PART 4

PHYSIOLOGY AND BIOCHEMISTRY OF VEGETATIVE PROPAGATION

ROOTING STEM CUTTINGS OF RADIATA PINE: ENVIRONMENTAL AND PHYSIOLOGICAL ASPECTS

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ABSTRACT

Seasonal changes in rooting promoters and inhibitors of stem cuttings of radiata pine (**Pinus radiata** D. Don) were followed and the influence of various environmental parameters on rooting examined. Hormonal changes occurred with season, but these are complex and are not yet well understood. Depletion of metabolic food reserves is considered to be important in determining health and survival, as rates of net photosynthesis of cuttings rapidly approached zero immediately after detachment and remained so until the cuttings had rooted. Alleviation of moisture stress is critical to rooting of cuttings and high humidities are required for at least the first week after setting. Cuttings take up water through the cut base of the shoot, through the basal foliage left in contact with the soil and probably through foliage wetted by dew or rain. Air temperatures of 20 to 25°C during the day provide rapid rooting, and it is thought that cold night temperatures of 5 to 10°C are advantageous. Photoperiod does not appear to influence rooting.

Further work is required to determine how pre-severance conditions influence the quality of shoots used as cuttings and to examine the effect of soil temperatures and night air temperatures on rooting performance. In the application of the results of this study the discussion concentrates on low-cost installations for use in forest nurseries to speed up and improve propagation.

INTRODUCTION

Methods have been developed empirically for rooting cuttings of radiata pine in the nursery of the Forest Research Institute at Rotorua. When set in open nursery beds stem cuttings taken in May -June from second- or third-order branches of healthy trees up to about 12 years old break dormancy, callus, and form roots some six to seven months later. Cuttings from older trees root well if girdled some weeks before severance (Thulin and Faulds, 1968). These methods are very successful at Rotorua but do not give reliable results in a number of forest nurseries in other parts of New Zealand.

During the past three years a series of experiments has been completed with the objectives of resolving the effects of environmental conditions on rooting processes and of improving propagation practices. The studies were conducted in growth cabinets at

Rotorua and in the Controlled Climate Laboratory of the Plant Physiology Division of the Department of Scientific and Industrial Research, Palmerston North. This paper reports the results of those experiments, parts of which still await formal publication.

The various influences that are known or thought to be important in root initiation and development have been reviewed by Cameron (1968). These include the age, health and genetic provenance of the parent tree, type of cutting, cold storage after severance, the season, the method of setting, and treatment after root initiation. Subsequently studies were made of the morphology of adventitious root formation (Cameron and Thomson, 1969), and of hormonal influences (Wilson and Cameron, 1972; Zabkiewicz, 1973). There are many indications that callus formation, root initiation, and root growth, although linked sequentially, are separate phenomena under different internal influences and affected in different ways by environmental conditions.

As these matters become better understood it should be possible to obtain better results by modifying the internal controls of rooting and by regulating the environments in which cuttings are set.

THE PHYSIOLOGY OF ROOT INITIATION

Hormonal Controls

There are conflicting statements concerning the effects of hormone applications on root formation; some report a definite stimulus, others no effect at all (*see* Cameron, 1968). Our own experience is that indole-butyric acid (IBA) has a positive effect on the rates of both callus formation and root initiation, possibly through direct morphogenetic action, possibly because of the way that IBA application substantially increases movement of photosynthates to the site of root initiation at the base of the cuttings (Cameron, 1970). It is presumed that endogenous auxins act similarly.

In a preliminary investigation on the biological activity of substances present in centrifugates from buds, extracts were made at different times of the year and partitioned by paper chromatography. One promoter and two inhibitors of rooting were found, as assessed by the mung bean rooting bioassay, and these varied in relative amounts with season. Similarly one fraction (most abundant during early spring, a time when cuttings are most difficult to root) inhibits root formation by hypocotyl cuttings of cotyledonary seedlings of radiata pine (Wilson and Cameron, 1972). Investigations by Zabkiewicz and Steele (1974) have confirmed these results. The next step will be chemical characterization of the biologically-active constituents.

Photosynthesis and Respiration

Girdling shoots by removal of a band of bark external to the xylem is a standard pre-severance treatment for successfully rooting cuttings from adult trees (Thulin and Faulds, 1968). Cameron *et al. (1974,* in prep.) measured changes in rates of photosynthesis and respiration in radiata pine cuttings, from the time of girdling, through detachment 4 weeks later, until well developed roots were formed some 6 months after detachment. All measurements were made under standard environmental conditions.

Girdling the shoot had no significant effect on the rate of net photosynthesis. However, detaching the shoot from the parent plant reduced net photosynthesis to a level only very slightly above the compensation point (approx. 0.3 mg CO_2/g leaf o.d./weight/hr). Rates remained low throughout the period of callus formation and initiation of roots. When roots started to emerge rates of net photosynthesis increased threefold.

Rates of respiration showed a progressive increase from time of severance, through the period of callus formation and root initiation, until at root emergence the rate of respiration of the whole cutting was some 40% greater than at severance.

Translocation of Photosynthates

At the time that cuttings are establishing new roots there is also active shoot growth and foliage extension. Therefore competition between different regions of the plant for available carbohydrates from both metabolic reserves and current photosynthates must be intense. To obtain a fuller understanding of the relative sizes and the location of the photosynthate sinks and of the relationship between different zones of foliage and the sinks, current photosynthates from old and recently expanded foliage were labelled with $^{14}CO_2$ (Cameron *et al.,* 1974).

The results indicated that approximately 75% of the photosynthate supplied to the roots came from the photosynthetic activity of old foliage. Young, recently expanded foliage contributed most of the photosynthate requirement for terminal shoot growth. It is expected that patterns of photosynthate distribution will be different under other environmental conditions(e.g., varying night temperatures, or moisture stress) but the study does demonstrate that the older foliage makes an important contribution to the growth of adventitious roots.

ENVIRONMENTAL INFLUENCES

Water Relations

The point is often made in literature that to survive and form roots a cutting must be able to maintain a healthy water balance and replenish water lost in transpiration. However, no studies are reported on the mode of water uptake by cuttings of conifers. At Rotorua it is normal practice to set cuttings with all foliage intact, pushing the detached 20-cm shoot 8-12 cm deep into the soil of the nursery bed. Four possible major pathways for water uptake seem likely—(1) through the cut base; (2) by diffusion into foliage immersed in the soil; (3) through the cuticle of that part of the stem in contact with soil; and (4) by entry through foliage exposed to the air and wetted by rain or dew. Preliminary results of a study by Rook *et al.* (1974, in prep.) indicate that little or no water passes through the stem cuticle and that most water uptake is through the cut base of the shoot and through foliage in contact with moist soil. Blocking either of these pathways caused significant reductions in rates of transpiration during the first two weeks after setting compared with normally-set cuttings; the latter also developed smaller water deficits. After five weeks there were no significant differences in rates of transpiration but the normally-set cuttings still maintained smaller deficits.

There has been considerable argument among propagators about whether or not to leave basal foliage intact when setting cuttings. The above results indicate that substantial amounts of water can move into cuttings through foliage in contact with wet soil, so leaving the foliage intact should aid rooting.

At the time of setting, cuttings are often in a condition of intense water stress (water deficits of −25 to −30 bars) so they need to lose relatively little water for levels of stress to become extreme, with lethal results. If good results are to be obtained strict precautions should be taken to reduce desiccation to a minimum before setting and then to replenish water lost by the cuttings. We found that spraying foliage with water maintained water deficits at substantially lower levels than those of unsprayed plants, a result attributable either to reduced rates of transpiration or to active water uptake through foliage. Preliminary results of experiments with tritiated water placed on leaf surfaces show that appreciable amounts of water can move into leaves in this way.

Temperature

The uncertain, often unsatisfactory, results obtained when the methods of cutting propagation developed empirically at Rotorua were applied in forest nurseries elsewhere was attributed to an environmental factor or factors. When climatic records for different nurseries were examined, wide differences in day and night temperature were apparent.

To investigate this effect, cuttings were set under different temperatures in controlled climate rooms at an arbitrary 65% relative humidity without any particular precautions to maintain a high moisture status in the cuttings. At all temperatures the cuttings desiccated severely and most of them died without forming roots. It was noted, however, that cuttings maintained best health and survived longest under conditions of cold night temperature (i.e., 5°C rather than 10 or 15°C) irrespective of day temperature (15, 20 and 25°C). Day/night temperature differentials *per se* did not appear to be important (Cameron, 1974 in prep.).

In a repeat experiment care was taken to maintain cuttings in as turgid a condition as possible, from collection until time of placement in climate rooms. Thereafter atmospheric water vapour pressure deficits were maintained at less than –1 mm Hg day and night for the first 2 weeks. The foliage of some cuttings was also sprayed daily with water. Measurements showed that humidity control in itself was sufficient to relieve the initial water stress. Thereafter vapour pressure deficits were increased to –1 mm Hg, day temperature, and –2 mm at night.

Cuttings set at 15°/5°C (day/night temperature) or 20°/10°C maintained better health than those at 25°/15°C. Those set at the highest temperature rooted first with some cuttings having roots at 10 weeks, compared with 16 weeks at 20°/10°C, 22 weeks at 15°/5°C and 24 weeks for cuttings set at the same time out-of-doors in the Rotorua nursery. At 25°/15°C only 57% eventually rooted, compared with more than 70% at 20°/10°C and 36% at 15°/5°C; these results were attributed to the development of fungal diseases at the highest temperatures and to lowered rates of metabolism at the lowest temperature regime (Cameron and Rook, 1974 in prep.). Measurements made during the experiment revealed that root temperature, based on the temperature of the soil in which cuttings were set, was substantially the same as air temperature.

The results were accepted as an indication that the temperature of the shoot, and probably the root, have important effects on both rate of rooting and cutting health. High temperatures hasten root development but favour fungal disease: low temperatures have opposite effects. An optimum temperature regime needs to achieve a balance between both effects.

Photoperiod

Because of the strong correlation between season and rooting processes, particularly the observation that under conditions of natural daylight no roots form until 'long day' conditions are achieved (November-December), and since removal of the terminal bud from cuttings can advance the time of rooting by two or three weeks, it was decided to investigate the importance of photoperiod as a controlling influence.

Environments were set up in climate rooms at Palmerston North to give conditions favourable, although probably not optimal, for root formation. The cuttings were given 8 hours of high intensity photosynthetic lighting, and varying conditions of photoperiod by means of a symmetrical extension of day length using low-intensity output incandescent lamps to give day lengths of 8, 12, 16 and 24 hours The cutting material used was taken from both young (8-yr-old) and mature (20-yr-old) trees.

At the time of writing the experiment was continuing. There were small differences between treatment in bud behaviour, cuttings in the 24 hour photoperiod having broken dormancy 1-2 weeks earlier than in the other day-lengths. A fair proportion of the cuttings had formed roots (some 12%) but no differences could be discerned between treatments in either rates of callus growth or root formation. A tentative conclusion is that photoperiod is not an important factor.

FURTHER PHYSIOLOGICAL AND ENVIRONMENTAL STUDIES

Pre-severance Conditions

Observations in nursery propagation work and experiments over several years in growth cabinets and controlled climate rooms have indicated marked differences in ease of rooting between clones and within clones. These differences have been attributable to the general health of the parent tree as affected by climate or other influences. For example, in the Rotorua district the summer of 1972-73 was one of low rainfall; as a result the cuttings collected in the autumn of 1973 were in a much poorer condition than those collected in 1972, a wetter summer. In 1973 only girdled cuttings rooted well in climate room experiments, and these maintained better health and exhibited a greater resistance to disease. This, and many similar observations, suggests that pre-severance conditions can have a critical effect on the success or failure of propagation work (Cameron, 1968).

It has also been shown that simple girdling of shoots 2 to 4 weeks before severance in autumn provides a significant increase in the proportion of current photosynthates that accumulates at the site of root initiation at the base of a cutting (Cameron, 1970); thus, the relative ease with which such cuttings could be rooted was attributed, in part, to a greater availability of carbohydrate reserves. It is probable that in adverse growing seasons carbohydrates and other metabolites necessary to support the cutting from severance to the formation of a fully functional root system become so depleted as to retard or preclude rooting. There is also a suggestion that the girdling pretreatment makes cuttings more resistant to adverse conditions of all kinds, including pathogen attack, and that changes other than simple accumulation of photosynthates occur during the period between girdling and severance. Such changes need to be studied in detail and quantified, and their physiological significance in rooting processes determined.

We plan to study the effect of climate and other manipulative pre-severance treatments on trees, using the large growth rooms now available at Rotorua. These will accommodate plants up to 7 m tall. Cuttings will be taken from the trees, set under standard reproducible conditions, and their performance followed. The main objective is to find out if it is possible to manipulate parent plants to provide cuttings which

Further Work in Controlled Environments

The experiments that have been completed in growth cabinets and climate rooms do not indicate clearly whether it is air temperature, or root temperature, or both that give the net promotion effect observed. Two attempts have been made in growth cabinets to obtain such information but both failed through technical faults. The work will be repeated, for until this matter has been resolved it will not be possible to prepare accurate specifications for the modified environments necessary to root cuttings predictably.

The experiments completed do not indicate clearly the best conditions of day and night temperatures. An environment of 20°/10°C gave the best results in an earlier experiment and there are indications that an even lower night temperature (say 5°C) would be an advantage (Hellmers and Rook, 1973).

Present intentions are first to resolve the relative effects of air and root temperature, using growth cabinets, and then to set up an experiment in climate rooms to investigate the effects of various day and night temperatures.

Chemical Aids to Propagation

As mentioned earlier attempts to use artificial hormones as aids in rooting cuttings have given conflicting results (Cameron, 1968). Root formation in cuttings is a complex phenomenon in which there are at least three distinct steps: callus formation; differentiation within callus resulting in root initiation; and root growth (Cameron and Thomson, 1969). It is postulated that each step is controlled in different ways by balances of hormones, inhibitors and other biochemicals. If this hypothesis is correct then it is unlikely that the simple application of any one synthetic auxin, applied against a background of natural growth regulators that vary with age of plant and time of year, will give consistent results. However, once an accurate assessment has been made of the complex of growth regulators present in cuttings then the possibility arises of using synthetic chemicals more precisely, whether singly or in combination, to stimulate rooting.

The need to alleviate water stress to a level within the capacity of the cuttings to replace water lost has already been emphasised in this paper. One of the possibilities of improving the water balance of the newly-set cutting is to spray the exposed foliage with an anti-transpirant. To be effective, this treatment must allow the cuttings to conserve water, but must not impair photosynthesis, which normally at this time is occurring at a very low rate. Equally important, the anti-transpirant must not prevent water uptake by the foliage.

Application of Research Results

Using controlled environment facilities to provide conditions conducive to rooting, this study has demonstrated that there are no inherent factors preventing stem cuttings of radiata pine from rooting within a few weeks. Cuttings rooted under normal, open-nursery conditions, as used at present, have to survive a 6-month period without an effective root system, a condition which restricts rates of transpiration and rates of carbon dioxide exchange to near zero under daylight conditions. As rooting occurs the terminal bud swells, needle primordia expand and elongate, and a typical 'rat-tail' type shoot develops, which is an indication of depleted metabolic reserves. The cutting continues growth in a condition of stress and starvation with an inadequate root system. Under standard nursery conditions in Rotorua, cuttings, from mature trees in particular, take two years to regain full health. Cuttings rooted under conditions of elevated air temperature, 25°/15°C in 10 to 12 weeks from setting, show no symptoms of nutritional stress and at 12 months resemble, in size and health, outdoor-grown cuttings that have been in the nursery for 2 years.

To apply these results to large-scale production of stem cuttings requires conditions which will result in rooting within a few weeks without the metabolic reserves of the cuttings being completely depleted.

The Tree Improvement research group at the Forest Research Institute is using two low-cost installations to provide conditions conducive to rooting cuttings: The systems are simple, inexpensive, easy to operate, and can be used singly or in combination. Polythene-covered boxes in a glass-house have been used with considerable success to root needle fascicles of pines. Large "cold-frames" have also been constructed over nursery beds and are being assessed for this work. Both of these systems provide cuttings with a more favourable climate to form callus and root by maintaining high atmospheric humidities, warm day temperatures and above-freezing night temperatures. A further degree of control can be provided by installing a simple, automated misting system and soil and air heating by buried and exposed heated cable with thermostat and time clock controls. When the spring temperatures become favourable for growth the cold-frames with their misting and heating systems can easily be removed; the rooted cuttings can then be treated as normal planting stock and grown out of doors for the remainder of the year.

The use of large removable, polythene cold-frames would appear to ensure the production of healthy, vigorous cuttings in one year at any New Zealand nursery, irrespective of the local climate. But the management and economics of this form of raising cuttings can be adequately appraised only when accurate prescriptions are available of optimum conditions for raising this type of planting stock and of how sub-optimum conditions affect ability to root.

Further studies are required before exact prescriptions can be written to provide optimum rooting, but at least some of the important factors affecting rooting are now more fully understood. This information largely endorses knowledge already gained through trial and error experiments in propagating other plants (Hartmann and Kester, 1968). The writers believe, however, that in attempting to establish cause and effect for the influences controlling the rooting of cuttings of radiata pine they are establishing a sound scientific basis, removing some uncertainties, and making possible a progressive improvement of methods.

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