susceptible than Lombardy poplar but need further testing for *Marssonina* resistance. The Lombardy poplar is being hybridised with rust-resistant *Populus deltoides* clones in an attempt to combine the fastigiate character with high rust-resistance.

A new tree willow hybrid, *Salix matsudana* × *S. alba* cl. 'NZ 1002', has been released and other tree willow hybrids are being tested in shelterbelts at the Centre.

Hybrids of the common 'pussy willow' *Salix discolor*' and other shrub willows have also been established in shelterbelts at the Centre.

Other genera established for shelter include, *Alnus*, *Betula*, *Casuarina*, *Eucalyptus*, *Tamarix* and *Olearia*. Many more genera are being tested for erosion control and any which appear to have promise for farm or horticultural shelter are retained and tested for this purpose.

RAPID PROPAGATION OF ASPENS AND SILVER POPLARS USING TISSUE CULTURE TECHNIQUES

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In New Zealand poplars are extensively used for soil conservation, farm forestry and for orchard shelter. In 1972 nearly 1 million poplars were planted.

In 1973, however, two species of poplar rust, *Melampsora medusae* and *M. larici-populina* became established in New Zealand. These fungi cause severe premature defoliation, which can result in branch dieback and even in death of the very susceptible poplar clones. Many of the most common poplar clones were affected and their continued cultivation became impossible. As a result poplar planting decreased dramatically in the following years until the first resistant clones selected by the National Plant Materials Centre (now part of the Aokautere Science Centre) became available in 1976.

It also had become apparent that a small number of the existing poplar clones were resistant to the rusts, notably *Populus alba* (silver poplar), *P. tremula* (European aspen), and *P. tremuloides* (American aspen). These poplars, besides being disease resistant, also possess a suckering habit making them valuable in soil conservation planting. However, the aspen pop-

lars, in particular, have not been widely used as they are difficult to propagate by hardwood cuttings. They can be propagated from root cuttings taken in winter or from softwood tip cuttings under mist in early summer. The number of propagules could be increased more rapidly by placing root cuttings in sphagnum moss in the glasshouse in winter and harvest the young shoots which develop from the preformed shoot initials in the root segments. These young shoots are then rooted under mist. This method was successfully used at the Aokautere Science Centre (2). However this production method was still too slow to satisfy the estimated demand of 200,000 silver and aspen-poplars which can be used annually in soil conservation plantings. Tissue culture methods can considerably speed up the production of these clones.

This paper describes the rapid method of silver and aspen poplar multiplication being used in the tissue culture laboratory at Aokautere Science Centre, Palmerston North. The techniques used here represent the development and practical application of the original work by Whitehead and Giles (6) of the Plant Physiology Division, DSIR.

The process of plant propagation *in-vitro* must proceed through three different steps known simply as stages I, II and III. The various steps in each stage are:

Stage I. In the first stage a sterile living explant is established in culture. Apical and axillary buds are removed from adequately chilled dormant shoots or actively growing shoots in spring through to mid-summer. Excised root sucker shoots have been used to initiate new cultures in autumn.

Buds are prepared for sterilisation by trimming off leaves whilst retaining a small piece of petiole attached to the stem. The buds are rinsed in ethanol prior to a 15 minute surface sterilisation in a solution of 0.15 to 0.3% sodium hypochlorite and 0.05% Tween 80. This is followed with three sterile distilled water rinses. The success of the sterilisation procedure depends in part upon the preculture growing conditions (1). A period of shoot growth in a glasshouse coupled with the application of Bavistan^{R} and Cuprox^{R} sprays prior to culture reduces microbial contamination of most explants to less than 5%. With the aid of a low-power stereo microscope the outer budscales are removed aseptically from the bud in a laminar air-flow hood. Excised buds 1 to 3mm are cultured on a solid agar medium using a modified Murashige-Skoog salts medium (5), plus a cytokinin to stimulate cell division. Refer to Table 1 for individual species media requirements. Alternatively, whole buds may be trimmed and placed onto the same medium as excised buds.

Table 1. The modified Murashige and Skoog medium for in vitro propagation of aspen and silver poplars:

Compound	mg/l	Compound	mg/l
NH_4NO_3	1650	Na_2 $Mo0_4$ $2H_20$	0.25
KNO_3	1900	$CuS0_4$ $5H_2O$	0.025
$CaCl_2$ $2H_2O$	440	$CoCl_2$ $6H_2O$	0.025
$MgSO_4$ $7H_2O$	370	Adenide Sulfate	20.0
KH_2PO_4	170	Nicotinic Acid	0.5
$MnSO_4$ $4H_2O$	22.3	Pyridoxin-HCl	0.5
EDTA	37.3	Thiamine-HCl	0.1
$FeSO_4$ $7H_2O$	27.8	Lysine	100
Zn SO_4	8.6	Inositol	100
H_3BO_3	6.2	Sucrose	20000
KI	0.83	Agar	10000

Hormone requirements (mg/l) of Populus species for in vitro propagation.

Species	Stage I	Stage II	Stage III
P. alba	BA.2	BA.2 NAA.02	IBA.2
P. alba x *glandulosa*	BA.5	BA.5 NAA.02	IBA. 5 NAA.1
P. alba x *tremula*	BA.5, 1	BA.5 NAA.02	IBA.2, .5
		BA.5 NAA.05	
P. canescens	BA.5, 1	BA.5 NAA.02	IBA. 2, .5
P. tremula	BA.5	BA.5	IBA.5 NAA.1
		BA.5 NAA.02	
P. tremuloides	BA.5	BA.5	IBA.5 NAA.1
		BA.5 NAA.02	

BA = benzyl adenine
NAA = naphthaleneacetic acid
IBA = indolebutyric acid

Cultures are incubated at 25°C under fluorescent lights (1 to 3000 lux) with a 16 hour photoperiod. Cultures are regularly checked for microbial contamination, clean explants are transferred to fresh medium after 3-4 days. The growth of bud explants is not uniform, some buds begin growth almost immediately whilst others required 6 to 8 weeks to commence growth. In contrast, shoots from root suckers invariably begin growth immediately.

The hormones in the growing medium control the growth and development of the explant. Basically the types of growth that may develop in this stage depend upon the auxin-cytokinin ratio. With a high auxin-cytokinin ratio in the medium unorganised callus is produced. The organised shoot growth and proliferation promoted by a low auxin to cytokinin ratio, is preferred to the production of undifferentiated callus. Although aspen callus has been differentiated into plantlets by Winton (7), Lester and Berbee in (3) reported irregular shoot production from callus coupled with cytological and morphological variation in plants produced from the same popular callus. Chromosomal abnormalities have been noted in other angiosperm cultures. *Prunus* (8) and *Acer* (4) callus show increasing abnormalities after several subcultures.

The first stage is completed when a sterile explant has commenced growth and possibly some shoot proliferation. When this is accomplished the shoots are transferred to stage II for bulking-up.

Stage II. In stage II, aspen and silver poplar multiplication in vitro is being achieved in two ways:

a) By the production of adventitious shoots from the explant surface in contact with a high cytokinin medium. A large number of small shoots and buds are produced. However, shoot elongation may be retarded, this makes subculturing difficult without the aid of a microscope.

b) Another system for propagule multiplication depends upon the enhanced axillary branching of shoots that can be stimulated by the appropriate combination of medium cytokinin and auxin (See Table 1). This system allows the regular subculturing of shoots 1 to 2 cm long onto fresh medium every 4 to 6 weeks. This proliferation process may be continued almost indefinitely, until the required number of shoots are produced.

An average multiplication rate of 10 shoots per culture per month can be attained, this would suggest more than 10^{10} plantlets per annum could be produced from one original bud. However, in practice, incubation space and nursery facilities could quickly become limiting factors. With the present facilities at Aokautere a daily production of up to 200 plantlets can be achieved. The number of proliferating cultures is kept at constant level by transferring 9/10 of the shoots to Stage III and placing the remaining 1/10 back onto fresh proliferation medium

Stage III. In this stage shoots 1 to 2 cm long are rooted in a simplified agar medium under lights ca 5000 lux. The best root initiation and growth is promoted by using both IBA and NAA in the rooting medium. Callus production in this rooting medium has been reduced by deleting the lysine, inositol and adenine sulphate from the basal medium.

Good root development on 80% of the shoots is obtained in 10 to 14 days, whereafter plantlets can be hardened off. Rooted shoots are then prepared for transfer from agar to a potting medium. To allow for some adaptation to the glasshouse light and temperature regime, plantlets are placed in the glasshouse for two days before being taken out of the agar. Most of the agar is sloughed off plantlet roots prior to potting in a soilless growing medium. Plantlets are sprayed with a systemic fungicide and placed under intermittent mist for 7 to 14 days. During the hardening off period it is particularly important to control water loss by intermittent mist or very humid conditions as plantlets fresh out of culture are unable to remain turgid. Intermittent

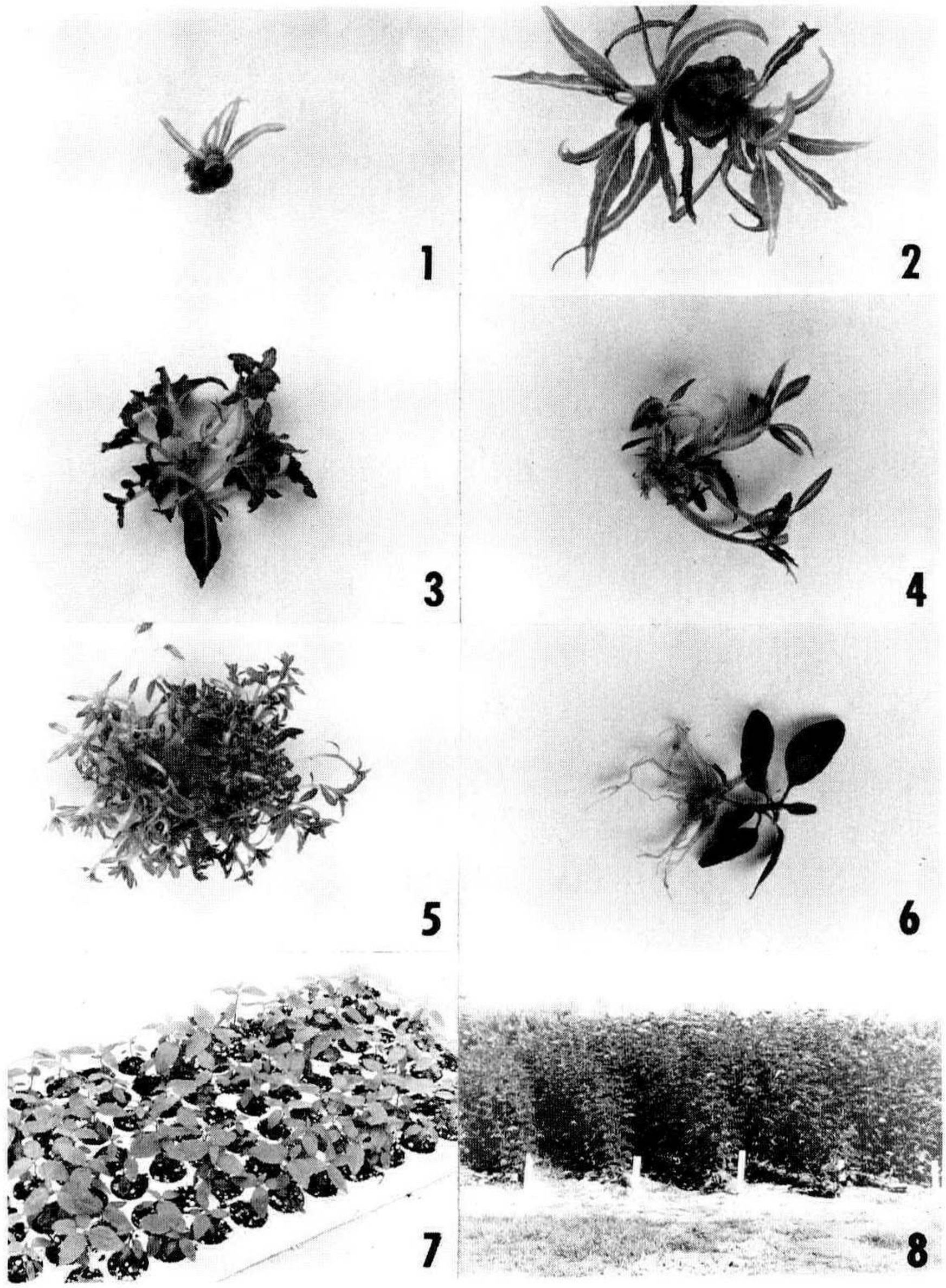

Figure 1. *In vitro* propagation of poplar plantlets and nursery trees from vegetative buds.

1. Growth of *Populus tremula* bud 2 weeks after excision (0.3 cm diam.).
2. Development of axillary buds from *P. alba* × *glandulosea* bud explant after 4-6 weeks (1.5 cm diam.).
3. Enhanced axillary branching of *P. canescens* (2 cm diam.).
4. Adventitious shoot production at the base of *P. tremuloides* shoot (1 cm long).
5. Advanced shoot proliferation of *P. alba* × *nigra* (5 cm diam.).
6. Rooted plantlet of *P. alba* × *glandulos* ready for hardening-off (1.5 cm long).
7. Hardened-off plantlets of *P. alba* × *glandulos* 4 weeks after leaving the laboratory (5-7 cm high).
8. Nursery trees of *P. alba* × *glandulosa* almost 2 m high 4 months after planting out.

mist and photoperiod extension in short days allows 90% of the plantlets to be weaned successfully. The young plants are then held on an unmisted bench for 7 days, during which time extensive root development takes place.

Finally, plants are potted into peat pots and grown-on in the glasshouse until about 10cm high; they are then hardened off outside prior to field planting. They then grow rapidly, attaining 1.5 to 2 meters within 3 months after planting in the field (Figure 1).

SUMMARY AND CONCLUSIONS

The vegetative propagation of difficult-to-propagate poplars has been achieved using tissue culture techniques.

The rapid bulking up of new clones allows quicker introduction of this material into new and existing planting programmes. It is planned to have 20,000 plants of two new *P. alba* x *glandulose* clones available for Catchment Board plantings in 1979. All the plants produced to the present time appear identical to the stock plants.

Besides the in vitro propagation of poplars, the sterile plant cultures have provided a very useful way to import new clones with minimal risk of disease entry. It is envisaged that the international exchange of sterile plant material in this manner will become of increasing importance to plant propagators throughout the world.

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