## MACRO AND MICRO PROPAGATION OF LEYLAND CYPRESS

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**Abstract.** Conventional propagation of Leyland cypress by stem cuttings is briefly reviewed; results are rarely consistent or reproducible. An alternative approach using micropropagation is outlined. Shoot tips (30 mm) from the lower branches of a 9-year-old hedge of 'Leighton Green' rejuvenated spontaneously after 3 months in Woody Plant Medium (WPM), or in WPM supplemented with 0.1 mg l<sup>-1</sup> NAA, IBA, or TBA, or 2 mg l<sup>-1</sup> BA or 2iP The juvenile shoots were maintained on WPM for shoot elongation, or on WPM containing 2 mg l<sup>-1</sup> BA plus 0 1 mg l<sup>-1</sup> NAA, for bud proliferation. Roots were induced in 0 4% agar medium containing either 1 mg l<sup>-1</sup> NAA or 1 mg l<sup>-1</sup> IBA. Although results are encouraging, further work is required to perfect the technique

#### INTRODUCTION

Leyland cypress, × Cupressocyparis leylandii (Jack. & Dallim.) Dallim., a spontaneous hybrid between Cupressus macrocarpa Hartw., and Chamaecyparis nootkatensis (D.Don) Spach., was first described by Jackson and Dallimore (5). Separate hybridisations have resulted in several clones, some of which have received cultivar names (6, 9). Leyland cypress has become increasingly popular for a range of uses due to its fast growth and environmental adaptability. Propagation has been by stem cuttings, but marked inconsistency of rooting, especially in the "green" clones, has directed attention to the possibilities of micropropagation techniques.

# MACROPROPAGATION

An extensive investigation of conventional propagation of Leyland cypress was made by members of the IPPS in the early 1970s and reported by Howard (4). This and later work (8) showed the strong influence of local factors on the rooting process and highlighted the lack of understanding of the plant mechanisms responsible. Published results covering most of the factors perceived as influencing rooting, including selection and pre-treatment of cuttings, time of year, rooting media, temperature and lighting conditions etc., reveal a range of results that are, overwhelmingly, neither consistent nor reproducible (Sturrock, unpublished). Some apparently refute physiological expectation: thus, records of better root initiation and faster root growth at lower rather than higher medium temperatures are paradoxical, and must indicate strong interactive effects among rooting factors.

Such complexity confounds inherent differences in rooting that may exist among Leyland cypress clones. A concensus view, nevertheless, suggests that rooting is easier in 'Haggerston Grey', 'Stapehill', and 'Ferndown' than in 'Leighton Green' and 'Naylor's Blue'. However, an experiment at Lincoln showed that the latter clones may, in some circumstances, root as well as or better than 'Haggerston Grey' (Figure 1). These graphs show that apparent differences among clones relate, at least partially, to differences in rates of rooting with the date of examination determining clonal rooting percentages. This result confirms an earlier conclusion reached by Deen (3) based on Dutch work. Within a single clone, rooting may take anywhere from five weeks to seven months. Undue delay in root initiation may have precluded clones with other desirable attributes from fuller commercial exploitation, e.g. 'Naylor's Blue' which has a tree branch structure superior to most other clones.

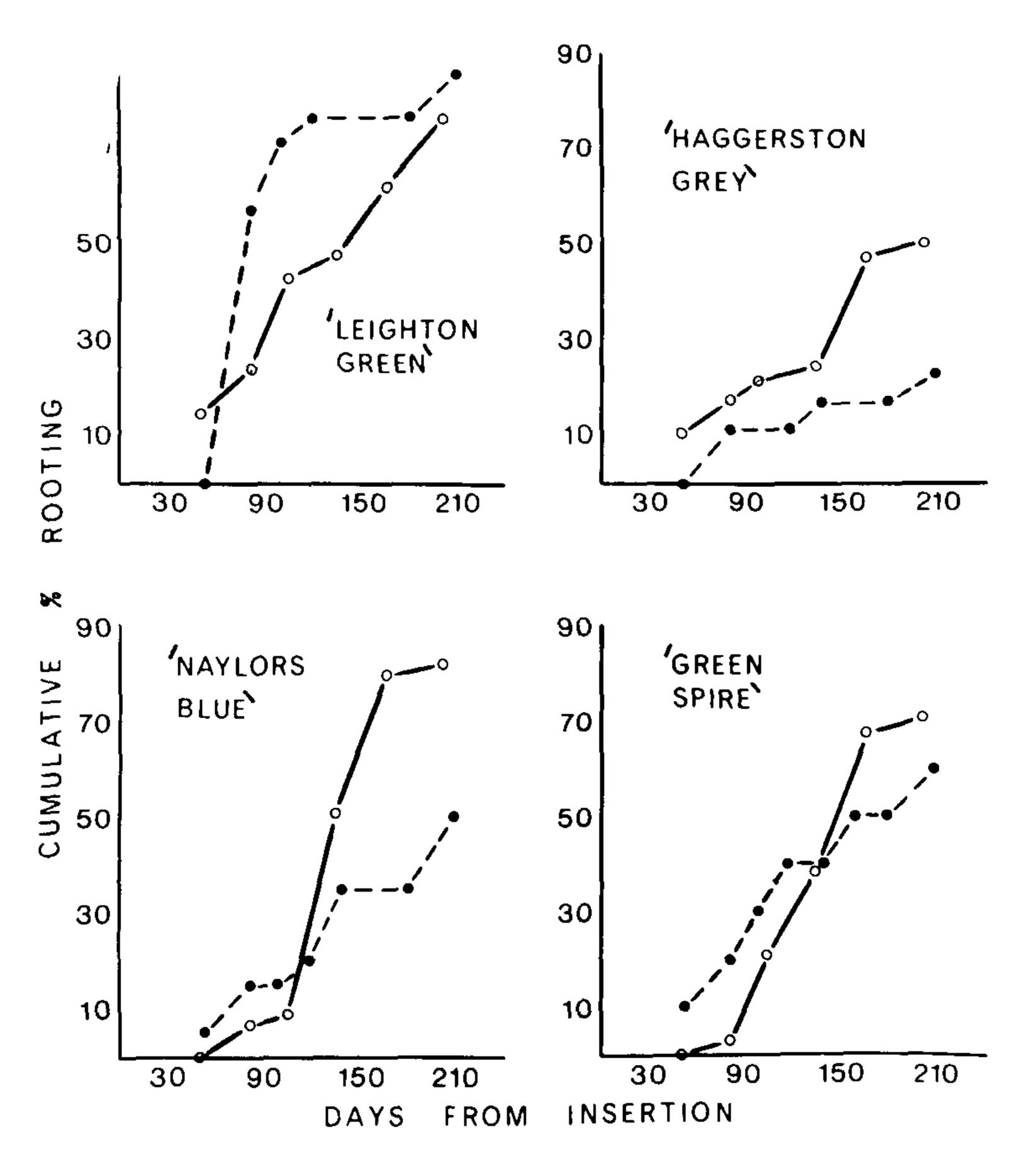


Figure 1. Cumulative rooting percentages in cuttings of four Leyland cypress clones inserted in spring (solid line) and autumn (dashed lines)

Number of cuttings per treatment = 40

Successful micropropagation would be especially useful with such recalcitrant clones and generally allow greater reliability in mass production of Leyland cypress.

## **MICROPROPAGATION**

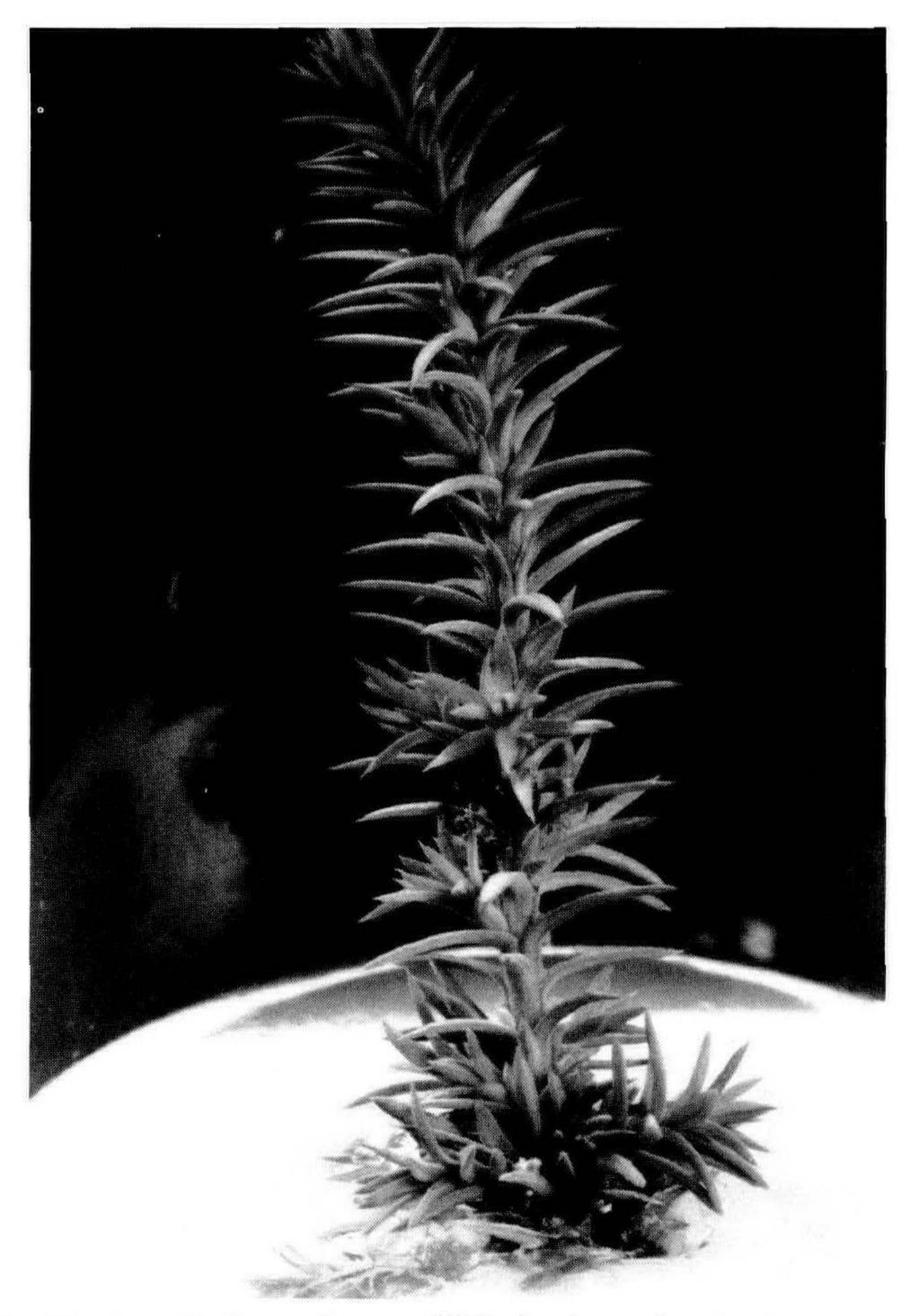
Culture methods. Shoot tips (30 mm) were taken in April (autumn) from the lower branches of a 9-year hedge of 'Leighton Green'. Following surface sterilisation in 0.1%  $HgCl_2$  for 45 min. they were placed in Woody Plant Medium (WPM) (7) with various added growth substances then cultured in a growth room at 24 °C with a 16 hr photoperiod of 28  $\mu$ m<sup>-2</sup> s<sup>-1</sup>. Cultures were made in 100 ml Erlenmeyer flasks with cotton wool bungs, containing 50 ml of the medium. There were five replicates in each treatment with a subculture time of 12 weeks. The material has been in culture for 4 years.

Experiments and results. Forty percent of the apices were lost because of bacterial or fungal contaminants. The use of 0.01, 0.1, 1.0, and 10 mg l-1 indoleacetic acid (IAA), indolebutyric acid (IBA), naphthyleneacetic acid (NAA), 2,4-dichlorophenoxyacetic acid (2,4-D), or trichlorophenoxybutyric acid (TBA) failed to induce rooting although profuse callus was induced in the two higher concentrations.

Juvenile foliage was formed in a number of treatments and the orthotropic form present in the main axis of the parent tree was restored.

This juvenile form occurred in the basic WPM medium as well as in this medium containing 0.1 and 1.0 mg l<sup>-1</sup> NAA or TBA. It appeared also in subsequent treatments including 2 mg l<sup>-1</sup> benzyladenine (BA) or 2-isopentyladenine (2iP), with or without 0.1 or 1.0 mg l<sup>-1</sup> NAA or IBA. Callus was often produced basally with axillary buds growing out through it.

The number of juvenile shoots was increased by inducing lateral bud growth in a medium containing 2 mg l<sup>-1</sup> BA + 0.1 mg l<sup>-1</sup> NAA, and shoot elongation was encouraged by alternate passages in basic WPM During the year, summer growth reached a maximum of 60 mm; in winter, growth was reduced to as little as 10 mm. The proliferating buds on decapitated stumps were a useful source of clonal material (Figure 2).



**Figure 2.** The juvenile form of an established culture showing lateral bud growth.

During the investigation, a possible alternative source of shoot material was noted as tiny adventitious buds developing on the swollen leaves of the basal buds of shoots in 3 ppm of a cytokinin. This has been noted in other gymnosperms (1, 2). However, they proved unsuitable substitutes for lateral shoots because of difficulty in culturing.

Roots have only been induced in soft 0.4% Difco agar medium containing 1 mg l<sup>-1</sup> NAA or IBA. They developed after 6 weeks on shoots that had already acquired a callused base (Figure 3). They were thick, robust organs but their presence did not enhance the growth of shoots. Growth equalled that of unrooted shoots, about 25 mm in 3 months.

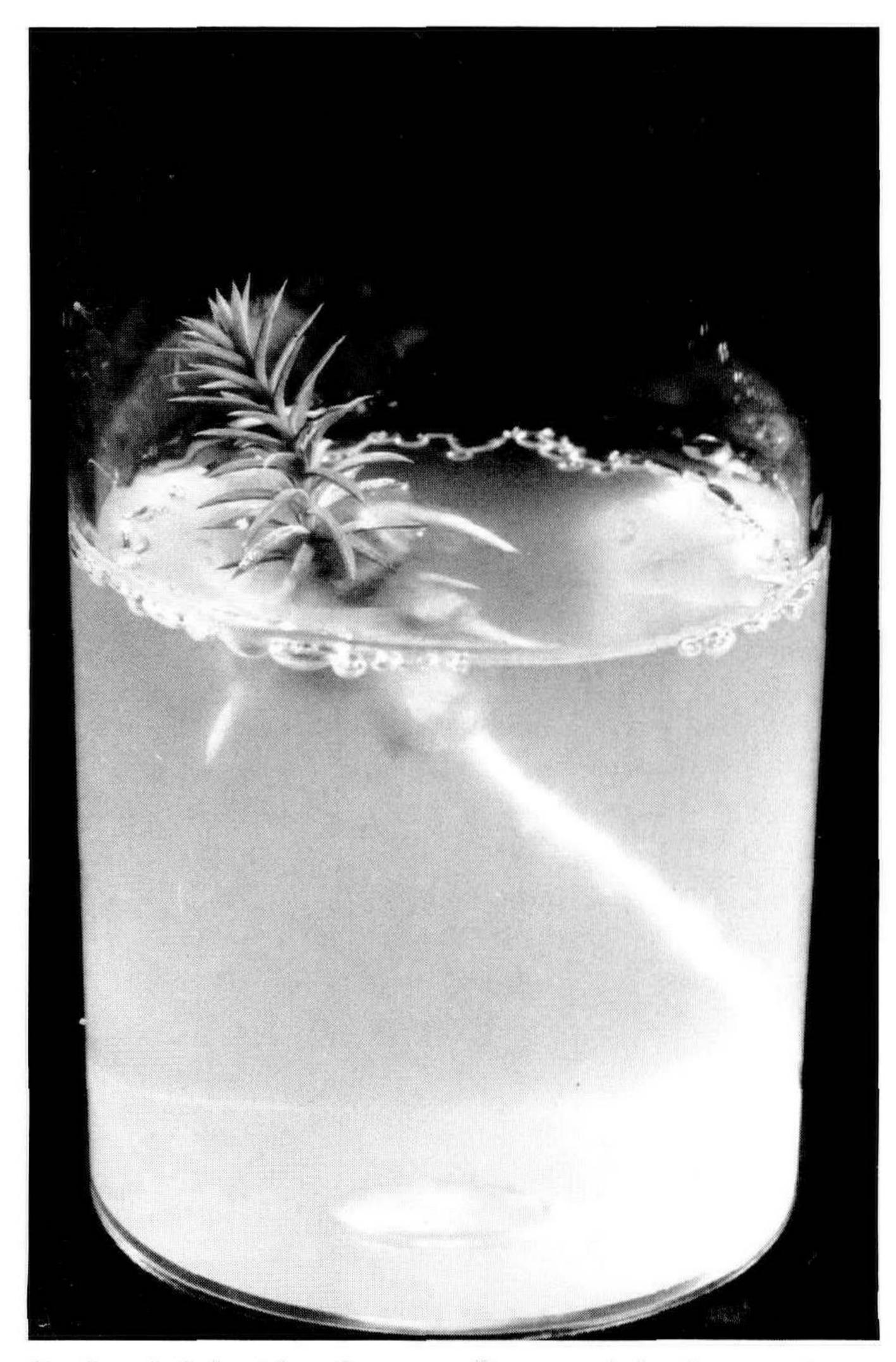


Figure 3. A rooted shoot in soft agar medium containing 1 mg per litre NAA.

Rooted specimens have just been transferred to soil and their suvival and growth have yet to be assessed.

# CONCLUSIONS

The problems that arise with macropropagation of Leyland cypress viz: inconsistency and a long rooting period, have not yet been overcome by micropropagation. Although this first attempt at micropropagation has met with qualified success, further work is needed to refine the technique and test its practicality.

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