

and Forest Insects. U.S. Dep. Agric; Gen. Tech. Rep. PSW-48, 1982. — ICONOMOU, N., VALKANAS, G. and BUCHI, J.: Composition of gum turpentine of *P. halepensis* and *P. brutia* grown in Greece. *J. Chromatog.* 16: 29—33, 1964. — KAYACIK, H.: Pines in Turkey and an investigation about their geographical distribution. *Orman Fakültesi Degrisi* 4: 44—64, 1954. — KAYACIK, H.: Untersuchungen über die geographische Verbreitung der türkischen Kiefernarten. *Orman Fakültesi Degrisi* 8: 1—10, 1963. — KAYACIK, H.: Pines in Turkey and an investigation about their geographical distribution. *Orman Fakültesi Degrisi* 23: 147—160, 1973. — KIRKPATRICK, J. B., SIMMONS, D. and PARSONS, R. F.: The relationship of some populations involving *Eucalyptus cypellocarpa* and *E. globus* to the problem of phantom hybrids. *New Phytol.* 72: 867—876, 1973. — LOUKAS, M., VERGINI, Y. and KRIMBAS, C. B.: Isozyme variation and heterozygosity in *Pinus halepensis* MILL. *Biochem. Genet.* 21: 497—509, 1983. — MELVILLE, R.: The distribution of land around the Tethys Sea and its bearing on modern plant distribution. *In: ADAMS, C. G. and AGER, D. V. (Eds.) Aspects of Tethyan Biogeography* 7: 291—312, 1967. — MELZACK, R. N., GRUNWALD, C. and SCHILLER, G.: Morphological variation in Aleppo pine (*Pinus halepensis* MILL.) in Israel. *Israel J. Bot.* 30: 199—205, 1981. — MELZACK, R. N., SCHILLER, G. and GRUNWALD, C.: Seed size, germination, and seedling growth in *Pinus halepensis* MILL. and their relation to provenance in Israel. *Leaflet. Dep. Fore Agric. Res. Org. Ilanot* 72, 1982. — MELZACK, R. N., SCHIL-MIROV, N. T.: The Genus *Pinus*. Ronald Press Co. New York, N. Y. 602 pp, 1967. — MIROV, N. T., ZAVARIN, E. and SNAJBERK, K.: Chemical composition of the turpentine of some Eastern Mediterranean pines in relation to their classification. *Phytochemistry* 5: 97—102, 1966. — NAHAL, I.: Le pin d'Alep: Etude taxonomique, phytogéographique, écologique et sylvicole. *Ann. Ec. Eaux. For.* 19: 473—686, 1962. — NAHAL, I.: The Mediterranean climate from a

biological viewpoint. *In: CASTRI, F. D. (Ed.) Ecosystems of the World* 3: 63—86. Elsevier, Amsterdam, 1981. — NEI, M.: Molecular Population Genetics and Evolution. North Holland Publ. Co., Amsterdam-Oxford. 288 pp, 1975. — OPPENHEIMER, H. R.: Mechanism of Drought Resistance in Conifers of the Mediterranean Zone and the Arid West of the U.S.A. Final Report on Project No A10-FS 7, Grant No FG-Is: 119. The Hebrew University of Jerusalem, Faculty of Agriculture, Rehovot, 1967. — PALMBERG, C.: Geographic variation and early growth in south-eastern semi-arid Australia of *Pinus halepensis* MILL. and the *P. brutia* TEN. species complex. *Silvae Gent.* 24: 150—160, 1975. — PANETOS, C. P.: Natural hybridization between *Pinus halepensis* and *P. brutia* in Greece. *Silvae Gent.* 24: 163—168, 1975. — PANETOS, C. P.: Monograph of *Pinus halepensis* MILL. and *P. brutia* TEN. *Ann. Forest. Zagreb* 9: 39—77, 1981. — PAPAIOANNOU, J.: Über Artbastarde zwischen *Pinus brutia* TEN. und *Pinus halepensis* MILL. in Nordostchalkidiki (Griechenland). *Forstwiss. Centbl.* 58: 194—205, 1936. — PELIZZO, A. and TOCCI, H.: Indagini preliminari su semi e semanzali di *Pinus halepensis* e *P. brutia*-*P. eldarica*. *Ann. Inst. Sper. Selv. Arezzo* 9: 111—129, 1978. — SAID, R.: The Geological Evolution of the River Nile. Springer, New York, N.Y. 1982. — SCHMIDT, W.: Das Ostwestgefälle der Kiefernrasen, Neue Einblicke und Methodenvorschläge für internationale Versuche. *Intersylva* 3: 473—494, 1943. — SCHRÖCK, O.: Die Untersuchung der phototropischen Reaktion als Auslesemethode bei Kiefernämlingen auf Gradschaftigkeit. *Züchter* 28: 320—323, 1958. — SWOFFORD, D. L. and SELANDER, R. B.: BIOSYS-1: A FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *J. Hered.* 72: 281—283. — WEINSTEIN, A.: Effect of seed origin on early growth of *Pinus halepensis* MILL. and *P. brutia* TEN. *La-Yaaran* 32: 25—30, 1982.

## Reliable Plantlet Formation from Seedling Explants of *Populus tremuloides* (Michx.)

By S. R. WANN and D. W. EINSPAHR

The Institute of Paper Chemistry,  
Appleton, Wisconsin 54912, USA

(Received 15th March 1985)

### Abstract

Seedlings have been ignored in tissue culture of *Populus* species due to the relative ease of obtaining or regenerating suitable explants from older individuals. Cotyledon and hypocotyl cultures of *Populus tremuloides* exhibit remarkable morphogenic capabilities that can be manipulated to form shoots, roots, or callus. Plantlets are obtained by production of multiple adventitious buds under a high cytokinin-to-auxin ratio, followed by shoot elongation and root formation. To date, over 600 propagules have been hardened into soil, making this a reliable procedure. Investigation of four different quaking aspen crosses reveals substantial variation in organogenic features both within and between crosses. This can be exploited to establish shoot cultures of individual seedlings, yielding a continuous harvest of rootable shoots at monthly intervals for up to five subculture periods. Such a system might find applications in isolation and proliferation of unique individuals from conventional and nonconventional breeding practices.

**Key words:** Aspen (*Populus*), Micropropagation, Seedling explant culture, Plantlet.

### Zusammenfassung

Weil bei Pappelarten relativ leicht von älteren Individuen durch Gewebekultur gute Explantate erzielt werden können, wurden bisher Pappel-Sämlinge als Ausgangsmaterial vernachlässigt. Hypokotyl- und Kotyledonenkultu-

ren von *Populus tremuloides* zeigen bemerkenswerte Fähigkeiten zur Manipulation, um Wurzeln, Sprosse oder Kallus zu bilden. Plantlets sind durch Herstellung multiplexer Adventivknospen bei einem hohen Cytokinin: Auxinverhältnis zu erhalten, wobei Wurzelbildung und Triebverlängerung folgen. Da bisher über 600 Abkömmlinge im Boden eingewöhnt werden konnten, dürfte dies ein zuverlässiges Verfahren sein. Die Untersuchungen von vier verschiedenen Aspen-Kreuzungen zeigen wesentliche Unterschiede in organogenetischen Merkmalen, sowohl innerhalb der Kreuzungen als auch zwischen diesen. Dies kann ausgenutzt werden, um Sproßkulturen einzelner Sämlinge zu erhalten und ständig neue bewurzelungsfähige Sprosse in monatlichen Intervallen bis zu fünf Einzelkulturperioden zu erzielen. Ein solches System könnte bei der Isolierung und Vermehrung besonders guter Individuen aus konventionellen und unkonventionellen Züchtungspraktiken Anwendung finden.

### Introduction

Of all the commercially important forest species that have been propagated *in vitro*, none have enjoyed the success of members of the genus *Populus*. Indeed, the first woody species produced from callus using tissue culture techniques was *P. tremuloides* (WINTON, 1970). Since that time, there have been reports of poplars regenerated from sporophytic (VENVERLOO, 1973; WINTON, 1971; WOLTER, 1968) and gametophytic (anther) callus (SATO, 1974; WANG *et al.*,

1975) as well as from somatic tissue-derived suspension cultures (DOUGLAS, 1982). Protoplasts have also been isolated and cultured from differentiated (VERMA and WANN, 1983; for recent review see AHUJA, 1984) and dedifferentiated (DOUGLAS, 1983) tissues.

Throughout most of the earlier reported work, *in vitro* manipulations of poplars have circumvented a critical problem associated with tissue culture of woody species. This problem is the apparent recalcitrance of mature tissue to *in vitro* techniques. This recalcitrance has confined most tissue culture work, particularly in gymnosperms, to juvenile explants. Due to the ease of vegetative propagation in poplars, rejuvenation of mature trees has provided suitable material to establish tissue cultures with a high morphogenic potential. Recently, reports of successful micropropagation of plants from 17- to 40-year-old aspen (AHUJA, 1983) have suggested that rejuvenation may be unnecessary.

These factors have served to preclude *in vitro* work on seedlings of *Populus* species. However, we are interested in tissue culture of aspen seedlings for a number of reasons. Briefly, these are:

1. Aspen seedlings, even from full-sib crosses contain a considerable amount of phenotypic (and presumably genotypic) variation (DICKMANN and STUART, 1983). A tissue culture method might be used in conjunction with selection pressure to conveniently rogue seed lots for desirable characteristics.

2. Certain unconventional aspen breeding procedures [use of heat-treated (WINTON and EINSPAHR, 1968) or irradiated (RUDOLPH, 1978) pollen and interspecific crosses (STETTLER and BAWA, 1971)] are characterized by poor seed set and germination. A tissue culture method would make it possible to proliferate these few individuals for a more detailed examination of the potential of these techniques.

3. Aspen seedlings are an excellent source of protoplasts (VERMA and WANN, 1983). Examination of culture conditions that afford morphogenesis from seedling explants could serve as a starting point for protoplast culture. Due to our interest in the above mentioned areas we wish to describe a reliable method of micropropagation of *P. tremuloides* from seedling explants.

## Material and Methods

### Plant Material

Full-sib crosses (designated XT-3-83, XT-2-84, and XT-20-82) were made in March and April employing a cut branch technique (WETTSTEIN, 1933). The branches were collected from The Institute of Paper Chemistry arboretum near Greenville, WI. Male and female flower buds were forced in a greenhouse and seed was produced by hand pollinating with a small brush. Seed was collected 21–22 days after pollination. For the cross employing dead or damaged pollen, the material was subjected to a temperature of 50° C for 3.5 min. using a previously described pollen oven (WINTON and EINSPAHR, 1968).

### Production of Sterile Seedlings

Seeds were sterilized by a two step procedure. In the first step, seeds were stirred with a 0.02% solution of 8-quinolinol sulfate (chinosol) for 5 min. After rinsing with sterile water (3 × 50 mL) the seeds were treated with 0.525% sodium hypochlorite (10% v/v commercial bleach) for 5 min. In both steps Tween-20 (0.4% v/v) was employed as a wetting agent.

Following a rinse with sterile water (6 × 50 mL), seeds

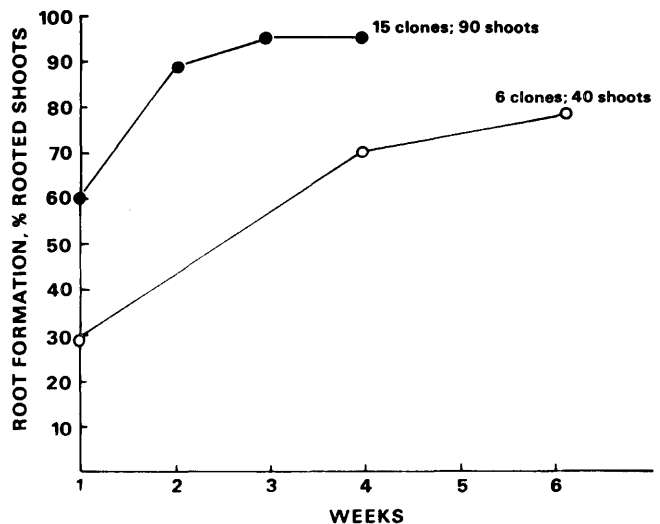


Figure 1. — Effect of sucrose addition (open circles = no sucrose; Closed circles = 1% sucrose) on root formation. (XT-0-24-80; 1/3 MS + 0.1 mg/L IBA).

were plated on 1% agar-water (Bacto; Difco) and incubated at 2,000 lux<sup>1</sup>) and 22° C. After 4 days, explants were taken to establish cultures.

### Shoot Differentiation

Cotyledons and hypocotyls were excised and placed (8 seedlings/9 cm Petri dish; 7 dishes/treatment) on a variety of media. This constituted the protocol for replicated studies of shoot differentiation within and between crosses that were subjected to a one-way ANOVA with Duncan's multiple range test ( $p < 0.05$ ). After 4 weeks at 22° C and 3,000 lux, morphogenesis was scored as the percentage of explants producing shoots. In addition, the number of shoots/explant with a length  $\geq 1$  cm was also recorded. In all subsequent work, MS medium (MURASHIGE and SKOOG, 1962) containing 0.1/1.0 mg/L NAA/BA (naphthaleneacetic acid/6-benzyl adenine) was employed to initiate shoot differentiation. The pH of the medium was adjusted to 5.8 prior to autoclaving, and the medium was solidified by the addition of 0.8% agar.

To elongate the shoots and multiple buds that lagged behind in development, the explants were transferred to 1/2 × MS (macro- and microelements) medium containing 0.3 mg/L BA and 1.5% sucrose. The pH and agar concentration were as above. After 3–4 weeks, the first crop of 1–2 cm shoots was harvested and transferred to a root initiation medium. The remaining explants were transferred to fresh medium to stimulate continued shoot formation and elongation.

### Root Formation

Root formation was achieved by placing excised shoots in 1/3 × MS (macro- and microelements) medium containing 0.1 mg/L IBA ( $\gamma$ -indole butyric acid) and 1% sucrose. The pH was adjusted to 6.7 and 0.65% agar was used for solidification. Incubation was at 22° C and 3,000 lux. The first roots appeared as early as 6 days, and by 3 weeks plantlets were ready for transfer to soil (see Figure 1).

### Plantlet Transfer

Plantlets were removed from the medium and most of the agar was carefully removed from the roots. The leaves from the lower half of the stem were removed, and the

<sup>1</sup>) All light regimes 16/24 h; cool-white fluorescent.

Table 1. — Effect of Media and Growth Regulator Regime on Shoot Proliferation Characteristics of Seedling Explants (XT-3-83).

Medium <sup>z</sup>	NAA/BA, mg/L	Ratio (BA/NAA)	Shoot Induction, %		Shoots/Explant	
			Hypocotyls	Cotyledons	Hypocotyls	Cotyledons
MS	0.01/1.0	100:1	95 <sup>a</sup>	47 <sup>b</sup>	6.1 <sup>b</sup>	4.4 <sup>a</sup>
	0.1/1.0	10:1	96 <sup>a</sup>	80 <sup>a</sup>	5.9 <sup>b</sup>	4.8 <sup>a</sup>
	1.0/1.0	1:1	61 <sup>b</sup>	48 <sup>b</sup>	3.1 <sup>c</sup>	3.1 <sup>b</sup>
	0.01/0.1	10:1	65 <sup>b</sup>	29 <sup>c</sup>	1.5 <sup>d</sup>	1.7 <sup>b,c</sup>
	0.1/0.1	1:1	50 <sup>b</sup>	15 <sup>c,d,e</sup>	1.1 <sup>d</sup>	2.0 <sup>b,c</sup>
LM	0.01/1.0	100:1	84 <sup>a</sup>	20 <sup>c,d</sup>	6.8 <sup>b</sup>	5.0 <sup>a</sup>
	0.1/1.0	10:1	95 <sup>a</sup>	19 <sup>c,d</sup>	7.3 <sup>a</sup>	4.4 <sup>a</sup>
	1.0/1.0	1:1	31 <sup>c</sup>	4.6 <sup>d,e</sup>	3.5 <sup>c</sup>	1.4 <sup>b,c</sup>
	0.01/0.1	10:1	66 <sup>b</sup>	5.4 <sup>d,e</sup>	1.3 <sup>d</sup>	1.6 <sup>b,c</sup>
	0.1/0.1	1:1	32 <sup>c</sup>	0.86 <sup>e</sup>	1.0 <sup>d</sup>	0.14 <sup>c</sup>

<sup>z</sup> MURASHIGE and SKOOG, 1962; LITVAY, *et al.*, 1981.

a, b, c, d, e Within a column, means followed by a common superscript are not significantly different by one-way ANOVA with DUNCAN's multiple range test ( $p < 0.05$ ).

plantlet was planted to this point. A soilless mixture composed of sand:peat:perlite (1:1:1) contained in Spencer-Lemaire Rootainers (175 cc) book planters was used. The plantlets were watered with a systemic fungicide, (Benomyl, 1 gm/L) and sealed in plastic bags to maintain a humid environment. The bags containing the plantlets were kept shaded in a greenhouse for 4 weeks at 3,000—5,000 lux. After 4 weeks, plantlets were removed from the plastic bags and kept under the ambient conditions of the greenhouse. In early summer 1984, 50 of these tissue culture-produced trees were planted at the Institute's arboretum near Greenville, WI.

### Results and Discussion

#### Sterile Seedling Production

The seed sterilization conditions were optimized with respect to germination. Employing higher concentrations of sodium hypochlorite and/or longer time intervals had adverse effects on germination and seedling vigor. The most typical indicator of harsh sterilization conditions was that root development of the seedlings was severely attenuated.

#### Shoot Proliferation

A limited study to examine the effects of growth regulators on shoot differentiation and shoot number/explant

was undertaken with the two media formulations employed. The number of shoots per explant was not entirely indicative of the proliferative ability of the explant, as many buds had not elongated at the time of scoring in some treatments. The results of this study are depicted in Table 1. In general, a greater percentage of hypocotyl segments gave rise to multiple adventitious buds, and the number of buds obtained was more uniform than for cotyledonary explants.

The effects of growth regulators can be divided into two groups based on the cytokinin regimes. The lower level of BA (0.1 mg/L) was characterized by production of 1—3 shoots that appeared without visible callus formation a week after the cultures were initiated. This response was relatively insensitive to the auxin levels employed, but at the higher level (0.1 mg/L NAA) extensive root formation replaced shoot differentiation.

The higher level of cytokinin (1.0 mg/L) resulted in cultures that proliferated callus which subsequently differentiated shoots after 3—4 weeks. The number of shoots obtained was similar for the lower levels of auxin (0.01 and 0.1 mg/L), but at 1.0 mg/L NAA, extensive parenchymatous callus formation predominated instead of the smooth, nodular callus that gave rise to shoots. This effect was clearly seen when loblolly pine medium (LITVAY *et al.*,

Table 2. — Organogenic Behavior of Four Different Aspen Crosses.

Cross No.	Shoot Induction Frequency, %	Elongation Frequency, %	Root Formation Frequency; Clones, %	Root Formation Frequency Ramets, %
XT-3-83	80 <sup>a</sup>	84 (51) <sup>y</sup>	96 (43)	94 <sup>z</sup> (43)
XT-0-24-80 <sup>w</sup>	69 <sup>a</sup>	75 (56)	94 (42)	88 (42)
XT-2-84	51 <sup>b</sup>	13 (60)	75 (8)	84 <sup>z</sup> (6)
XT-20-82 <sup>x</sup>	39 <sup>b</sup>	23 (22)	100 (5)	60 (5)

<sup>w</sup>Open-pollinated cross.

<sup>x</sup>Heat-treated pollen cross.

<sup>y</sup>Value in parentheses indicates the number of explants for which the frequency was determined.

<sup>z</sup>Minimum 6 ramets/clone.

a, b Within this column, means followed by a common superscript are not significantly different by one-way ANOVA with DUNCAN's multiple range test ( $p < 0.05$ ).

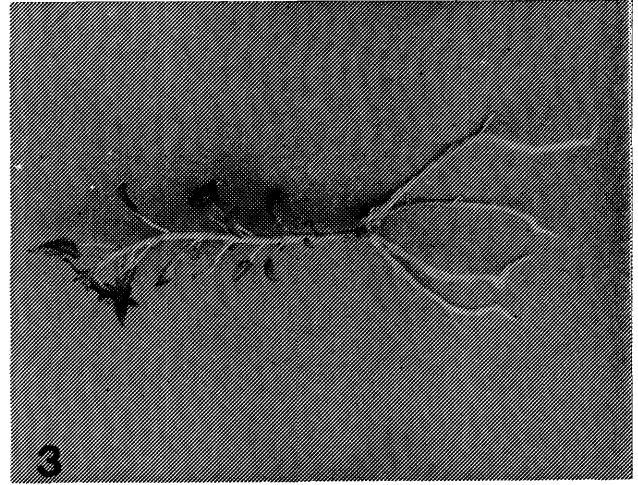
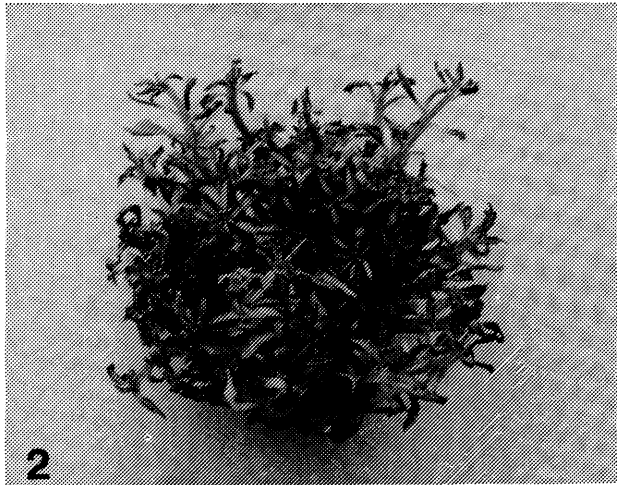


Figure 2. — Shoot culture established on elongation medium (8 weeks).

Figure 3. — Rooted plantlet ready for transfer to soil.

Figure 4. — Tissue culture trees 14 weeks after establishment in soilless medium.

1981) was employed. At 1.0/1.0 mg/L NAA/BA the shoot induction frequency drops significantly for both explants when compared to MS, and callus formation occurs almost exclusively from cotyledons. We have previously observed this propensity for LM to proliferate callus at the expense of differentiation, and BORNMAN has suggested that the low level of calcium in LM (22 vs. 440 ppm in MS) may

be the reason behind a similar suppression of differentiation in *Picea abies* (BORNMAN, 1983).

In spite of this, when LM was compared to MS at the two growth regulator levels that gave rise to the greatest bud proliferation (0.01/1.0 and 0.1/1.0 mg/L NAA/BA) there is little difference between the two media. Although MS and LM produced comparable numbers of shoots at the

time of evaluation, MS containing 0.1/1.0 mg/L NAA/BA was used in all subsequent work as it consistently produced the greatest number of buds that could subsequently be elongated.

Examination of several crosses on shoot proliferation medium suggests that there is a genetic component to *in vitro* behavior (see Table 2). Within a seed lot, two responses are seen on this medium — parenchymatous callus proliferation or nodular callus that differentiated buds. Significant differences in shoot induction frequency occurred between the normal diploid crosses and the heat-treated pollen cross which was severely impaired with respect to organogenesis.

In addition to differences in induction frequency, the ability of the buds to develop into rootable shoots also varied between seedlots. In particular XT-2-84 and XT-20-82 yielded a high percentage of leafy buds that never elongated and developed stems.

In each seed lot, a number of explants were exceedingly prolific. For those explants, shoot cultures could be established on elongation medium that produced a continuous harvest and multiplication of rootable shoots for up to 5 monthly subcultures, (see Figure 2).

#### Shoot Elongation

Transfer of cultures that produced rootable shoots to 1/2 MS containing 0.3 mg/L BA stimulated elongation of those buds that had lagged behind in development. In addition, this treatment caused axillary bud elongation in existing shoots which could also be excised and rooted. By taking care during excision to leave a portion of the shoot behind, this "microcoppicing" became an effective way to perpetuate certain seedlings in a juvenile state. Under these conditions, full-sib clones were established in which a varying number of ramets (1 to 60 ramets) had been rooted.

#### Root Formation

Root formation was facile, perhaps because of the juvenile nature of the material, since woody cuttings of quaking aspen are considered difficult to root (EINSPAHR and WINTON, 1977).

The inclusion of a carbon source (1% sucrose) significantly enhanced the rate and frequency of rooting within clones (see Figure 1). When sucrose was employed in the medium, the level of IBA was kept at 0.1 mg/L. At 1.0 mg/L IBA, a large mass of basal callus formed at the end of the shoot that later differentiated roots.

In the normal diploid crosses, most of the derived clones produced roots (see Table 2). The rooting ability of the ramets within a clone was also insensitive to differences in parentage. For the heat-treated pollen cross, all of the 5 clones examined produced roots but at lower frequency. In this cross, stunted, knobby roots were formed that reduced subsequent survivability in soil.

#### Plantlet Transfer

Figure 3 shows a representative plantlet that can be readily transferred to a simple, soilless medium and hardened without the need of expensive growth chambers or intermittent misting. In one study, the survival after 2 months in this medium was 115/134 individuals (86%) for XT-0-24-80 and 109/119 (91%) for XT-3-83. At the time of transfer, several plantlets exhibited characteristics that seemed to be artifacts of the tissue culture system (i.e., vitrified or lanceolate leaves, or a rosette pattern of the terminal bud). However, once established in the soilless medium these features were quickly lost and the plants

grew and developed in a manner that was indistinguishable from quaking aspen seedlings (see Figure 4). Although ploidy level was not determined, plantlets resembled seedlings from the crosses in which they were derived. While examination of gross features like leaf shape suggests this is a clonal method of propagation, this assumption is currently under examination by establishing shoot cultures from a single cotyledon and growing the remainder of the seedling. In this way a direct comparison of propagules with the donor plant will be made.

#### Conclusions

Aspen seedling explants display a remarkable capacity for morphogenesis that can be manipulated by hormonal regimes to give roots, shoots, or callus. Organogenesis appears to have a strong genetic component both within and between crosses, and differences between clones in organogenic behavior has been previously reported (AHUJA, 1983) for *Leuce* poplars. This feature can be exploited to isolate and establish shoot cultures that have to date provided a continuous source of rootable shoots. The suppression of all organogenic features in the heat-treated pollen cross suggests that this treatment caused some manner of alteration. Although the ploidy level was not determined, use of heat-treated pollen has been reported to give rise to aneuploidy and mixoploidy (WINTON and EINSPAHR, 1968), which may account for its reduced capacity for organogenesis. The three clones derived from this cross that have been established in soil do not exhibit phenologies or growth rates different from the normal diploid crosses.

The reproducibility of this micropropagation technique has enabled its utilization in examining the role of polyamine synthesis inhibitors on differentiation (FEIRER *et al.*, 1984). The use of this technique for isolation and propagation of unique individuals from conventional or non-conventional hybridizations may be applicable if selection does not coincide with the lack of organogenic capacity within and between crosses.

#### Acknowledgements

The authors wish to thank G. WYCKOFF, R. ARVEY and J. LORENZ for their care of the tissue culture plants. One of the authors (SRW) wishes to thank the member companies of The Institute of Paper Chemistry for financial support during this work. Portions of this work were used by SRW as partial fulfillment of the requirements for the Ph.D. degree at the Institute.

#### References

- AHUJA, M. R.: Protoplast research in woody plants. *Silvae Genetica* 33: 32-37 (1984). — AHUJA, M. R.: Somatic cell differentiation and rapid clonal propagation of aspen. *Silvae Genetica* 32: 131-135 (1983). — BORNMAN, C. H.: Possibilities and constraints in the regeneration of trees from cotyledonary needles of *Picea abies in vitro*. *Phys. Plant.* 57: 5-16 (1983). — DICKMANN, D. and STUART, K. W.: The Culture of Hybrid Poplars in Eastern North America. Hickory Hollow Associates, Dansville, MI. 168 p. (1983). — DOUGLAS, G.: Protoplast isolation from totipotent cell-cultures of *Populus* hybrid TT32. In: Proc. 5th Intl. Cong. Plant Tissue and Cell Culture. FUJIWARA, A. (ed.) Tokyo. p. 605-606 (1982). — EINSPAHR, D. W. and WINTON, L. L.: Genetics of Quaking Aspen. U.S. Dept. Agric. For. Ser. Res. Paper WO-25, p. 8 (1977). — FEIRER, R. P., MIGNON, G. and WANN, S. R.: Effect of spermidine synthesis inhibitors on *in vitro* plant development. *Plant Physiol.* (Supplement) 75: 103 (1984). — LESTER, D. T. and BERBEE, J. G.: Within-clone variation among black poplar trees derived from callus culture. *For. Sci.* 23: 122-131 (1977). — LITVAY, J. D., JOHNSON, M. A., VERMA, D. C., EINSPAHR, D. W. and WEYRAUCH, K.: An Improved Medium for the Culture of Loblolly Pine Cell Suspensions. *Inst. Paper Chem. Tech. Paper Series*, 114: 17 p. (1981). — MURASHIGE,

T. and SKOOG, F.: A revised medium for rapid growth and bioassay of tobacco tissue cultures. *Phys. Plant.* 15: 473–497 (1962). — RUDOLPH, T. D.: Seed yield and quality in *Populus tremuloides* following pollination with gamma-irradiated pollen. *Can. J. Bot.* 56: 2967–2972 (1978). — SATO, T.: Callus induction and organ differentiation in anther culture of poplars. *J. Japan Forestry Soc.* 56: 55–62 (1974). — SRETTLER, R. F. and BAWA, K. S.: Experimental induction of haploid parthenogenesis in black cottonwood. *Silvae Genetica* 20: 15–25 (1971). — VENERLOO, C. J.: The formation of adventitious organs. I. Cytokinin induced formation of leaves and shoots in callus culture of *Populus nigra* L. 'Italica'. *Acta Bot. Neerl.* 22: 390–398 (1973). — VERMA, D. C. and WANN, S. R.: Isolation and high yield of protoplasts from quaking aspen seed-

lings and cultured loblolly pine cell suspensions. In: 6th Intl. Protoplast Symposium. POTRYKUS, I. *et al.* (eds.). *Experientia Suppl.* 10–11 (1983). — WANG, C. C., CHU, Z. C. and CHU, C. C.: The induction of *Populus* pollen-plants. *Acta Bot. Sinica* 17: 56–59 (1975). — WINTON, L. L.: Shoot and tree production from aspen tissue cultures. *Amer. J. Bot.* 57: 904–909 (1970). — WINTON, L. L.: Tissue culture propagation of European aspen. *For. Sci.* 17: 348–350 (1971). — WINTON, L. L. and EINSFAHR, D. W.: The use of heat-treated pollen for aspen haploid production. *For. Sci.* 14: 406–407 (1968). — WETTSTEIN, W. V.: Die Kreuzungsmethode und die Beschreibung von F1 Bastarden bei *Populus*. *Zeit. für Züchtung Pflanzzüch.* 18: 597–626 (1933). — WOLTER, K. E.: Root and shoot initiation in aspen callus cultures. *Nature* 29: 509–510 (1968).

## Genetic variances and interactions in 9-year-old Douglas-fir grown at narrow spacings

By R. K. CAMPBELL, R. M. ECHOLS and R. W. STONECYPHER<sup>1)</sup>

(Received 26th March 1985)

### Summary

The variance structure (additive-genetic, plot, within plot, and interactions) in a population of unselected Douglas-fir was studied; 54 control-pollinated families grown at three close spacings (30, 60, 90 cm) in two plantations were used. Significant genotype  $\times$  spacing interaction occurred only for height of 5-year- and volume of 9-year-old Douglas-fir and could be attributed to measurement scale. Plantation  $\times$  genotype interactions occurred in diameter, height, height increment, branch length, volume, and dry weight of stem. Traits fell roughly into three individual-tree heritability ( $h^2$ ) classes. The lowest group ( $h^2 \approx 0.15$ ) included total height at younger ages, height increment and branch length; the medium group ( $h^2 \approx 0.20$ ), 9-year height, diameter, volume, and dry weight of stem; and the highest group ( $h^2 \approx 0.48$ ), specific gravity.

Close spacing appears to have the potential for improving effectiveness of early selection for total height by increasing the age-age correlation as hypothesized by FRANKLIN (1979). Close spacing, however, apparently did not shift the genetic variance structure from the juvenile to the mature phase. Coefficients of variation for additive-genetic, plot, and within-plot effects were similar to corresponding coefficients calculated for tests in young loblolly pine, ponderosa pine, and Douglas-fir grown at much wider spacing.

**Key words:** Genetic variations, plantation spacing, growth, growth rate, Douglas-fir, *Pseudotsuga menziesii*.

### Zusammenfassung

Es wurde die Varianzstruktur (additiv-genetische, Parzellen-, innerhalb der Parzellen und Interaktions-Varianz) in einer Population unselektierter *Pseudotsuga menziesii* studiert. Hierzu wurden 54 kontrolliert bestäubte Familien, die in 3 engen Pflanzen-Abständen (30, 60, 90 cm) auf zwei Versuchsflächen herangewachsen waren, benutzt. Signifikante Genotyp  $\times$  Standraum Interaktionen gab es nur für die Höhe im Alter 5 und das Volumen im Alter 9 der Douglasien und konnten der Meßskala zugeschrieben werden.

<sup>1)</sup> The authors are Research Geneticist, USDA Forest Service, Pacific Northwest Forest and Range Experiment Station, 3200 Jefferson Way, Corvallis, Oregon, USA; Research Forester (Retired), USDA Forest Service, Pacific Southwest Forest and Range Experiment Station, Berkeley, California, USA; and Research Advisor, Tree Improvement, Forestry Research Center, Weyerhaeuser Company, Centralia, Washington, USA.

Versuchsfläche  $\times$  Genotyp Interaktionen traten beim Durchmesser, bei der Höhe, dem Höhenzuwachs, der Astlänge, dem Volumen und dem Stammrockengewicht auf. Die Merkmale fielen grob in 3 Einzelbaum-Heritabilitäts- ( $h^2$ )-Klassen. Die niedrigste Gruppe ( $h^2 \approx 0.15$ ) enthielt die Gesamthöhe im jüngeren Alter, den Höhenzuwachs und die Astlänge; die mittlere Gruppe ( $h^2 \approx 0.20$ ) die Höhe im Alter 9, den Durchmesser, das Volumen und das Stammrockengewicht, die höchste Gruppe ( $h^2 \approx 0.48$ ) das spezifische Gewicht.

Der enge Abstand schien die Fähigkeit zu besitzen, die Effektivität für eine Frühselektion auf die Gesamthöhe durch Erhöhung der Alter zu Alter Korrelation zu verbessern, wie Franklin 1979 hypothetisch angenommen hat. Ein geringer Pflanzenabstand verschob jedoch augenscheinlich nicht die genetische Varianzstruktur von der Jugend- zur Altersphase. Additiv genetische Variationskoeffizienten und Parzellen-Variationseffekte, sowie die innerhalb von Parzellen waren entsprechenden Koeffizienten ähnlich, die in Tests mit jungen *Pinus taeda*, *Pinus ponderosa* und *Pseudotsuga menziesii* errechnet wurden, die mit viel größerem Abstand aufgewachsen waren.

### Introduction

In a previous study of 3-year-old, nursery-grown Douglas-fir (*Pseudotsuga menziesii* [MIRB.] FRANCO var. *menziesii*) seedlings with a widest spacing of 18 cm  $\times$  18 cm, spacing-genotype interactions in stem diameter and volume were attributed to effects of measurement scale (CAMPBELL and WILSON 1973). In this paper, we report an experiment involving 9-year-old cross-pollinated families grown in two plantations at square spacings ranging from 30 to 90 cm.

The experiment tested interaction but also provided information on genetic and environmental variances for several traits, and on age-age correlations for stem height. Variance structures for stem height have been shown to change with age in Douglas-fir (NAMKOONG *et al.* 1972), ponderosa pine (*Pinus ponderosa* LAWS.) (NAMKOONG and CONKLE 1976), loblolly and slash pines (*P. taeda* L., *P. Elliotii* ENGELM.) (FRANKLIN 1979). These changes apparently give rise to characteristic time trends in variances, heritabilities, and age-age correlations (FRANKLIN 1979). FRANKLIN suggested that trends might be manipulated by silviculture, one factor being close spacing, to increase the age-age correlation and thereby make early selection more