PROPAGATION OF SOME TROPICAL AND OTHER FOREST SPECIES

JANETTE E. INGLIS

Oxford University Field Station
Wytham, Oxford

The tropical pines, *Pinus caribaea* Morelet and *Pinus oocarpa* Schiede are important tropical forest plantation trees. They are particularly fast growing and will tolerate poor soil. The timber can be used for poles, sawn, pulped, or used for resin production. These species seed profusely and in natural conditions reproduction is entirely from seed. Clonal propagation in forest tree species is becoming more widely practised as techniques for rooting cuttings improve; research is in progress with micropropagation. A spectacular increase in productivity has already been achieved through exploitation of the potential available in tree to tree genetic variation.

The work discussed here aims to increase the yield of propagative material from selected clones of these species by treatments with cytokinin and other growth regulators, followed by improved techniques of micropropagation, stem cuttings, and grafting.

Bud induction on ortet (parent plant). The main problem with vegetative propagation of pines is the limited quantity of suitable cutting material produced by the ortet. Constraint has been a severe restriction to progress. This is a limiting factor in the specialised application of the techniques. Aging of the ortet is also a problem because rooting ability declines severely before a tree is large enough to exhibit its economically important traits.

Synthetic hormones, as used successfully at Wye College by Whitehill and Schwabe (8) on *Pinus sylvestris* were applied to *P. caribaea*. Decapitated 2½ year-old seedlings, were used in this study to induce interfascicular shoots.

The treatments applied were:

a) BAP, 200mg/litre. (BAP = benzyladenine)
b) BAP, 200mg/litre + TIBA, 50mg/litre. (TIBA = triiodobenzoic acid)
c) TIBA, 50mg/litre.
d) BAP, 100mg/litre.
e) GA_3, 50mg/litre. (GA_3 = gibberellic acid)
f) BAP 100mg/litre. + GA_3 50mg/litre.

These were made up in a wetting agent, 3% glycerol containing 0.0125% Tween 20 for all treatments.

The seedlings were sprayed to run-off at 5-day intervals for one month. New interfascicular buds appeared within 18 days from the first application of BAP, 100 ml/litre. These also developed more quickly than those in the other treatments. Gibberellic acid ultimately stimulated production of longer shoots but they were fewer in number and bud development was inhibited.

Rooting of cuttings and needle fascicles. The induced buds were left to grow out into shoots large enough to provide cutting material. This material was used for a further experiment to determine the best size of cutting. Shoots were graded into small shoots (less than 50mm in length), medium shoots (50 to 80mm), and large (80 to 125 mm) Cuttings were prepared with a small heel of the main stem and the lower needles were stripped.

Hormone treatments were applied to promote rooting. An application of IBA (0.8%) in talc was compared with IBA 2500 ppm +IAA 250mg/litre in 50% ethanol as a quick-dip. The cuttings were kept under mist, arranged in eight randomised blocks. The base temperature was set at 30°C and air temperature was maintained at 20 to 30°C giving a temperature within the containers of 25°C. Analysis of variance showed that both rooting and the number of roots were increased by the application of 0.8% IBA in talc, especially so with larger cuttings.

This indicates that a larger amount of clonal material can be produced from a single plant of *P. caribaea* simply by inducing interfascicular shoots with BAP sprays. To ensure successful rooting, shoots should be selected which are at a minimum of 50 mm. in length.

Since this trial, studies have been made on the application of similar treatments to *P. oocarpa* using one-year-old seedlings of six different provenances. In certain provenances of *P. oocarpa* basal coppice shoots are produced naturally. These are physiologically juvenile and will provide good cutting material. In addition mature trees will produce coppice shoots after felling and while the main stem is in normal active growth.

The overall cutting material yield is therefore provided by a combination of apical shoots, basal coppice shoots, and needle fascicles in varying extents. Apical shoots and coppice shoots are taken as normal stem cuttings, using 0.8% IBA in talc either inserted under mist or, more recently, good results have been achieved by rooting cuttings under polythene. This system used an insulated propagation bench with basal heat incorporating a pulse-ratio controller to maintain a temperature of 25°C. in 50:50 washed silver sand and perlite rooting medium.

Needle fascicles can also be rooted successfully. The best results were obtained in trials with a basal dip of Synergol at 2500 ppm. Rooting is possible but the percentage take is not yet good enough to be considered an economic method of production. Although, if it were, a vast amount of propagation material would be available. Another obstacle is that, although a fascicle can root, very few go on to produce an apical bud, therefore little further growth is achieved beyond rooting.

To overcome this problem trials have been run using applications of BAP singly or in combination with TIBA and Alar (dimethylaminosuccinamide) to 1, 3, and 5-year-old seedlings of both *P. oocarpa* and *P. caribaea* to encourage fascicles to produce an apical bud. Treatment of the current year's growth has shown promising results, especially with BAP.

This material has been used for propagation experiments both by direct rooting and micropropagation, the latter since December, 1984. The explant material has included apical tips from coppice shoots or the mainstem, needle fascicles, and buds induced by BAP application.

The same media as used successfully by David, et al. (1,2,3,4,5), for *Pinus pinaster* have been chosen. Four stages of media are required. In addition to the shoot elongation medium, activated charcoal is included at 20 g/litre plus de-proteinised coconut water at 10 ml/litre. Coconut water is included following the suggestion of Konar and Singh (7) who obtained good results with this in studies of *Pinus wallichiana*. Results so far with *P. caribaea* and *P. oocarpa* show that better elongation has been achieved by including coconut water.

Induced mainstem buds and induced fascicle buds from ortets sprayed with BAP and Alar were transferred directly onto elongation medium. This omits the bud induction stage of the medium. Interestingly, buds take the same time to be induced *in vitro* as they do on the ortet. This is 18 to 20 days for the ortet and 21 days on agar.

A small trial was conducted to find out whether bud induction of fascicles *in vitro* would be increased by priming the ortet with BAP before transferring the fascicles to the bud induction medium. The results showed that the percentage bud induction of fascicles from an ortet primed with BAP was 78%, compared with only 40% bud induction in fascicles from an untreated ortet.

Preparation of the needle fascicles for micropropagation involves selection of material from the treated current year's growth when they are strong enough to withstand handling. They are carefully excised with a scalpel close to the stem and the papery sheath is removed to aid surface sterilisation. Surface sterilisation of *Pinus* is successful, using 0.1% mercuric chloride for one minute followed by three 1 min. rinses of sterile distilled water. Once inserted into the bud induction medium they are kept under continuous light at 28°C, while on the shoot elongation medium they are kept at 25°C with a daylength of 16 hours at 2000 lux followed by eight hours dark at 21°C. These conditions also apply to apical tip material.

Etiolation of induced buds. Etiolation of BAP-induced buds may be an alternative method of developing this material for propagation. Comparisons of black polythene with double layers of 2 mm Netlon mesh to shade trees with induced buds have been made. Further work is required on this subject. However, the best results at present are from using double layers of Netlon.

Grafting of *Pinus caribaea* and *Pinus oocarpa*.

Grafting of *P. caribaea* and *P. oocarpa* is used with some success in forest nurseries in the areas of tropical afforestation as the natural environment provides perfect warm and humid conditions. A disadvantage is that more demand is made on clonal material for scions. *P. oocarpa* and *P. caribaea* are graft-compatible, coppice shoots being a useful source of scion material.

This year we obtained a license to import scion material for grafting to the field laboratory at Wytham. The scions were collected from selected trees in Zimbabwe and consisted of coppice shoots and shoot tips from the current year's growth. The scions were kept fresh by wrapping them in wet muslin inside cool boxes and they showed no signs of deterioration on arrival. Grafting took place within 48 hours of collection in Zimbabwe. A cleft graft was used, secured by Rapidex ties. Each graft was enclosed in a polythene bag to maintain humidity and a brown paper bag over this to ensure complete shade for each graft. The understocks we are using are a combination of 5-year-old *P. caribaea* seedlings and 1-year-old *P. caribaea* and *P. oocarpa* seedlings, pot-grown at all times under glasshouse conditions. The percentage take this year is 70%. It is hoped that this grafted stock will provide suitable material for micropropagation and we aim to form a British clone bank of selected trees which could then be returned to the tropics of afforestation. This work is at a preliminary stage of development and it is in cooperation with Dr. Richard Barnes of The Unit of Tropical Silviculture of the Commonwealth Forestry Institute in Oxford.

***Picea sitchensis* — Sitka spruce.** There is an immense interest in Sitka spruce at present for afforestation in Britain. The Foresty Commission and the private sector aim to produce a combined output of one million plants of selected clones per year by 1988. Breeding this species over several years has resulted in the selection of particularly promising clones. Production from cuttings has been accepted as uneconomic but it is now considered essential to exploit the potential of selected trees. Cuttings from superior stock ultimately provide an increased yield of cuttings of elite trees which will outweigh any initial increased costs of production from cuttings compared with seed production.

This year the methods mentioned previously to induce interfascicular buds to *Pinus spp.* were applied to Sitka spruce. From March the 2-year-old seedlings were kept under glasshouse conditions with supplementary lighting of 16-hours using florescent warm white lighting. Temperatures were maintained at 15 to 20°C. In this trial all resting buds were recorded and each branch numbered; the tips of each branch were pruned back. A spraying programme began at the beginning of May, with applications at five days and on five occasions.

The results were recorded in mid-June this year. The best results were from using BAP at 200 mg./litre (Table 1). With this treatment buds were also induced at the base of each branch and clusters of buds appeared at the tips of pruned shoots. There was also a dramatic difference in bud size with the application of BAP at 200 mg./litre these being 4 to 5mm in diameter at the first recording after treatment compared with 2 mm average size for the control and other treatments.

Table 1. Results of the effect of BAP, ALAR, and TIBA on *Picea sitchensis* to induce buds.

Growth regulator	Untreated Mean number buds /branch	Treated Mean number buds /branch	Difference between mean number of buds
1. Control.	1.18	3.97	2.79
2. BAP + ALAR + TIBA	6.95	8.77	1.82
3. BAP + ALAR	6.11	8.29	2.18
4. BAP	1.40	6.02	4.62

Treatment rates:
1. No treatment
2. BAP, 200 mg/litre + TIBA 50 mg/litre + Alar 500 mg/litre
3. BAP, 200 mg/litre + Alar 500 mg/litre
4. BAP, 200 mg/litre

At the present, size and number of shoots in this treatment is also the greatest, but development needs to be assessed again at a later date. This work will be repeated on a larger scale to allow fuller statistical analysis of what have so far been very encouraging results. Cutting material can be increased on Sitka spruce by hedging, being pruned back in late autumn and again lightly the following spring. For abundant new growth the hedges can be shaded to induce etiolation. This will provide seasonal cutting material, whereas an alternative system of treating trees with BAP under cover may produce cutting material throughout the year.

SUMMARY

1.) *Pinus caribaea* and *Pinus oocarpa* respond to treatments of BAP on 1, 2 and 5-year-old seedlings to induce interfascicular buds.

2.) Induced buds can be used for:

a.) Micropropagation explants; this allows the first stage medium to be omitted.

b.) The induced buds can be etiolated into elongated shoots for cuttings.

3.) Needle fascicles also respond to treatments of BAP on 1 and 5 year-old seedlings and will form an apical bud for elongation *in vitro*.

4.) Cuttings from basal coppice shoots and etiolated shoots from induced buds root well with 0.8% IBA in talc under polythene with basal heat.

5.) Because micropropagation of gymnosperms is a slow process and rooting *in vitro* is difficult quicker techniques of reproducting clonal material could be:

a.) Etiolation of induced buds on the ortet for cuttings.

b.) Elongation of induced buds *in vitro*, followed by subsequent rooting as cuttings in sterile medium using conventional methods.

6.) *Picea sitchensis* responds to BAP 200 mg/l sprays on 2-year-old seedlings to induce a higher yield of potential cutting material — but further work is required.

LITERATURE CITED

1. David, A. 1972. Éffets de diverses solutions minerales sur la proliferation des tissus de Pin maritime en culture *in vitro*. *CR. Acad. Sci.* 275:2857-60.
2. David, A. 1979. Manifestations de diverses potentialites organogenes et micropropagation vegetative chez le Pin maritime (*Pinus pinaster* Sol.) *Ann. Rech. sylvicoles, AFOCEL*, Domaine de l'Etancon, 77370 Nangis, France, 57-75.
3. David, A. and H. David. 1977. Manifestations de diverses potentialities organogenes d' organes ou de fragments d'organes de Pin maritime *Pinus pinaster* Sol.) en culture *in vitro*. *CR. Acad. Sci.* 284: 627-30.
4. David, H., K.I. Semukali, and A. David. 1978. Obtention de plants de Pin maritime (*Pinus pinaster* Sol.) a partir de brachyblastes ou d'apex caulinaires de tres jeunes sujets cultives *in vitro*. *CR Acad. Sci.* 287:245-248.
5. David, A. and M.J. Thomas 1979. Organogenese et multplication vegeta-

tive *in vitro* chez les Gymnospermes. *L'Annee Biol.* 18: 381-416.

6. Inglis, J.E. 1984. Effects of some growth substances on the promotion and rooting of interfascicular shoots in *Pinus caribaea*. *The Plant Propagator*, 30 (3) 4-6.
7. Konar, R.N. and M.H. Singh. 1980. Induction of shoot buds from tissue cultures of *Pinus wallichiana*. *Z. Pflanzenphysiol* 99: 173-77.
8. Whitehill, S.J. and W.W. Schwabe. 1975. Vegetative propagation of *P. sylvestris*. *Plant Phys.* 35: 66-71.

HALF-HARDY PERENNIALS

IVAN DICKINGS

Notcutts Nurseries Ltd. Woodbridge Suffolk. IP12 4AF

Half-hardy perennials are a much neglected range of plants which I think should be more widely grown. The variation in habit, flower colour, and foliage is quite considerable. They always create a lot of interest in my garden and visitors are continually asking where they can obtain them. At the moment, there are only a few specialist nurseries who grow them in a reasonable range. It was the difficulty in obtaining these plants which originally prompted me to collect them in the hope that the company which I work for would make them available through their garden centres, and I am pleased to say that this is now happening. We now sell some kinds as spring and summer bedding plants in 9 cm pots.

There are people who, when told that these plants are only half hardy and will probably die during the winter, dismiss them outright, but the same people are quite happy to spend considerable amounts of money each spring on annuals and geraniums. All of these plants are very useful for planting between newly-planted shrubs to give a display while the shrubs are getting established. They can be planted with established plantings to give a longer period of interest, and they can also be used as bedding plants. They also look very good when planted in pots, troughs and urns, adding colour to the terrace or patio.

Most of the plants are very easy to propagate from cuttings taken during late summer and these are ready for sale the following spring. The following plants are not hardy in my part of the British Isles but, no doubt, in some more sheltered, warmer parts of the country they are perfectly hardy. Below is a list of some of the species which I have grown in my own garden during the past 4 to 5 years:

Helichrysum petiolatum: A vigorous plant with wooly, heart-shaped