In vitro Root Suckering of Aspen (*Populus tremuloides*)

Kathryn Louis[1], **Chris V. Hanson and Wesley P. Hackett**
University of Minnesota, Department of Horticultural Science, 1970 Fowell Avenue, St. Paul, Minnesota 55108

Carl A. Mohn
University of Minnesota, Department of Forest Resources, 1530 No. Cleveland Ave., St. Paul, Minnesota 55108

The development of an in vitro protocol for the growth of roots and the subsequent production of microshoots from the suckering of the roots of two clones of *Populus tremuloides* (quaking aspen) was studied. Root growth was greatest with attached shoot tips. Adventitious bud initiation from root explants was greatest using Murashige and Skoog medium, supplemented with 3% sucrose and 1.0 mg/liter thidiazuron. The addition of 0.01 mg/liter NAA did not enhance adventitious bud initiation. Shoot development and elongation after adventitious bud initiation was achieved by growing the adventitious bud clusters on medium supplemented with 0.1 mg/liter BA for 8 weeks (2 cycles). Rooting and acclimation of the root-derived shoots was the same as traditional shoot-tip-derived shoots.

INTRODUCTION

Because of additional applications, the forest industry demand for aspen has increased during the past few years. At one time it was used primarily for pulp and paper. Now it is in demand for waferboard, oriented strand board and packing crates (Adams and Gephart, 1989; Prosek, 1988).

To meet these demands and to keep abreast with anticipated increases, clonal forestry is now being considered in the United States. Aspen does not propagate well by cuttings, so historically, foresters have planted non-selected seedling trees. The primary restriction to large-scale field planting of cloned, superior aspen is the shortage of readily available, affordable propagules. Tissue culture of aspen using the traditional shoot-tip method can be accomplished, but it is prohibitively expensive. Foresters will only plant superior material if the plantlet price is near the price of seedling produced material (currently $150-250 per thousand).

Root-sucker propagation is most often used to clonally propagate aspen. Roots are dug in the spring, washed, fungicide treated, and placed in sand. After 10+ days, shoots emerge from the roots which are removed and rooted as cuttings. This method works, but it is limited by the amount of available root material and appears to be only seasonally successful.

This paper discusses our investigations of the use of this natural root-suckering phenomenon in a micropropagation program. If we could grow isolated roots in vitro indefinitely, and regenerate microshoots on demand from those roots, we could micropropagate aspens without seasonal restrictions or the availability of root material. This paper reports the biological feasibility of this technique.

---

[1] Minn vitro, Inc., 15 West 36th Street, Minneapolis, Minnesota 55408

However, further research is still underway to optimize the methodology and yield. After optimization, cost evaluations will be conducted.

MATERIALS AND METHODS

In Vitro Shoot-Tip Cultures. *Populus tremuloides* clones 3 and 17A were selected as test subjects because they are considered superior genotypes with potential commercial value. Clone 3 is considered to be "easy" and clone 17A "difficult" to propagate using standard shoot-tip tissue culture procedures. Actively growing, greenhouse-derived, shoot-tip cultures of the two clones were established using standard commercial protocol. Throughout this study, we used Murashige and Skoog (MS) Medium supplemented with 3% sucrose, with the pH adjusted to 5.7. Proliferation-stage medium was supplemented with 0.5 mg/l benzyladenine (BA). Microshoot-elongation medium was supplemented with 0.1 mg/l BA. Individual shoots were then selected and used in subsequent root growth and for vitro root-suckering studies.

Root Growth Studies. To initiate adventitious root growth, individual shoots of clone 3 were transferred to medium without growth hormones. After approximately 10 days when the roots reached 10 mm in length, the root/shoot unit was subjected to treatments based on preliminary results which indicated the attached shoot and/or darkness may be important for continued root growth. To test these possibilities, half of the shoots were trimmed to 2 mm before all were placed vertically onto a filter paper support in a Magenta GA7 vessel containing liquid medium supplemented with 1.0 nM naphthalene acetic acid (NAA). Half of each group (trimmed or non-trimmed) was placed in the dark and the other half remained in the light. Ten explants per treatment were used and root growth was measured every 3 days for 30 days.

In Vitro Root Suckering. Roots from microshoots were harvested and placed onto adventitious-bud-initiation medium supplemented with BA at 0.0, 0.5, 1.0, and 1.5 mg/l, or thidiazuron (TDZ) at 0.0 0.01, 0.1, and 1.0 mg/l. Both BA and TDZ were tested with or without 0.01 mg/liter NAA. Root-explant length was 0.5, 1.0, 2.0, and 4.0 cm. Explants remained on the initiation medium for 4 weeks followed by 8 weeks (2 cycles) on shoot-development medium containing 0.1 mg/l BA. Ten explants were tested per treatment and each treatment was replicated.

Rooting and Acclimation. Microshoots from root explants were harvested, rooted, and acclimated using standard ex vitro protocol. The microshoots were stuck into a peat : perlite : vermiculite mix (1 : 1 : 1, v/v/v) in 288 plug trays, covered with a Jiffy Dome, and placed under 16 h light period supplied by CW fluorescent lamps. Acclimation was accomplished by replacing the solid Jiffy Dome after 3 weeks with a ventilated Jiffy Dome.

RESULTS

Root Growth. Root growth was greatest when the root remained attached to the stem, whether or not the explant received light. The poorest root growth was from roots grown in the light without attached stems (Fig. 1).

Table 1. Influences of TDZ and NAA on the mean number of adventitious bud clusters and microshoots derived from *Populus tremuloides* root explants.

Initiation treatment	Mean number of adventitious bud clusters		Mean number of microshoots	
	Clone 3	Clone 17A	Clone 3	Clone 17A
0.01 TDZ	0.18	0.10	0.20	0.04
0.01 TDZ + NAA	0.11	0.13	0.06	0.04
0.1 TDZ	0.19	0.38	0.19	0.12
0.1 TDZ + NAA	0.19	3.31	0.06	0.16
1.0 TDZ	0.30	0.39	0.06	0.82
1.0 TDZ + NAA	0.26	0.31	0.12	0.42

Note: TDZ concentration is mg/l.
NAA = 0.01 mg/l.

In Vitro Root Suckering. Data on root suckering (adventitious bud initiation and microshoot development) were extremely variable and were not suitable for statistical analysis. To assess the data for trends that would assist in further study the data were pooled whenever possible and are presented in that format in this report.

No adventitious buds formed on explants on control medium or medium containing BA. Only explants grown on medium supplemented with TDZ produced adventitious buds. There was also no effect of explant length on whether or not it had the capacity to form adventitious buds. Adventitious-bud formation and microshoot development for both clones is shown in Table 1. Root explants of both clones 3 and 17A produced the greatest number of adventitious bud clusters when treated with 1.0 mg/l TDZ and was not enhanced by the addition of NAA.

Clone 3 microshoot development after 8 weeks on developmental medium (0.1 mg/l BA) was best if adventitious buds were initiated on 0.01 or 0.1 mg/liter TDZ (Table 2). Clone 17A microshoot development was best when adventitious bud were initiated on 1.0 mg/l TDZ.

Rooting and Acclimation. Rooting and acclimation of the microshoots was 92% for clone 3 and 96% for clone 17A.

DISCUSSI0N

The poor growth of roots without attached shoots may be due to the lack of a shoot-produced substance.

The initiation treatment that resulted in the greatest number of adventitious-bud clusters did not result in the greatest number of microshoots for clone 3, but did result in the most microshoots for clone 17A. The lower number of microshoots that developed from clone 3 explants may be due to a carryover inhibition by the high

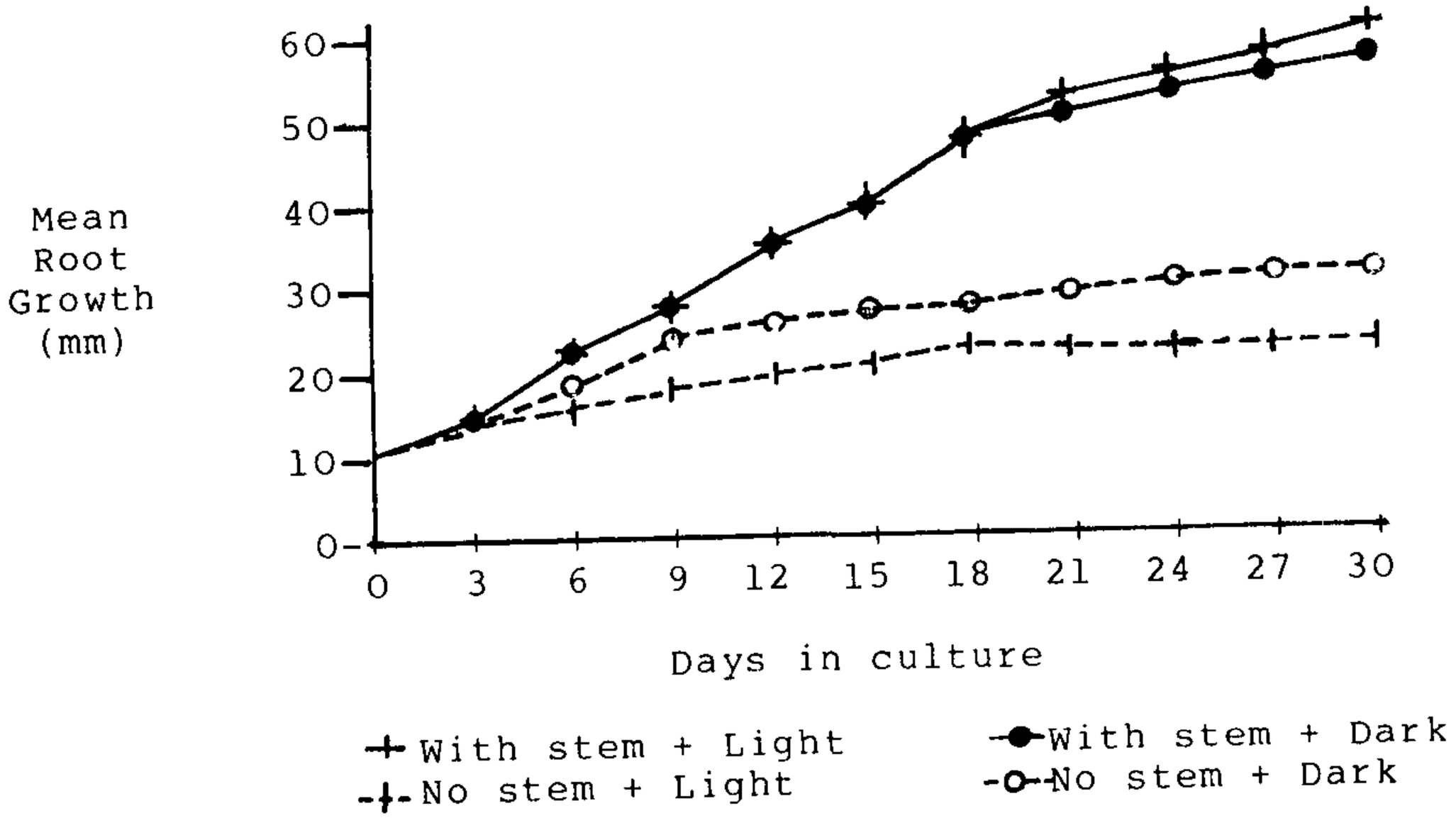

Figure 1. *In vitro* root growth of *Populus tremuloides* Clone 3.

level of TDZ or by a competition among the numerous adventitious buds. The addition of NAA did not increase the number of microshoots that formed on the root explants. This is contrary to adventitious-shoot development when leaf-segment explants are used in hybrid poplar (Lee-Stadelmann et al, 1989).

Rooting and acclimation were the same as for the traditional shoot-tip micropropagation technique.

The initiation of adventitious buds from root explants and the subsequent microshoot development, although variable at this time, appears to be quite feasible. This *in vitro* method is not limited by a shortfall of field harvested roots or by seasonal variations of in situ root suckering.

Acknowledgement. Funding for this project approved by the Minnesota Legislature ML 1991, Chapter 254, Art. 1, Sec. 14, Subd. 7g as recommended by the Legislative Commission on Minnesota Resources from the Minnesota Future Resources Fund.

LITERATURE CITED

Adams, R.D. and **J.S. Gephart**. 1989. Introduction to symposium and the aspen resources study. USDA Forest Service, North Central Experiment Station. General Technical Report NC-140.

Lee-Stadelmann, 0.Y., S.W. Lee, W.P. Hackett, and **P.E. Read**. 1989. The formation of adventitious buds in vitro on micro-cross section of hybrid *Populus* leaf midveins. Plant Sci. 61:263-272.

Prosek, C. 1988. Aspen supply—a DNR perspective. Proc. of Minnesota's Timber Supply: Perspectives and Analysis, Cong. Staff Paper, Series Report no. 64.