table>

*July 7th observations, percentages based on number of individuals rooted.*
Aside from the initial rooting problems, survival was good, ranging from 78 to 100%. The lowest survivors were for the December treatments in the Styroblocks. In fact, the survival progresses from low to high from December through February. Whether or not this trend holds any significance could not be tested. The "alba x bigtooth" hybrid had slightly less survival (87% overall average) than the triploid hybrid (94% overall average), although both are good. There was little difference between the survival due to growth containers with 92% survival in the Styroblocks and 91% survival in the shoe boxes. In October, the average height for AG-1-60 was 3-1/2 feet and for XT-Ta-14-58, S-3 was 3 feet and the survival for both was approximately the same as in July, see Fig. 2.

From the results listed, it can be said that aspen rooted root sprouts can be forced into dormancy and over-wintered at a small (4-6 inches) size without adverse effects by using the conditions described. Two questions arose from the use of this system. First, what can be done to improve the initial rooting and survival in the fall and early winter starts? Second, would the use of high light intensity, short days and a high fertility level allow rooted root sprouts to be stored with soft buds and green leaves prior to transplanting? Since the work in this project has shown the year-round vegetative propagation of aspen clones can be accomplished, the answers to the above two questions would simply give a better understanding and improve the economic practicality of this system.

PRODUCTION INFORMATION

Several questions arose after Report One was released regarding production information. The first try in any new system generally results in inefficiencies and, if production figures from that study are used, per se,
some poor assumptions will be made. Therefore, it is logical to pool all past experiences to develop a "ball park" approximation of the production costs in terms of time and space. These costs will vary depending on the greenhouse involved and size of the program. A reduction in costs can be obtained with further mechanization and development of new efficiencies as the system is used.

Suckering efficiency can best be demonstrated using AG-1-60 suckering information from Phase One of this project. The "alba x bigtooth" material sprouted less prolifically than the triploid hybrid. The roots used were 404 lineal feet collected from 400 healthy one-year-old line-out stock in the IPC nursery. The number of suckers excised from the spare roots of the 400 trees was 2325. Obviously, a clonal propagation system with geometrically expanding production could be initiated from one tree. The limiting factor aside from growing facilities then becomes the condition of the growing stock, as healthy trees have abundant root systems but poor growing stock provide little extra roots.

The greenhouse space required for propagating 1000 root sprouts with the shoe box system is a ten-square-foot surface for roots and a 40-square-foot surface for rooted sprouts. Assuming 80% of the excised sprouts reach plantable size, 1.5 feet, in the first year, approximately 4.0-man hours are needed to produce 1000 plants. This time covers collecting roots, dipping the roots in captan solution, cutting them in lengths, waxing the ends, excising the sprouts and all other greenhouse, transplanting, nursery care and fall lifting. About 25% of the time is for root preparation, 35% for sprout rooting and greenhouse care, 20% for transplanting and 20% for nursery care and lifting. Increasing
production to several thousand trees could affect savings of more than ten man hours per thousand through efficiency and further mechanization.

Nursery bed space required for 1000 plants is about 110 square feet, or about 9 plants per square foot of bed. Closer spacing results in reduced individual plant growth.

The system used in Phase Two requires more handling but less greenhouse space because dormant stems can be moved outside. The rooting efficiency of the sprouts during the late fall and early winter also adds to the cost, indeterminate at this time, of man hours necessary per thousand plants.

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