COMMERCIAL CONIFER MICROPROPAGATION

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Since 1980 Canadian Forest Products Ltd. has been working with Clay's Nurseries to develop a practical and cost effective tissue culture method for mass propagating conifer trees. The immediate and long range goal of the research project is to be able to utilize the *in vitro* cloning technique to rapidly mass produce species that are slow to propagate by traditional methods, and to clone genetically superior trees obtained through selection, breeding, or genetic engineering in the future.

Micropropagation: Advantages and Disadvantages. The tremendous potential benefits of vegetative clonal propagation in the genetic improvement and mass production of forest trees have been fully recognized and critically discussed in recent years (1,2, 3,5,6,8,9,11,12,13,14,15). The most significant of these is the capture of all the genetic gains obtained through breeding and selection. Micropropagation and rooted cuttings are the two most important vegetative propagation methods that can be employed in the operational production of forest tree propagules for reforestation.

The main advantages that micropropagation has over rooted cuttings, wherever these methods can be applied include the following:

1. Only a small amount of source plant material is required. Once a genotype is established *in vitro*, it can be rapidly bulked up and maintained over a long period of time in a small amount of space.
2. Micropropagation is a much more rapid mass production method once a suitable protocol is developed for a species, by virtue of the high *in vitro* multiplication rate of the plant material.
3. Some conifer species whose cuttings cannot be rooted with ease may lend themselves more readily to *in vitro* clonal propagation.

The major drawbacks of micropropagation, in comparison to rooted cuttings, when both are used in operational production are:

1. It is still, at the present time, a more labourious method requiring a stringently controlled environment.
2. In addition to the *in vitro* plantlet production stage, an acclimatization procedure is usually required, where the micropropagules are given special treatments in order to

adjust them to the normal plant growth environment.

3. It may take years of research and much capital investment in order to develop a cost effective micropropagation production system.

Having taken into consideration all the pros and cons of micropropagation both in theory and through practical experience, we have persisted in our research. We are now close to the point of making it a fully viable alternative to both seedling and rooted cutting production methods in the case of Alaska yellow cedar (*Chamaecyparis nootkatensis* [D. Don] Spach). This is a species that exhibits an indeterminate growth habit. This makes the small amount of explant materials required for tissue culture inoculation available all year around under a controlled environment.

Since the last report (7) on our micropropagation research work, we have brought yellow cedar into its first operational production phase. The following is a report on the materials and methods used.

MATERIALS AND METHODS

Source Plant Materials. Shoot tips 3 to 5 cm. long with radially symmetrical morphology were obtained from 20 hedged plants 4 to 7 years old. These were raised from seeds of four different seed lots originally collected from the following areas in British Columbia: Morseby Island, Harrison Lake, Campbell Lake, and the Fraser Valley.

Surface Sterilization. The source plants were treated with various fungicide sprays (Benlate, Rovral, and Captan) for four weeks prior to explant collection. The explants were then surface sterilized according to procedures previously described (7). Damage due to oxidation by polyphenolic compounds was reduced with the use of blue light in the first week after inoculation.

***In vitro* Treatment.** Modified MS media (7,10), with a reduced ammonium nitrate level, were used for shoot induction and elongation. Cultured shoots were allowed to multiply and grow for 12 weeks and then subcultured on new media. Roots were subsequently induced to form plantlets. (See Figure 1)

Acclimatization Procedure. Plantlets growing in 313A Capilano containers were put through the acclimatizatiion process under a mist tent in the greenhouse for six weeks. (See Figure 2) During this period light intensity was gradually stepped up from 2000 to 20,000 lux by use of layers of shade cloth. High humidity conditions were maintained by a mist system operating initially on a 16 min. cycle with 6 sec. mist burst to protect the young plantlets from desiccation. Temperature was kept at 20° to 23°C. Pathogen problems such as damping off, botrytis, and fungus gnats were controlled with Benlate, Captan, and Diazinon treatments. The medium

Figure 1. *In vitro* rooted yellow cedar plantlet.

Figure 2. Yellow cedar plantlets in 313A Capilano containers under a mist tent.

used was a 3:1 peat/vermiculite mixture, as has been used by the B.C. Ministry of Forest Nurseries. The plantlets were fully acclimatized after six weeks.

Nursery Phase. The acclimatized plantlets were allowed to grow on to one year old until they were ready for field planting. After the acclimatization step, the plantlets were grown under growth regimes corresponding to those used for containerized propagules by the B.C. Ministry of Forest Nurseries.

RESULTS AND DISCUSSIONS

Results from the early phase—the period between 1980 and 1985 of our research on micropropagation of coniferous species have been reported (7). In the last three years, we have greatly increased our *in vitro* shoot multiplication rate in yellow cedar. We are now able to induce the formation of an axillary bud from almost every single axil of the needles on the explant shoot (see Figure 3). Microshoots have been rooted at 80% efficiency level. Micropropagated yellow cedar have grown to a height of 85 cm and a root collar diameter of 5 mm (see Figure 4) in 15 months when grown in 1 gal. containers. Some of these yellow cedar plants are now growing in a demonstration plot at a permanent site at Clay's Nursery.

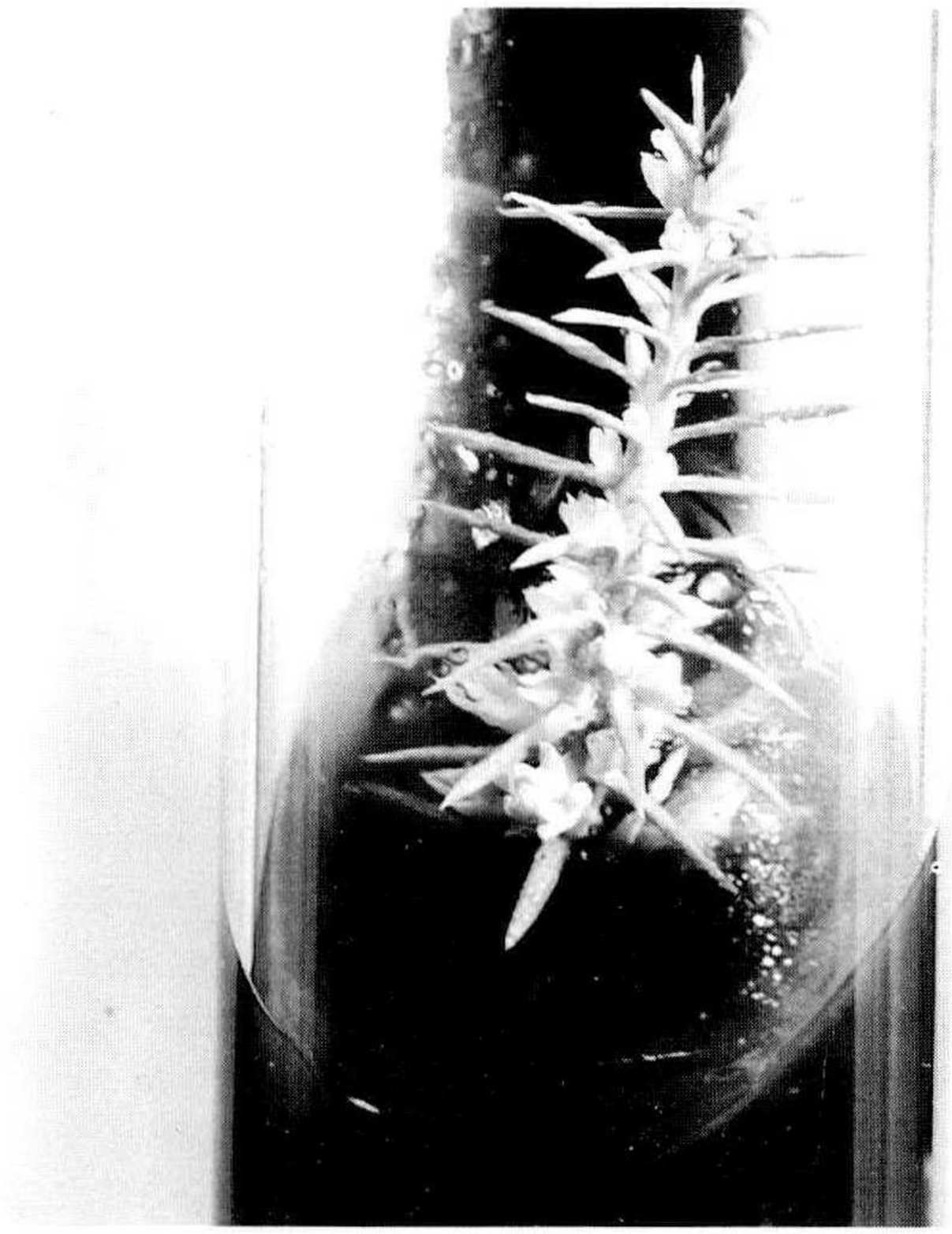

Figure 3. Axillary bud formation in axils of yellow cedar.

Figure 4. Fifteen month old micropropagated yellow cedar grown to a height of 85 cm with a root collar diameter of 5 mm.

So far no apparent difference in growth rate has been observed among yellow cedar micropropagules, rooted cuttings, or seedlings. Currently we are carrying out experiments to evaluate greenhouse performance of yellow cedar micropropagules by comparing several important shoot and root morphological traits of these propagules with those of rooted cuttings and seedlings from the same seed source. Results from these are forthcoming and will be presented in our next report. Assessment of long term field performance of our yellow cedar micropropagules will begin next spring.

Other conifer species that we have been working on in the hope of developing a similar micropropagation production system in the future include Douglas fir (*Pseudotsuga menziesii* [Mirb.] Franco), white spruce (*Picea glauca* [Moench] Voss), and Sitka spruce (*Picea sitchensis* [Bong.] Carr). These species have a determinate growth

habit and are apparently much more recalcitrant to *in vitro* treatments than yellow cedar. However, we have achieved some very encouraging results. In the case of Douglas fir, we have produced many plantlets from 12 year old source plants but have difficulties in getting them acclimatized to greenhouse growth conditions. In white spruce we have succeeded in inducing an enormous amount of bud differentiation from 7 and 8 year old materials, but these buds have so far failed to elongate. Sitka spruce, a recent addition to our tissue culture research project has shown some bud differentiation in culture. An *in vitro* clonal production system for Sitka spruce will have great economic implications to the B.C.'s forest industry since the long term soluton to our serious weevil problem in Sitka spruce is believed to be in the planting of weevil resistant trees (4).

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LITERATURE CITED

1. Aitkin-Christie, J. 1984. Micropropagation of *Pinus radiata*. *The Plant Propagator* 30(3):9–11
2. Amerson, H. V., L. J. Frampton, Jr., S. E. McKeand, R. L. Mott and R. J. Weir. 1985. Loblolly pine tissue culture: Laboratory, greenhouse, and field studies. *In*, Tissue Culture in Forestry and Agriculture. Henke, R. R., K. W. Hughes, M. J. Constantin, and A. Hollaender, eds. Plenum Press, New York. pp. 271–287.
3. Arnold, R. and J. A. Cleed. 1985. Raising and managing radiata pine vegetative cuttings for production forests. *Aust. For.* 48(3):199–206.
4. Borden, H. J. 1988. Personal communication, Dept. of Biological Sciences, Simon Fraser University, Burnaby, B.C. Canada.
5. Durzan, D. J. 1985. Tissue culture and improvement of woody perennials: An overview. *In*, Tissue culture in Forestry and Agriculture. Henke, R. R., K. W. Hughes, M. J. Constantin, and A. Hollaender, eds. Plenum Press, New York. pp. 233–256.
6. Hasnain, S. and W. Cheliak. 1986. Tissue culture in forestry: economic and genetic potential. *Forestry Chronicle* 62(4):219–225.
7. Kurz, M. L. 1985. Tissue culture of conifers. *Proc. Inter. Plant Prop. Soc.* 35:206–216.
8. Hasnain, S., R. Pigeon and R. P. Overend. 1986. *Forestry Chronicle* 62(4):240–245.
9. Libby, W. J. 1980. What is a safe number of clones per plantation? *In*, Resistance to Diseases and Pests in Forest Trees. *Proc. Third International Workshop on the Genetics of Host-Parasite Interactions in Forestry*, Wageningen, the Netherlands, 14–21 Sept. 1980. Heybroek, H. M., B. R. Stephan and K. Von Weissenberg, eds. Centre for Agric. Publishing and Documentation, Wageningen. 1982. pp. 342–360.
10. Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant* 15:473–497.
11. Smith, D. R. 1986. Forest and nut trees 1. Radiata pine (*Pinus radiata* D. Don). *In*, Biotechnology of Tree Improvement for Rapid Propagation and Biomass Energy Production. BAJAJ. Y.P.S. ed. Springer-Verlag, Berlin. pp. 274–291.

12. Smith, D. R., K. Horgan and J. Aitken. 1980. Micropropagation—A new aid in tree improvement? *What's New in Forest Research*. 87:4 For. Res. Inst., Rotorua, New Zealand.

13. Timmis, R. 1985. Factors influencing the use of clonal material in commercial forestry. *In*, Crop Physiology of Forest Trees. Proc. *Inter. Conf.* on Managing Forest Trees as Cultivated Plants in Finland. July 1984. Tigerstedt, P. M. A., P. Puttonen, and V. Koski. eds. pp. 259–272.

14. Von Arnold, S. and T. Eriksson. 1986. Forest and nut trees 2. Norway spruce (Picea abies L.). In, Biotechnology of Tree Improvement for Rapid Propagation and Biomass Energy Production. BAJAJ. Y.P.S. ed. Springer-Verlag, Berlin. pp. 291–310.

15. Zobel, B. and J. Talbert. 1984. Applied Forest Tree Improvement. John Wiley and Sons, Inc., New York. pp. 309–344.

A PRELIMINARY REPORT ON VEGETATIVE PROPAGATION OF CALIFORNIA LIVE OAKS FOR DISEASE RESISTANCE[1]

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Since about 1981, a branch dieback of oaks caused by *Diplodia quercina* has become rather widespread in California. This disease is most severe during dry years. More recently, twigblights caused by at least two fungi: *Cryptocline cinerescens* and *Discula quercina*, have also become a serious problem in California. These fungi cause most damage during wet years. The diseases occur in landscaped as well as non-landscaped areas of California and can be serious on *Quercus agrifolia*, *Q. lobata*, *Q. kellogii*, *Q. chrysolepis* and *Q. wislizenii*. They have also been recorded on *Q. douglasii*, *Q. robur* and *Q. suber*.

Sixteen native oak species are recognized in California. These belong to three subgenera: the intermediate oaks, the black oaks, and the white oaks. However, extensive hybridization within each subgenus has been well documented, resulting in highly variable intermediate types. Noticeable differences in disease susceptibility and levels of insect attacks of individual trees have been observed. For instance, it is quite common to see two *Q. agrifolia* trees side by side, one with severe infection of twigblight, the other with a negligible amount. It is customary in California to produce oaks

[1] Poster Presentation