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Anatomical and Physiological Factors Affecting Adventitious Root Formation in *Pinus radiata* (D.Don) Cuttings.

by

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B.Agr.Sc. (Hons.), Tas

Submitted in fulfilment of the requirements for the

degree of Doctor of Philosophy

Dept of Agricultural Science

UNIVERSITY OF TASMANIA
HOBART

February 1997

Declaration

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Mark P. Krstic

5 / 2 / 1997

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List of Abbreviations

AACC	American Association of Cereal Chemists
ABA	Abscisic acid
AOAC	Association of Official Analytical Chemists
ARF	Adventitious root formation
BSA	Bovine serum albumin
CG	Callus growth
DHZR	Dihydrozeatin riboside
DW	Dry weight
GA_n	Gibberellic acid A_n
GC	Gas chromatography
GC-MS	Combined gas chromatography - mass spectrometry
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
IRGA	Infra red gas analysis
LM	Light microscopy
LSD	Least significant difference
LWP	Leaf water potential
MPa	Mega pascal
PFD	Photon flux density
PGR	Plant growth regulator
ppm	Parts per million
RIA	Radioimmunoassay
SRG	Scale of Root Growth
TIBA	2,3,5-Triiodobenzoic acid
TG	Top Growth
TMS	Trimethylsilyl
ZR	Zeatin riboside

Summary

Pinus radiata is an important plantation forest species in Tasmania and other regions of the world. The growth of *P. radiata* from vegetative propagules has been demonstrated to have many advantages over the conventional seedling method of propagation. The major problems associated with the vegetative propagation of *P. radiata* by cuttings are the seasonal variation in the percentage of cuttings forming roots and the loss of rooting ability with increased stock plant age. This study investigated the anatomical and physiological factors affecting adventitious root formation in *P. radiata*..

Initial investigations using controlled environment conditions indicated that anatomical and/or physiological factors rather than the propagation environment were mainly responsible for the seasonal variation in the percentage of *P. radiata* cuttings forming roots. Most of the mortality observed in cuttings occurred prior to callus formation, highlighting the time of adventitious root development which needed to be investigated further.

In controlled environmental conditions (20°C, 95% RH, 14 hour photoperiod), callus initiation was observed approximately 4 weeks after excision and root initiation was observed at approximately 11 weeks after excision. The examination of endogenous plant growth regulator concentrations indicated that the change in the concentration of auxin during the callus formation period may be an important indicator of rooting success in *P. radiata* cuttings. In those cuttings which exhibited a high rooting percentage, a transient increase in auxin concentration was observed in the basal region of the cuttings 2 and 3 weeks after excision. This was not observed in cuttings which exhibited a low rooting percentage.

The concentration of carbohydrates in the basal stem region of the cuttings at the time of excision related poorly to the rooting ability. However, the maintenance of sucrose concentrations in the basal tissue above approximately 15mg/g DW was considered important for the successful rooting of cuttings.

The maintenance of adequate water relations in the cuttings during ARF was demonstrated to influence the rooting percentage. In those cuttings which survived and rooted, the leaf water potential was maintained above a value of -2.50MPa. However, in those cuttings which failed to survive the leaf water potential was below -2.50MPa in the week prior to cutting necrosis.

In summary, the ability of the cutting to react to excision was considered more important than the absolute levels of these physiological factors at the time of excision. The physiological basis of these findings is discussed and investigated further, from which recommendations are made for improved management practices in commercial *P. radiata* cutting nurseries.

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I. General Introduction

I. General Introduction

The high demand for quality pulp and timber products in modern society has resulted in an increased reliance and production of forestry related products. Traditionally, the pulp and timber was obtained from native forest stands. However, an increased environmental awareness about the need to preserve areas of native forest, where endemic flora may be rare, has resulted in the introduction of government legislation in many countries to protect these areas from forestry activities. In comparison to native forest stands, a higher yield of pulp and timber is generally achieved under a plantation forestry system. In plantation forestry, many different tree species have been used as a source of pulp and timber, including *Pinus radiata*. Plantation forestry aims to maximise the economic return from an area of land by optimising yield, quality, management and sustainability parameters.

Most of the pulp and timber used in modern society is obtained from plantation forests. Plantation forests are often managed intensively and therefore growers often research new ways of maximising the economic yield. One such way of increasing the economic yield of *P. radiata* plantations is to use vegetative propagules as transplants. Vegetative propagation in forestry has been used for many years, not only as a propagation tool, but also as a means for increasing the rate of genetic improvement in breeding programs. It has been known since the late 1920's that *P. radiata* can been propagated relatively easily from cuttings. However, the commercial production of rooted cuttings on a large scale, as a source of transplants in plantation forestry, was not adopted until the early 1980's. At this time research in Australia and New Zealand proved conclusively that there were significant advantages in using cuttings as transplants, compared with seedlings. It was demonstrated that the use of rooted cuttings as transplants, compared with the use of seedlings, could result in increased yields of high quality end product (Klomp and Menzies, 1988; Menzies and West, 1982; West, 1988). Similar research also demonstrated that the management of cutting plantations was significantly easier than conventional seedling plantations (Menzies *et al.*, 1985).

According to a survey conducted by Ritchie (1991) more than 65 million coniferous cuttings were being produced around the world annually. Half of this

total production occurs in Japan, where valuable sugi (*Cryptomeria japonica* D.Don) cultivars have been vegetatively propagated for at least five centuries (Ritchie, 1991). Another 10 million or more cuttings of *P. radiata* are being grown annually in Australia and New Zealand (Ritchie, 1991). In 1992 eleven nurseries, located in south-eastern Australia, produced over 3.3 million cuttings (Duryea and Boomsma, 1992). The current (1996) number of *P. radiata* cuttings placed in the soil (referred to as 'set' here after) in Australia is estimated at over 9.0 million (N. McGeary, pers. comm., 1996). In Tasmania, the current production of *P. radiata* cuttings is estimated at 300, 000 (S. Hetherington, pers. comm., 1996). However, Australian Newsprint Mills (ANM) aims to produce over 2.5 million cuttings annually, in Tasmania by the year 1999 (S. Hetherington, pers. comm., 1996).

In Tasmania, the propagation of *P. radiata* cuttings was first trialed in 1993 by the local forestry company, ANM. ANM acted on the advice of a Victorian forest company Amcor Plantations Pty. Ltd.. Initially, only 100, 000 cuttings were set. The methods and technology employed to propagate cuttings in the nursery were essentially identical to those employed in other forestry nurseries located throughout both Australia and New Zealand.

The factors affecting the propagation of *P. radiata* by cuttings have been researched intensively in Australia and New Zealand during the past 50 - 60 years. Seasonal and maturational problems are considered some of the most important factors influencing the successful propagation of *P. radiata* cuttings under normal field nursery conditions (Fielding, 1954; Fielding, 1969; Jacobs, 1939; Thulin and Faulds, 1968). In large scale commercial cuttings operations these factors are managed to maximise the propagation success. However, there is currently a lack of scientific knowledge about how these factors influence the success of propagation. It is desirable to have an understanding of the physiological, biochemical and morphological changes associated with adventitious root formation (ARF) to aid in the management of large scale cuttings nurseries.

The aims of the present study were to:

1. Describe in detail the morphological and anatomical changes associated with ARF in *P. radiata* cuttings propagated under Tasmanian field conditions.

2. Examine the influence of cutting environment on the rooting success in *P. radiata*.

3. Examine the changes in endogenous plant growth regulator concentrations in *P.radiata* at the time of cutting excision and during ARF.

4. Examine changes in endogenous carbohydrate concentrations during ARF.

5. Examine changes in water relations during ARF.

6. Examine the photosynthetic capacity of *P. radiata* cuttings during ARF.

7. Investigate the effect of cultural practices on ARF in *P. radiata* cuttings with the aim of enhancing the large scale commercial production at times in the season when propagation is difficult.

II. Literature Review

II. Literature Review

II.1 Introduction

1.1 Introduction of Plant Species being Investigated

1.1.a Botanical Description of *Pinus radiata* D. Don

Pinus radiata D.Don (Monterey pine) is an evergreen tree which belongs to the family Pinaceae, the largest family of Gymnosperms. The family contains 9 genera and approximately 200 species, most being natives of the northern hemisphere (Curtis and Morris, 1975; Mabberley, 1989). Trees are monoecious, usually 12-35 metres high when mature, with fissured, hard and scaly bark. The branches are arranged into regular whorls, with three needle-like leaves (7-14cm long; 1-2mm wide; triangular in transverse section) arising from dwarf shoots. Numerous male cones occur at the base of each year's growth, replacing the dwarf shoots. The female cones occur singly or in whorls replacing long branches and take approximately 2-3 years to ripen. The ripe female cones (8-14cm long) are usually asymmetrical, ovoid-conical in shape when closed, and almost spherical and light brown in colour when open (Curtis and Morris, 1975). The seed is approximately 6mm long with broad oblong wings approximately 3 times as wide as they are long (Curtis and Morris, 1975; Dallimore and Jackson, 1966).

1.1.b Origin and History.

P. radiata is an introduced species in Tasmania, Australia, having been planted for shelter belts and as a source of softwood. The trees grow rapidly and provide timber of medium quality which may be used for plywood, weatherboards, flooring, packing cases and pulp-wood source (Curtis and Morris, 1975).

P. radiata is a native of Monterey County, California, where its distribution is limited to a very small area of hilly ground near the sea. It was introduced to England by Douglas in 1833 (Dallimore and Jackson, 1966).

1.1.c Importance in Forestry.

The principle advantages of *P. radiata* over other forestry species is its rapid growth when young and its ability to withstand considerable exposure to strong sea winds (Dallimore and Jackson, 1966). It grows best in light, moist, but well-drained loamy or sandy soil (Curtis and Morris, 1975; Dallimore and Jackson, 1966). However, it can grow well even on dry, infertile, sandy soils (Dallimore and Jackson, 1966; Neilsen, 1990). It is susceptible to a small number of introduced insects (e.g. *Sirex*) and diseases (e.g. *Dothistroma* and Spring yellow needle cast) and has a low susceptibility to browsing damage (Neilsen, 1990). It produces light wood (400-450kg per m^3) which is soft, brittle, with conspicuous resin ducts, fine medullary rays, and often very wide annual rings (19-31mm apart) (Dallimore and Jackson, 1966). The heartwood is light brown or brownish-purple and sapwood is white or pale yellow in colour, comparable to *Pinus pinaster* in quality (Dallimore and Jackson, 1966). Although the timber is inferior in quality to that of several other species, there are many purposes for which it can be utilised, notably indoor work in building construction and box-making (Curtis and Morris, 1975; Dallimore and Jackson, 1966)

P. radiata is of little commercial value in its native country, but is an important timber tree species in several countries to which it has been introduced, notably, South Africa, Australia, and New Zealand, where it has produced stands of 425 m^3 per hectare at 25 years of age (Dallimore and Jackson, 1966).

1.1.d Seedling Propagation

In modern plantation forestry the use of nursery grown and conditioned transplants is considered normal practice for most forest species. Nursery grown transplants of *P. radiata* have traditionally been propagated from seed. The seed is collected from open or control-pollinated mature female cones which have been harvested from existing plantations or specialised seed orchards. The *P. radiata* seed is usually subjected to a stratification and/or a fungicide treatment prior to mechanical sowing into a nursery bed of fine tilth at a defined sowing rate in spring. In nurseries located in north-eastern Victoria, Australia, seedling emergence can be expected to occur between 10 to 16 days (at >70% germination) after sowing if adequate soil moisture is maintained (Minko and Craig, 1976). The spring sown seedlings are allowed to grow in the nursery for 7 - 9 months, or until they reach a

plantable size (approximately 30cm in height). The physiological and environmental factors effecting coniferous seedling growth has previously been reviewed by Lavender (1984). While growing in the nursery the seedlings are usually 'conditioned' for transplanting by utilising various mechanical treatments (undercutting, wrenching, topping and lateral root pruning). The mechanical treatments used to condition seedlings aim to produce 'hardy' seedlings, with a compact fibrous root system and an ideal root:shoot ratio able to best survive the stress of transplanting.

1.2 Importance of Vegetative Propagation in *P. radiata*

The propagation of *P. radiata* from seed is a well understood and established technique for the commercial production of this species for use in plantation forestry. With the relative abundant production of improved seed from breeding programs, it appears pertinent to ask, why grow *P. radiata* from cuttings? Research during the past 50 - 60 years has demonstrated that the use of *P. radiata* cuttings as transplants has four main advantages over conventional seedling transplants; (1) genetic, (2) morphological, (3) managerial and (4) economic (Shelbourne, 1986).

1.2.a Genetic Advantages

It was generally considered that one of the main advantages of using cuttings as transplants, as opposed to seedlings, would be the propagation of genetically superior clones. However, because of the loss of rooting ability associated with plant ageing, the propagation of mature *P. radiata* trees by cuttings is difficult. The rejuvenation of mature clones to allow them to be vegetatively propagated is not yet possible, but may be an option in the future.

In control-pollinated (CP) orchards where crosses are made artificially between small numbers of the very best progeny-tested parents, the number of individual plants produced from the CP seed may be increased using vegetative multiplication (Shelbourne, 1986). In CP clones, the traits of commercial interest are usually field tested prior to their use in establishing clonal plantations. Therefore, vegetative multiplication allows higher parental selection pressures to be used at the breeding stage, and speeds the realisation of genetic gain achieved with time (Chaperon, 1989).

1.2.b Morphological Differences

A number of studies have compared the growth rate of *P. radiata* seedlings and cuttings in the field (Brown, 1974; Field, 1934; Fielding, 1969; Shelbourne and Thulin, 1974; Sweet and Wells, 1974). Most report similar growth rates (measured as height growth/unit time) in both seedlings and cuttings set from juvenile (ie less than 8 years old) stock plants (Brown, 1974; Field, 1934; Fielding, 1969). However, slower growth rates (height and diameter) were observed in cuttings collected from older stock plants (ie greater than 15 years old) (Brown, 1974; Fielding, 1969; Sweet and Wells, 1974). Sweet and Wells (1974) stated that the loss in growth rate associated with ortet (stock plant) age would result in increased times from planting to canopy closure, when compared with trees of seedling origin. In conclusion, it appears that cuttings collected from juvenile stock plants have similar growth rates as seedlings, however, if cuttings are collected from mature stock plants then a loss in growth rate can be expected.

It has been observed that *P. radiata* raised as cuttings, even from unselected trees, produce straighter trees with fewer defects, such as butt sweep, crooked or forked stems than trees of seedling origin (Menzies *et al.*, 1985; Menzies and Klomp, 1988; Thulin and Faulds, 1968). More specifically, cuttings collected from older stock plants tend to produce straighter trees than those collected from juvenile stock plants (Shelbourne and Thulin, 1974). Cuttings also tend to have branches that are thinner and more perpendicular to the stem in comparison to trees of seedling origin (Fielding, 1969; Pawsey, 1971; Tufuor, 1973). This thin branching habit results in smaller knots and a higher recovery of stress graded sawn timber and veneer (Spencer, 1987). Also, trees grown from cuttings generally have less stem taper and thinner bark than those grown from seedlings (Eldridge and Spencer, 1988; Fielding, 1970; Pawsey, 1971; Sweet and Wells, 1974). These factors are all a reflection of a more advanced maturation state.

In the early stages of growth cuttings are reportedly less susceptible to *Dothistroma pini*, a plant pathogen, than seedlings (Burdon and Bannister, 1985; Carson, 1988) . However, as the plantation ages both seedlings and cuttings have been found to be equally susceptible (Carson, 1988) Cuttings are also less prone to toppling than seedlings because of their reduced 'sail' area and more rigid root system (Mason and Trewin, 1987). A higher percentage survival has been reported for cutting transplants (95%) in the field compared with seedling transplants (83%) (Cameron, 1993).

In *P. radiata*, the wood properties of cuttings collected from mature stock plants differ significantly to those observed in seedling or juvenile cutting origin trees (Cown, 1988; Nicholls, *et al.*, 1974). In cuttings collected from mature trees the propagules had longer tracheids (especially near the pith), larger spiral grain angles and a lower wood density, compared with seedling origin trees of equivalent age (Cown, 1988). However, Cown (1988) also demonstrated that cuttings collected from juvenile parents (up to 7 years old) have similar wood properties to those trees grown from seedlings.

1.2.c Managerial and Economic Advantages

The cost of producing cuttings as transplanting stock from open-pollinated (OP) seed is approximately 2 - 5 times the cost of producing seedlings (Menzies *et al.*, 1985; Menzies *et al.*, 1988) . However, the high costs associated with the production of genetically superior CP seed has meant that cuttings collected from nursery stool beds are cheaper to produce than control pollinated seedlings (Menzies *et al.*, 1992).

Cuttings are usually planted at a wider spacing than seedlings, because of their better growth form, increased resistance to toppling and better field survival (Mason and Trewin, 1987; Menzies *et al.*, 1985). Although the cost of producing cuttings is higher than that for seedlings, the establishment costs are reduced because of the reduced planting density and transplanting labour costs. The cost of establishing seedling or cutting plantations ($/Ha) then becomes similar (Cameron, 1993). Compared with seedling plantations, cutting plantations require less labour to thin and prune because of their lower planting density and thin branch habit (Cameron, 1993). The straighter stem, reduced taper, thinner bark, smaller knots and better wood properties of cutting trees translate into a higher percentage of superior quality harvestable end product.

1.3 The Vegetative Propagation of *P. radiata*

P. radiata may be vegetatively propagated in a number of ways, (1) by stem cuttings (Cameron, 1968; Fielding, 1954; Fielding, 1969; Jacobs, 1939); (2) by fascicle cuttings (Menzies *et al.*, 1986) or (3) in tissue culture (Biondi and Thorpe, 1982; Kumar *et al.*, 1988; Washer *et al.*, 1977). The method used will depend on, (1) the cost per plant, (2) the multiplication rate required and (3) the availability of

equipment and technology. There is a negative correlation between the cost of producing a plant and multiplication rate. Stem cuttings are the cheapest to produce while tissue culture plants are the most expensive to produce. However, the multiplication rate is the highest in tissue culture compared with the propagation by stem cuttings. The vegetative propagation of *P. radiata* on a commercial scale is achieved using stem cuttings. The lower costs of production per plant and the ability to utilise existing farm machinery and equipment make this the most common method of propagation. The propagation of *P. radiata* by stem cuttings will be the method discussed here after.

The propagation of stem cuttings firstly requires the collection of suitable cutting material. Cuttings may be collected from existing field plantations or from specialised nursery grown and hedged stock plants. The major problems associated with the propagation of *P. radiata* by cuttings are the seasonal variation in the percentage of cuttings forming roots and the loss of rooting ability associated with plant ageing (Fielding, 1954; Fielding, 1969; Jacobs, 1939). Therefore, the age of cutting material and the timing of cutting collection are very important in determining the success of the commercial operation (discussed later in Section II.2.3 and Section II.2.4 of this thesis). Stem cuttings, usually between 7-12cm in length and 3 - 8mm in diameter, are collected from the parent plant and placed approximately 5cm deep into the nursery soil. The cuttings are usually misted periodically (5 minutes/hour) to reduce the rate of transpiration and maintain cell turgor during adventitious root formation (ARF). During ARF various herbicide and fungicide treatments are applied to control weeds and plant pathogens respectively.

Cameron and Thomson (1969) observed that cuttings set in open bed nurseries during the early-winter months developed basal callus tissue by mid-late spring, and by early summer >60% of cuttings had formed roots or visible root primordia. They concluded that cuttings remain dormant for approximately 4-5 months prior to any visible activity occurring. Once a root system has been formed the normal seedling practice of lateral pruning and undercutting is conducted. The mechanical treatments aim to produce a 'hardy' transplants with a compact fibrous root system, as with seedlings, to best survive the stress of transplanting.

II.2 Stock Plant Effects

2.1 Genetic Effects

Adventitious root formation in cuttings is thought to be under strong genetic control (Haissig, 1986; Haissig and Riemenschnieder, 1988). A proportion of the variation in the rooting ability of stem cuttings may be attributed to the biochemical and physiological processes which occur in the cutting during development and are ultimately affected by genetic differences within and between species (Leakey *et al.*, 1992).

Fielding (1954) demonstrated that cuttings taken from *P. radiata* trees, particularly over ten years of age, exhibit large differences in the ease with which they can be propagated from cuttings. At the New Zealand Forest Research Institute, both family and clonal differences were observed in a trial which was established to investigate the effect of different genotypes on the rooting of *P. radiata* cuttings (M. Menzies, pers. comm., 1996). In this trial, cuttings were collected from stock plants which were physiologically 1.5-3 years old. The trial examined 10 families, with five clones per family and at three different times in the season (June, mid-July and August). The variation in rooting between families and clones was the greatest in August and the smallest in mid-July. This indicated that genetic differences in the rooting percentage of *P. radiata* occurred in juvenile material at different times in the season. In *Pinus banksiana* ARF varies markedly between cuttings from half-sib families within good and poor-rooting groups (Haissig, 1986). Also, the rooting response to applied auxin has been demonstrated to differ between families (Haissig, 1986). In Norway Spruce cuttings (*Picea albies*) the heritability for rooting in 30-year-old untreated cuttings was calculated to be 0.7 (Mergen, 1960 cited by Haissig and Riemenschnieder, 1988). Mergen (1960) concluded that rooting percentages and volume of roots produced by Norway spruce cuttings were genetically controlled. The variable nature often observed in the rooting of cuttings indicates that many metabolic pathways are involved in ARF, and that a genetic deficiency in one pathway may result in loss of rooting ability.

2.2 Environmental Effects

Traditionally, little emphasis was placed on the stock plant environment. It wasn't until the effects of etiolation were known that attention was focussed on the effect of stock plant environment on rooting of cuttings. Now many environmental effects are known to influence the rooting of cuttings in many plant species.

2.2.a Temperature

There has been no research in *Pinus* spp. which has investigated the effect of stock plant temperature on the subsequent rooting of cuttings. This is surprising considering that seasonality differences occur in the rooting of *P. radiata* cuttings (discussed later in Section II.2.4) (Fielding, 1954; Fielding, 1969; Jacobs, 1939). The effect of stock plant temperature on the rooting of cuttings has been reviewed by Andersen (1986) and Moe and Andersen (1988). Both stress that temperature effects other environmental parameters and also the rate of growth and development in the stock plant. For example, leaf temperature could be 12-15°C higher than the surrounding air temperature under irradiance as compared with darkness (Andersen, 1986). Given the problems in experimental technique, research by Heide (1964) in *Begonia* spp. demonstrated that there was little difference in the rooting success of cuttings taken from stock plants grown at different temperatures. Fischer (1981) cited by (Andersen, 1986) observed no difference in root number or days required for root emergence in pea cuttings, where stock plants were grown at 15, 20 or 25°C.

Although little research has been conducted, a tentative conclusion is that stock plant growing temperature plays a minor role in the subsequent rooting of cuttings.

2.2.b Light

Perhaps the first evidence that incident light received by the stock plant was involved in the rooting of cuttings came from etiolation experiments (Reid, 1923 cited by Maynard and Bassuk, 1988). Etiolation is the phenomenon exhibited by green plants when grown in darkness (Abercrombie *et al.*, 1980). Etiolation was found to accelerate the rooting of cuttings. Banding, or the localised exclusion of light from the region of ARF has similar effects to etiolation (Maynard and Bassuk, 1988). Also, the intensity, duration and quality of the incident light received by the stock plant has been demonstrated to influence the subsequent rooting in some species of cuttings (Andersen, 1986; Moe and Andersen, 1988).

2.2.b.1 Irradiance

In *P. radiata* the influence of stock plant irradiance on the subsequent rooting of cuttings has not been investigated. However, the influence of stock plant irradiance on the rooting of cuttings has been reviewed in other plant species (Andersen, 1986; Harmann and Kester, 1983; Moe and Andersen, 1988). The level of irradiance experienced by the stock plant may inhibit or delay rooting, promote rooting, or have no effect (Moe and Andersen, 1988). A high level of irradiance ($40Wm^{-2}$) experienced during the growth of the stock plant has been demonstrated to significantly reduce the rooting of *Pinus sylvestris* cuttings, compared with stock plants grown at a low level of irradiance ($8Wm^{-2}$) (Hansen *et al.*, 1978). Stromquist and Eliasson (1979) reported similar findings with *Picea abies* (Norway spruce). The carbohydrate content of *Pinus sylvestris* cuttings grown under a high level of irradiance was greater than those cuttings grown under the low level of irradiance (Hansen *et al.*, 1978). Hansen *et al.* (1978) stated that it was possible that the higher carbohydrate content observed in cuttings collected from stock plants grown at high levels of irradiance is supraoptimal to ARF.

In other plant species, eg *Chrysanthemum morifolium* and *Begonia* spp., increasing the level of irradiance to the stock plant has resulted in increased rooting of cuttings (Moe and Andersen, 1988). Similarly, there are examples where the level of irradiance experienced by the stock plant has no influence on the subsequent rooting success of the cuttings, eg *Ligustrum* x *vicary* (Knox and Hamilton, 1982 cited by Moe and Andersen, 1988).

2.2.b.2 Photoperiod

In *P. radiata* the influence of stock plant photoperiod on the subsequent rooting of cuttings has not been investigated. The effects of stock plant photoperiod on the rooting of cuttings from other plant species has been reviewed previously (Andersen, 1986; Moe and Andersen, 1988). Only a few investigations have considered the effects of stock plant photoperiod on the subsequent rooting of cuttings. According to a review by MacDonald (1969) the rooting of woody ornamental stock plants was always inhibited by short-days (SD), while long-days (LD) stimulated the rooting of cuttings. This finding was supported by Smith and Wareing (1972), who demonstrated that SD given to stock plants significantly reduce the number of roots in *Populus* cuttings. However, there are exceptions to the rule,

where SD may stimulate rooting, eg *Chrysanthemum* (Andersen, 1986). In conclusion, when considering the seasonal nature of rooting (Section II.2.4) a photoperiodic link does appear possible. However, the physiological basis and mechanism of action are poorly understood.

2.2.b.3 Light Quality

There is a lack of published information on the effect of light quality during stock plant growth of *P. radiata* on ARF of cuttings . Moe and Andersen (1988), in their review of the effects of light quality on stock plants and the subsequent rooting of cuttings, indicated that the exposure of stock plants to far-red (FR) light may, as opposed to red (R) light may stimulate rooting. However, in *Phaseolus mungo* the rooting of cuttings taken from seedlings grown under FR light was completely inhibited (Gupta *et al.*, 1977). In *P. radiata* the rooting is enhanced when cuttings are taken from the lower rather than the upper region of the stock plant (Fielding, 1969). This may be attributed to the increased level of FR light found lower in the canopy due to the filtering of R light by the overlying leaves. However, the effects of lowered light intensity and differences in ontogenetic ageing may be responsible for this enhanced rooting lower in the canopy.

2.2.c Water Relations

The effect of stock plant water relations and the subsequent rooting of cuttings can be divided into two main groups; (1) water stress and (2) water logging.

2.2.c.1 Water Stress

The growth of water-stressed *P. radiata* trees may be significantly reduced compared with plants grown under low water-stress conditions (Green and Mitchell, 1992). However, the effect of stock plant water relations on the subsequent rooting success remains unknown in *Pinus* spp.. In pea cuttings Rajagopal and Andersen (1980a) demonstrated that there was a reduction in root numbers associated with increasing stock plant water stress, especially in stock plants grown under low levels of irradiance (16 Wm^{-2}). Although, they did find that the rooting may be stimulated by a small amounts of water stress in stock plants grown under high levels of irradiance (38 Wm^{-2}). The reduction in root numbers associated with increasing stock plant water stress has been weakly correlated to the increased ABA content of the leaf tissue (Rasmussen and Andersen, 1980; Rajagopal and Andersen, 1980b).

2.2.c.2 Water Logging

Some intact plant species can often develop adventitious roots in response to waterlogging. The effect of water logging on ARF in *P. radiata* has not been investigated. However, the influence of stock plant water logging on ARF has been investigated in many other plant species (Andersen, 1986; Moe and Andersen, 1988). The ecological significance of ARF in waterlogged conditions may be that it assists in the survival of the plant when the normal roots are suffering oxygen deficiency (Jackson and Drew, 1984 cited by (Visser *et al.*, 1995). Kawase (1965) has suggested that ethylene production in the waterlogged root was responsible for the induction of adventitious roots.

2.2.d Mineral Nutrition

The mineral nutrition of *P. radiata* stock plants and the relationship with the subsequent rooting of cuttings has not been investigated. The effect of stock plant mineral nutrition and the subsequent rooting of the cuttings has previously been reviewed (Andersen, 1986; Moe and Andersen, 1988). In general, it appears that stock plants which are fertilised suboptimumally produce cuttings which root the best (Andersen, 1986; Moe and Andersen, 1988). Cuttings from nursery grown stock plants of *Hypericum, Rosa* and *Rhododendron* which had been grown in stone wool (Grodan) with a lower concentration of the nutrient solution (0.05%) rooted better than those from strongly fertilised stock plants (0.2%) (Knoblauch, 1976 cited by Andersen, 1986). In summary, the available evidence suggests that lowering the mineral nutrition of the stock plants encourages rooting of the cuttings.

2.3 Stock Plant Age

2.3.a Effect of Stock Plant Age

The age of the parent plant has, for a long time, been known to influence the subsequent rooting of cuttings. Fielding (1954), Fielding (1969), Jacobs (1939), Mirov (1944) and Thulin and Faulds (1968) have demonstrated that the ease with which cuttings of *P. radiata* can be rooted declines with the age of the parent stock plant. Fielding (1954) reported data from experiments in which the rooting success of cuttings fell from 88% for cuttings from 3 year-old stock plants to 68% at age 5 years, and 11% at age 26 years. However, Thulin and Faulds (1968) reported a high

rooting percentage in cuttings taken from 9 year-old stock plants (100%) and only a small reduction in cuttings taken from 20 year-old stock plants (70%). Although there are some inconsistencies in these reports, the general effect of increased parent stock plant age is to lower the rooting success of cuttings. Fielding (1969) speculated that the differences observed in the rooting success of cuttings collected from the same aged parent plants could have been due to different parent genotypes, physiological age (Section II.2.3.b) and environmental growth conditions.

2.3.b Physiological Age

In many woody plant genera there is a clear morphological distinction between the juvenile stage and a later mature stage in the growth of the stock plant. In *P. radiata*, there are differences between juvenile and mature foliage. The juvenile foliage contains a higher proportion of primary needles to secondary fascicle leaves (M. Menzies pers. comm., 1994). The rooting of the cuttings from juvenile and mature stock plants is known to differ greatly (Section II.2.3.a). However, differences in the stage of maturity can exist on the same tree. For example in *P. radiata* cuttings taken from a position high in the crown are known to root poorly in comparison with cuttings taken lower in the crown (Fielding, 1969). The differences in the rooting of these cuttings has also been related to the ageing phenomena. The cuttings collected higher in the crown are physiologically older than those taken near the base, therefore the differences in rooting may be attributed to the difference in physiological age. Different trees may age more rapidly than others, which may be why differences in rooting success exist between trees of the same chronological age. Libby *et al.* (1971) developed a maturation model for the rooting of cuttings based on the distance between the position of the cutting on the stock plant and the ground base of the stock plant. The further away from the base of the stock plant that the cutting was collected, the less likely that those cuttings would strike, because the cuttings were physiologically older (Libby *et al.*, 1971).

Fielding (1969) reported that hedged *P radiata* stock plants of seedling origin remained physiologically juvenile in that cuttings taken from them rooted more readily than those taken from unhedged stock plants of the same chronological age. Hedging appears to arrest physiological ageing in *P. radiata* by allowing juvenile foliage to continue growing near the base of the stock plant. However, the physiological age of the hedged stock plant can not be maintained in a juvenile state indefinitely, and ageing still occurs, but at a much reduced rate.

2.4 Season of Collection

The time in year when cuttings are collected from the stock plant has been demonstrated to influence the rooting success of *P. radiata* stem cuttings (Fielding, 1954; Fielding, 1969; Jacobs, 1939; Menzies *et al.*, 1988). Cuttings which are collected during the winter months when the apex is in the dormant period of growth appear to root better than those taken during spring or summer when the stock plant is growing rapidly. Fielding (1954), in Canberra, demonstrated that stem cuttings taken between April and July (late-autumn, mid-winter) rooted well (99-82%), while those taken in November rooted poorly (10%). Contrary to this evidence, Menzies *et al*. (1988) demonstrated that fascicle cuttings taken from juvenile stock plants and set under mist rooted well all year round. This may mean that the environmental conditions experienced during propagation are more important than the physiological condition of the cuttings at the time of collection. In fact, Fielding (1954) suggested that the dry-hot condition experienced during the summer months is one of the major factors responsible for the reduction in rooting success. However, where cuttings are collected and set in field nurseries there does exist a consistent seasonal variation in the rooting ability of cuttings. Whether it is due to the physiological status of the stock plant or the environmental conditions experienced during propagation remains unknown.

It is often recommended that cuttings be taken from fully turgid stock plant, ie. early in the morning or in cloudy weather (Hartmann and Kester, 1983). The timing of cutting collection (diurnal variation) has not been investigated in *P. radiata* or any other plant species. However, it does appear likely that it may be related to the water relations of the stock plant at the time of cutting collection (Section II.5.1).

II.3 Post Cutting Excision Effects

3.1 Environmental Effects

3.1.a Temperature

P. radiata cuttings set under field conditions are inherently predisposed to ambient temperature fluctuations. It is extremely difficult, if not impossible, to separate the effect of temperature on ARF from a number of other environmental influences occurring concurrently in the field (Moe and Andersen, 1988). The investigation of temperature effects has been confined to few controlled environment studies, where increasing the mean temperature of propagation resulted in faster rates of ARF, or conversely less time to root (Cameron and Rook, 1974). Cameron and Rook (1974) reported unpublished results where *P. radiata* cuttings propagated in a day/night temperature regime of 25°/15°C had produced roots at 10 weeks, compared with 16 weeks at 20°/10°C and 22 weeks at 15°/5°C. This result would not appear surprising as the rate of many plant metabolic processes is controlled by temperature. Cameron and Rook (1974) reported that at 20°/10°C more than 70% of cuttings formed roots, compared with 57% at 25°/15°C and only 36% at 15°/5°C. However, Cameron and Rook (1974) stated that the interpretation of the effects of temperature on the percentage rooting in *P. radiata* was complicated due to fungal infection at higher temperatures.

The use of bottom heat mist beds is not only wide-spread in the propagation of *P. radiata* but also for most other plant species (Eldridge and Owen, 1986; Hartmann and Kester, 1983; Janick, 1986; Mirov, 1944). In *P. radiata*, bottom heat to maintain the temperature of the rooting media in the zone of root initiation at approximately 22°-25°C and glasshouse air temperatures between 18°C and 25°C are frequently used (Mirov, 1944; Eldridge and Owen, 1986). In most plant species, the use of bottom heat to maintain the temperature of the rooting medium at approximately 24°C facilitates rooting by increasing the rate of cellular divisions in the rooting area, while misting reduces the temperature of the aerial portion of the cutting thus lowering the rates of transpiration and respiration (Janick, 1986). Apart from Cameron and Rook (1974) no other research has investigated the effect of temperature on ARF in cuttings of *P. radiata*. In other herbaceous and woody plant

species basal temperature is considered to play an important role in the rate and success of ARF in cuttings (Mencuccini *et al.*, 1988). Fisher (1981) cited by (Andersen, 1986) demonstrated that pea cuttings took longer to root to a predetermined stage at lower temperatures, however, if the cuttings were allowed to continue growth, root number was tripled at 15°C compared with 25°C.

Temperature has been demonstrated to influence the rate of ARF in cuttings of *P. radiata* and other plant species. More specifically the temperature in the zone of root initiation and growth probably has the most influence on the rate of root development. However, it is difficult to separate the effect of temperature from a number of other environmental influences which can occur at the same time. The effect of temperature on the final rooting percentage still remains unknown, although differences were observed in *P. radiata* cuttings, the cause may not have been temperature related (Cameron and Rook, 1974). The measurement of percentage rooting in temperature experiments at a particular date may only reveal different rates of root development and not true percentage rooting.

3.1.b Water Relations

When a cutting is severed from the stock plant a severe stress situation is created. The natural route for water supply, ie the roots, have been removed, yet transpiration through the leaves continues (Hartmann and Kester, 1983). The loss of water from the leaves may reduce the water content of the cuttings to such a low level as to cause the cutting to die before ARF can occur (Hartmann and Kester, 1983). At the time of setting *P. radiata* cuttings are often in a condition of intense water stress (-2.5 to -3.0MPa) and if water loss continues, the stress will become extreme, ultimately fatal (Cameron and Rook, 1974). Cuttings lose water at a rate which is mainly dependent on the water vapour pressure difference between the leaf and the surrounding air surrounding (Andersen, 1986). This difference further depends to a considerable extent on the incoming and absorbed radiation which creates a temperature gradient (Andersen, 1986). To reduce the rate of transpiration in the leaves of the cutting to a minimum, the water vapour pressure of the air surrounding the leaves should be maintained as close as possible to the water vapour pressure in the intercellular spaces within the leaf (Hartmann and Kester, 1983; Andersen, 1986). Also, the level of irradiance absorbed by the leaves should be reduced (Hartmann and Kester, 1983; Andersen, 1986).

Cameron and Rook (1974) proposed that there are four possible pathways for water uptake in *P. radiata* cuttings; (1) through the cutting base; (2) by diffusion into the foliage immersed in the soil; (3) through the cuticle part of the stem in contact with the soil; (4) by entry through the foliage exposed to the air and wetted by rain, dew or misting. In experiments using tritiated water it was observed that most of the water entry into the cutting occurs through the base of the cut stem and through the foliage in contact with the moist soil (Cameron and Rook, 1974). Cameron and Rook (1974) considered this to be an important reason for leaving the foliage intact during propagation.

3.1.c Light

In all types of plant growth and development light is of major importance as the source of energy in photosynthesis. In the rooting of cuttings, the products of photosynthesis are important in the formation and development of a new adventitious root system. The effects of light on the rooting of cuttings can be due to light intensity (irradiance), photoperiod (day length) and light quality (Hartmann and Kester, 1983).

3.1.c.1 Irradiance

The influence of irradiance[1] or light intensity on ARF in *P. radiata* cuttings during propagation has not been investigated. However, the influence of irradiance during the rooting period has been reviewed in other plant species (Andersen, 1986; Hartmann and Kester, 1983; Loach, 1988). Loach (1988) reported that much of the research describing the relationship between the rooting of cuttings and irradiance experienced during propagation failed to consider the importance of stock plant irradiance (Section II.2.2.b). The level of irradiance (8 or $40Wm^{-2}$) experienced during the propagation of *Pinus sylvestris* (Scots pine) seedlings had no significant effect on either the number of roots/cutting or the percentage of cuttings with roots (Hansen *et al.*, 1978; Stromquist and Hansen, 1980). Similar results were observed in *Picea abies* (Norway spruce) cuttings, where differences in irradiances (8 or $40Wm^{-2}$) experienced during propagation had little effect on the subsequent rooting percentage (Bollmark and Eliasson, 1990; Stromquist and Eliasson, 1979).

However, Haissig (1990) demonstrated that low levels of irradiance (maximum $120\mu mol.m^{-2}.s^{-1}$) during the propagation of *Pinus banksiana* (Jack pine)

cuttings resulted in significantly lower rooting and reduced numbers of roots/cutting when compared with cuttings propagated under high levels of irradiance (maximum 900μmol.m^{-2}.s^{-1}). High levels of irradiance during propagation have been demonstrated to promote rooting in other plant species (e.g. *Pisum sativum* and *Phaseolus aureus*) (Eliasson, 1980; Jarvis and Ali, 1984). This may suggest that ARF is limited by the availability of current photosynthate (Andersen, 1986; Davis, 1988; Davis and Potter, 1981). This hypothesis gained support from experiments with photosynthesis inhibitors such as diuron (DCMU, a photosystem II inhibitor), where the rooting of cotyledon *Sinapsis alba* and *Raphanus sativus* cuttings was reduced using DCMU (Lovell *et al.*, 1972).

High levels of irradiance during propagation have been demonstrated to inhibit ARF in some plant species, e.g. *Weigella, Hibiscus* and *Viburnum* (Loach, 1979). Loach (1979) reported that cuttings propagated under high levels of irradiance usually have a lower water content than those under low levels of irradiance. The level of irradiance may also influence the temperature during propagation. This can complicate the interpretation of experiments designed to investigate the effect of irradiance on rooting. Etiolation of tissue at the site of adventitious root initiation has been demonstrated to stimulate the rooting of many plant species (Andersen, 1986; Eliasson, 1980; Hartmann and Kester, 1983).

No research has investigated the effect of irradiance on ARF in *P. radiata* cuttings. However, the level of irradiance experienced during the propagation of cuttings has been demonstrated to influence ARF in many plant species. The importance of stock plant irradiance on rooting percentage has previously been discussed (Section II.2.2.b) and should be taken into consideration when investigating the effects of irradiance during propagation.

[1] - Irradiance is considered the relative amount light in the form of photosynthetically active radiation (PAR; 400-700nm wavelength) per unit area (Hartmann and Kester, 1983).

3.1.c.2 Photoperiod

Because of the strong correlation between the season of cutting collection and the rooting success under field conditions, photoperiod was thought to have a controlling influence in the rooting of *P. radiata* cuttings through a phytochrome

response (Cameron and Rook, 1974). Under conditions of natural daylight, Cameron and Rook (1974) observed that cuttings did not form roots until 'long day' conditions are achieved in November-December. In a preliminary experiment, which investigated 4 different day lengths (8, 12, 16 and 24 hours) Cameron and Rook (1974) reported that photoperiod did not appear to influence the rate of callus growth or root formation of *P. radiata* cuttings, and that no significant differences were observed in rooting percentage. They concluded that photoperiod was not an important factor in the rooting of *P. radiata* cuttings.

The effect of photoperiod during propagation on ARF in other plant species has previously been reviewed (Hartmann and Kester, 1983; Andersen, 1986). Andersen (1986) concluded that long days during propagation appeared to promote the rooting of cuttings in general, however, the effect was not spectacular. This conclusion is in agreement with (Smith and Wareing, 1972), who demonstrated that *Populus* x *robusta* (poplar) cuttings propagated under long day conditions rooted more successfully and produced more roots/cutting, however, the effects of photoperiod were only significant on a few sample dates. The promotion of rooting under long days conditions may have resulted from the increased production of photosynthates.

3.1.c.3 Light Quality

No research has investigated the effect of light quality[1] on ARF in *P. radiata* cuttings during propagation. The effects of light quality on ARF in other plant species has previously been reviewed (Hartmann and Kester, 1983; Andersen, 1986). It appears that red light can inhibit ARF in some plant species more than blue light, it is unknown whether this effect is mediated through some phytochrome response (Hartmann and Kester, 1983; Andersen, 1986). Andersen (1986) stated that in view of the extremely complicated experimental procedures necessary to elucidate this problem it is not surprising that insufficient evidence has been presented to indicate whether phytochrome is involved.

[1] - Light quality refers to the spectral distribution of incident radiation received by the plant or plant part.

3.1.d Rooting Media

The large scale commercial production of *P. radiata* cuttings is usually conducted in field nursery sites. Cuttings are usually set in raised nursery beds of soil, and misted on a regular basis to prevent desiccation. The soil which supports the cutting during adventitious root formation (ARF) should be open textured, deep, and free draining (Fielding, 1969). Both Fielding (1969) and Jacobs (1939) described experiments where low rooting percentages were observed when cuttings were set in soil which was classed as a poor draining clay loam. In general, the soil media which supports the cutting throughout ARF should satisfy certain physical, chemical and biological criteria (Peate, 1989). The physical characteristics of the soil are usually considered to be of most importance during the early stages of ARF, before a root system has been formed. The soil media should have good aeration, high water holding capacity and good drainage characteristics for the developing root system which absorbs oxygen and releases carbon dioxide (Peate 1989). *P. radiata* cuttings have been raised successfully on soils with a pH range of 5.1-7.0 (Jacobs, 1939; Whiteman pers. comm., 1993).

Where *P. radiata* cuttings are propagated in a containerised form such as pots, trays or root trainer cells, etc, the type of rooting media used to fill these containers varies greatly. In examining the literature on rooting media used to propagate conifer cuttings, the standard rooting medium for many years was medium-course sand (Hitchcock 1928 cited by (Girouard, 1974). Experiments performed during the late 1930's demonstrated that a mixture of sand and peat moss of sphagnum origin in a ratio of 2:1 (v:v) was vastly superior to sand alone (Grace 1940, 1941, 1942 cited by (Girouard, 1974). Jacobs (1939) demonstrated that the type of rooting media used to propagate containerised *P. radiata* cuttings had a large influence on the subsequent rooting percentage. Jacobs (1939) recommended using soil mixed with rotted *P. radiata* sawdust (1:1 by volume) because it was cheap and easy to obtain in large quantities. Since Jacobs, many other researchers have used various combinations of rooting media, including peat, pumice, granulated bark, granite grit and other industrially manufactured materials such as perlite and vermiculite (Eldridge and Owen, 1986; Faulds and Dibley, 1989; Haissig, 1982c; Libby *et al.*, 1971; Smith and Thorpe, 1975a; Smith and Thorpe, 1975b; Summers, 1986). The type of material(s) used in the rooting medium will depend not only on their physical, chemical and biological characteristics but also the propagation environment, availability, reproducability and cost. It appears that the various

combinations of successful rooting media meet the appropriate physical characteristics, ie an optimal volume of gas-filled porespace(15-20%), an oxygen diffusion rate adequate for the needs of respiration and a reasonable water retention capacity (Andersen, 1986). Most modern rooting media contains a significant proportion of artificial media (perlite, vermiculite, etc), although often more expensive, they are able to provide the rooting media with better physical characteristics for ARF (Andersen, 1986).

The chemical requirements of rooting media are that they be low in salts, chemically stable during use, have a pH in the range of 4 - 6.5 and have some cation exchange capacity (Peate 1989). The rooting media should be free of biologically active substances and organisms such as weed seed, spores, insects, larvae, and any pathogenic materials (Peate 1989). However, beneficial effects of incorporating inoculum of mycorrhizal fungi in the rooting medium have been reported in other woody species (Massicotte *et. al.*, 1994). These areas, although poorly understood, are attracting more research attention.

3.1.e Carbon Dioxide

The response of intact higher plants to elevated levels of atmospheric carbon dioxide has been reviewed by (Wullschleger *et al.*, 1994). For intact plants it is well known that CO_2 enrichment usually results in increased photosynthesis and growth, reduced stomatal conductance and increased water use efficiency (Grant *et al.*, 1992). The effects of carbon dioxide enrichment during the propagation of *P. radiata* has not been investigated. However, the effects of carbon dioxide enrichment techniques have been used successfully in the propagation of other plant species by cuttings. ARF on both *Chamelaucium* and *Correa* cuttings maintained at high humidity in an enclosed fog tunnel was significantly enhanced when ambient carbon dioxide was increased from 350 to 800μbar (Grant *et al.,* 1992). Cuttings propagated under high levels of CO_2 displayed improved water relations, due to decreased rates of transpiration, compared with cuttings propagated under ambient levels of CO_2 (Grant *et al.,* 1992). The stimulation of rooting observed in both *Chamelaucium* and *Correa* cuttings under CO_2 enrichment could also be derived from improved carbohydrate relations, since significantly higher starch contents were observed in the high CO_2 environment (Grant *et al.,* 1992).

3.1.f Mineral Nutrition

The effects of mineral nutrition on ARF in *P. radiata* cuttings has not been investigated. Nitrogen deficiency symptoms have been observed in the new top growth cuttings produce during ARF (Menzies *et al.*, 1986). The current recommendation is to spray nitrogen deficient cuttings with a 2% urea solution applied at 400-500 L/Ha on a regular basis until symptoms disappear (Menzies *et al.*, 1986). However, it is not known if the nitrogen deficiency affects the rooting capacity of the cuttings.

Mineral nutrition of cuttings is considered to be of minor significance when compared with other factors such as parent stock plant age or season of cutting collection. The effects of mineral nutrition on ARF in cuttings from other plant species has been reviewed previously (Andersen, 1986; Blazich, 1988). The general consensus is that nutrients are essential to ARF in cuttings and the demands may differ during root initiation and root growth. However, the importance of various nutrients during ARF is poorly understood. Blazich (1988) speculated that N, P, K, Ca, Mn, Zn and B are important during both root initiation and root growth.

3.2 Cutting Size and Setting Depth

Commercial *P. radiata* cuttings nurseries generally require the collection of standard sized cutting material from parent plants. The typical size of cuttings set in commercial nurseries may range from 50-150mm in length and 2-8mm in diameter (Duryea and Boomsma, 1992; Fielding, 1954; Fielding, 1969; Libby and Conkle, 1966; Menzies, 1992; Menzies *et al.*, 1985; Menzies *et al.*, 1992). However, most nurseries set cuttings which have a shoot length of about 100mm long and a minimum diameter of at least 4mm (Duryea and Boomsma, 1992).

The effect of cutting dimensions on the rooting percentage and subsequent growth of *P. radiata* was investigated by Fielding (1954, 1969). Fielding (1954) concluded that there was no significant difference in the rooting percentage of shoots between 75-200mm in length. However, Fielding (1954) demonstrated that the smaller shoots (50mm in length) did display a reduced rooting capacity. Fielding (1969) concluded that shoot thickness, *per se*, had little effect on the rooting percentage of cuttings. However, Fielding (1969) noted that shoot thickness had a significant effect on the nursery growth of cuttings. Similar results were observed in

Picea abies (L.) Karst. (Norway spruce) and *Picea sitchensis* (Bong.) Carr. (Sitka spruce), where longer cuttings displayed a higher rooting percentage and a better quality root system (Mason *et al.*, 1992).

The depth at which *P. radiata* cuttings are set in the rooting media has been demonstrated to influence the rooting success (Jacobs, 1939; Fielding, 1954, 1969). The rooting percentage of cuttings was demonstrated to decline if the cuttings were set deeper than half their length into the rooting media (Fielding, 1954, 1969). The deeper set cuttings, although rooting poorly, grew better in the nursery (Fielding, 1954, 1969).

II.4 Morphological and Anatomical Events Associated with Adventitious Root Formation

4.1 Adventitious Root Formation

Roots that originate in locations other than from the embryo or as branches of the primary root system are termed adventitious (Abercrombie *et al.*, 1980). Adventitious roots may form spontaneously on intact plants, especially at the nodes of prostrate stems, and on rhizomes or stolons, or they may develop as a response to damage when part of the plant has been severed from the existing root system (Lovell and White, 1986). The ease with which adventitious root formation (ARF) occurs in plants varies tremendously between species, between cultivars, with age and with the nature of the plant organ or part being propagated (Lovell and White, 1986). In plants which require severance before ARF will occur, woody plants are generally considered more difficult to root than herbaceous plants (Lovell and White, 1986). The woody plants can be further divided, according to their ease of rooting, into two main groups; (1) easy to root plants with preformed quiescent root primordia in annual shoots, eg *Salix, Ribes* and *Populus* and (2) difficult to root plants without preformed root primordia in the stems, eg *Pinus* and *Abies* (Montain *et al.*, 1983; Blakesley *et al.*, 1991a). *Pinus radiata*, like most conifers, does not contain preformed adventitious root primordia and is considered difficult to propagate (Satoo, 1955; Hartmann and Kester, 1983). However, investigations during the past 60 - 65 years has indicated that *P. radiata* cuttings taken from parent trees less than five years old are easy to root (Field, 1934; Jacobs, 1939; Sherry, 1942; Mirov, 1944; Fielding, 1954; Libby and Conkle, 1966; Thulin and Faulds, 1968; Fielding, 1969).

4.2 Anatomical Stages of Adventitious Root Formation

Many researchers have attempted to divide ARF of *P. radiata* into a number of anatomical stages (Cameron and Thomson, 1969; Smith and Thorpe, 1975b). Although there is a lack of agreement about the exact number of stages involved and the terminology used, there are considered to be at least two main stages; (1) root initiation and (2) root growth, where root initiation must precede root growth

(Cameron and Thomson, 1969; Lovell and White, 1986). Also, there appears to be a lack of uniformity in the terminology used by many authors when discussing the processes of root initiation and root growth (Lovell and White, 1986).

4.2.a Root Initiation

Cameron and Thomson (1969) reported that root initiation in *P. radiata* appears to occur within the callus tissue which develops after a cutting has been removed from the parent plant. Callus tissue develops as an amorphous mass of rather large-celled, loosely arranged parenchyma cells which appear to originate from the cambium region (Cameron and Thomson, 1969). However, in *Pinus banksiana* (Jack pine) cuttings callus tissue appears to originate from cortical cells near the cut surface, grow and develop downward and around the exposed xylem (Montain *et al.*, 1983).

Root initiation in *P. radiata* stem cuttings is preceded by the appearance and directional development of strands of callus xylem (tracheid nests), located centripetally to callus meristems (Cameron and Thomson, 1969). Root initials appear to form in, or near, active callus meristems and give rise to root primordia that develop rapidly (Cameron and Thomson, 1969). Smith and Thorpe (1975b) found with 20-day-old *P. radiata* hypocotyl cuttings that primordial adventitious root initials developed from a single cell at the margin of a differentiating resin canal or in cortical tissue external to these resin canals. The first signs of initiation involved the expansion of this single cell and the concurrent swelling of its nucleus (Smith and Thorpe, 1975b). A similar result was observed in *P. banksiana* hypocotyl cuttings, where primordial root initials appeared to initiate adjacent to, or outside one of the four axial secondary xylem resin canals (Montain *et al.*, 1983). Montain *et al.* (1983) also noted that the root primordia were located in the transitional area between the lower portion of the intact hypocotyl and at the top portion of the callus parenchyma. The primordial initials that are activated may or may not become the future root primordium (Lovell and White, 1986). The induction and activation of a meristematic locus and the first cellular division at a specific location within the callus tissue represents the root initiation phase of ARF.

In *Pinus taeda* (Loblolly pine) an overabundance of callus may be produced without any roots being initiated (Reines and McAlpine, 1959 cited by Cameron and Thomson, 1969).

4.2.b Root Growth

Cellular division continues to occur within the primordial root initial until an organised structure called the root primordium is formed (Lovell and White, 1986). The vascular connections are formed between the root primordium and the existing vascular tissue of the plant (Lovell and White, 1986). Hoffman and Kummerow (1966) cited by Cameron and Thomson (1969) attributed the frequent mortality of apparently rooted *P. radiata* fascicle cuttings to a failure to establish functional vascular connections. In *P. radiata*, the root emerges from the callus produced at the base of the cutting, appearing first as small, white, finger-like protuberances which rapidly lengthen, remaining succulent and brittle (Jacobs, 1939; Cameron, 1968). At first the succulent roots does not possess a root-cap, root hairs, a regular vascular system, secondary roots, or any of the structures that are characteristic of a root (Cameron, 1968). Later the roots shrink, harden and become dark in colour, giving rise to lateral roots and a normal type of root structure (Cameron, 1968).

4.3 Barriers to ARF

The effect of various physical barriers to ARF in *P. radiata* has not been investigated. There has been a continuing debate in the literature over whether or not certain anatomical structures present physical barriers to ARF (Lovell and White, 1986). The restriction of ARF may occur as a result of obstruction by sclerenchyma bands, secretory canals, resin canals, or large volumes of induced vascular tissue occupying space in the sub-basal region which is thus unavailable for sites of root initiation (Lovell and White, 1986). Alternatively, the sites for root initiation may be not be the limiting factor but barriers preventing root primordium growth may be the critical factor (Lovell and White, 1986). Although some of these structures are considered barriers to ARF, there is no general relationship between those structures and the success of ARF (Lovell and White, 1986).

II.5 Physiological Changes Associated with Adventitious Root Formation

5.1 Water Relations

No research has specifically investigated the physiological changes in water relations of *P. radiata* cuttings during propagation. Cameron and Rook (1974) demonstrated that the cuttings may be in a condition of severe water stress at the time of setting (-2.5 to -3.0 MPa). Cameron and Rook (1974) concluded that prior to setting precautions should be taken to reduce dessication of the cuttings. Cuttings which are set in field nursery beds often develop symptoms of water stress, ie. drooping stem, soon after setting (Jacobs, 1939; Fielding, 1954; Fielding, 1969). Because the cuttings lack a root system they readily develop water deficits. For a cuttings to develop an adventitious root system cells must be maintained in a turgid state.

The water relations of cuttings during propagation has been reviewed previously (Loach, 1988). In psychrometric studies which examined changes in leaf water potential of *Ceanothus thyrsiflorous* Esch. cuttings propagated under clear polyethylene covers or mist Loach (1977) demonstrated that the mean leaf water potential decreased gradually until root emergence occurred, where upon the mean leaf water potential increased and was restored to 'normal' levels. However, there were considerable variations in the mean leaf water potential of cuttings between different sampling times. This was inversely correlated to the level of solar radiation (MJ) at the time of recording leaf water potentials (Loach, 1988). The cuttings propagated under the clear polyethylene displayed minimum water potential of -1.2 MPa, where as those propagated under mist displayed a minimum water potentials of -3.9 MPa (Loach, 1988). The rooting percentage of cuttings propagated under clear polyethylene and mist was 96% and 56% respectively. Loach (1988) provided further evidence that rooting is influenced by the degree of water stress developed by the cutting during propagation. The major problem with using leaf water potential as an indicator of the water status of cuttings, is that roots are initiated in the stem base of the cutting, whose water status may differ from that of the leaves (Loach, 1988). Also, the water relations of cuttings may be influenced

by other environmental parameters, including temperature, solar radiation levels and the vapour pressure deficit between leaves and the surrounding air.

5.2 Photosynthesis and Respiration

Cameron and Rook (1974) demonstrated that the net rate of photosynthesis in *P. radiata* cuttings was reduced to a level slightly above the compensation point shortly after detaching the shoot from the parent plant (approximately 0.3mg CO_2/g leaf oven dry weight/hour). The photosynthetic rate remained low throughout the period of callus formation and root initiation. When the roots emerged from the callus tissue the rate of net photosynthesis increased threefold (Cameron and Rook, 1974).

Changes in the rate of photosynthesis during adventitious root formation in other plant species has been reviewed previously (Davis, 1988). Davis and Potter (1981) reported similar patterns in net photosynthesis to those observed by Cameron and Rook (1974) for leafy pea cuttings. In pea cuttings the most significant reduction in net photosynthesis occurred within the first 24-48 hours after excision. The decline in net photosynthesis was postulated to be caused by stomatal closure (Davis, 1988). The closure of stomata may be a response to water stress which can result in reduced rates of net photosynthesis. However, water stress may directly influence the rate of net photosynthesis via non-stomatal effects (Farquhar and Sharkey, 1982 cited by Davis, 1988). The rate of net photosynthesis remained low in the pea cuttings until adventitious roots began to emerge from the cutting, where upon the rate increased. The increase in net photosynthesis may be mainly attributed to the alleviation of water stress and the re-opening of stomata (Davis, 1988). The rate of photosynthesis may be influenced by temperature, light (intensity and quality), water relations and many other environmental factors.

Cameron and Rook (1974) revealed that the rate of respiration in *P. radiata* cuttings showed a progressive increase from the time of excision, through the period of callus formation, until root emergence. The rate of respiration in the whole cutting was observed to be 40% greater than the rate prior to excision (Cameron and Rook, 1974).

II.6 Biochemical Changes Associated with Adventitious Root Formation

6.1 Plant Growth Regulators

Plant growth regulators (PGR's) appear to be involved in many aspects of plant growth and development, including ARF. There are five main groups of plant hormones; auxins, ethylene, cytokinins, gibberellins and abscisic acids. Most of the evidence for their involvement and importance in ARF comes from the application of exogenous PGR's, and observing the subsequent growth responses (Gaspar and Hofinger, 1988; Mudge, 1988; Van Staden and Harty, 1988).

Most of the literature regarding PGR involvement in the process of ARF considered a concentration change necessary to provoke a growth response. However, when attempting to understand the response of plants to PGR's it is important to also consider the issue of tissue sensitivity, reviewed by Trewavas (1981). Because of the anomalies in the literature regarding PGR concentration and the subsequent growth response Trewavas (1981) proposed that the concentration of the PGR may not be the limiting factor, but the sensitivity of the plant tissue to the PGR may be. Recent literature is beginning to address the issues of tissue sensitivity on a quantitative basis (Blakesley and Chaldecott, 1993). However, the 'concentration change' line-of-thought still dominates the bulk of the literature.

6.1.a Auxin

Auxin has received the most attention of all the PGR's with respect to its involvement in ARF. The relatively specific root promoting properties of applied auxin have led to the belief that these substances play a crucial role in the process of ARF in cuttings (Gaspar and Hofinger, 1988). A high level of endogenous auxin has been correlated with the initiation of adventitious root primordia (Jarvis, 1986; Gaspar and Hofinger, 1988; Blakesley *et al.*, 1991b). Auxins comprise the group of chemicals which consistently enhance the rooting in naturally responsive, or so-called easy-to-root plant species (Jarvis, 1986). However, none of these authors considered auxin the sole determinant in the rooting of cuttings.

6.1.a.1 Exogenous Applications

For many years, the only evidence of the involvement of auxin in the initiation of adventitious roots came from studies which used exogenous applications (Blakesley *et al.*, 1991b). Jacobs (1939) demonstrated that *P. radiata* cuttings treated with a commercial rooting hormone (Hortomone A, 3100ppm naphthalene-acetic acid, NAA) rooted significantly earlier, had a better root system and with a slightly higher percentage (54%) than the control cuttings (38%). However, there was no significant difference in the height growth of hormone-treated and control cuttings in the plantation (Jacobs, 1939). Jacobs (1939) concluded that the effect of the hormone treatment was not marked enough to make it commercially viable in the nursery. Libby and Conkle (1966) found similar findings with *P. radiata* cuttings, where the application of indole-3-butyric acid (IBA) increased the rate of root formation, the final percentage rooting and the number of roots per rooted cutting. Contrary to these findings, Fielding (1954) demonstrated that growth-regulating substances did not improve the rooting of cuttings from older trees sufficiently to justify their use. However, later work by the same author demonstrated that 0.8% IBA dip increased the production of roots on young trees, but did not have a significant effect on the rooting percentage (Fielding, 1969). In conclusion, it appears that the application of exogenous auxin-like compounds to cuttings generally increased the rate of ARF and the number of roots produced per rooted cutting. However, the effect of auxin on the final rooting percentage of *P. radiata* cuttings still appeared to be confusing. In *P. radiata* hypocotyl cuttings, Smith and Thorpe (1975a) demonstrated that ARF may be inhibited if IBA was not present during the phase of root initiation and early root primordium growth. However, once the root primordium had developed, transferring the cuttings from IBA to water had no significant effect on ARF (Smith and Thorpe, 1975a).

6.1.a.2 Endogenous Levels

Although the importance of auxin in ARF is well documented, there are relatively few reports in the literature which have specifically investigated the changes in endogenous auxin concentration during ARF. Indole-3-acetic acid (IAA) appears to be the natural physiologically active auxin found in most plant genera, including *P. radiata* (Janick, 1986; Shepherd and Rowan, 1967). In *P. radiata* there has been no research which has specifically investigated the changes in endogenous auxin during ARF. There are various reports which have correlated the level of

endogenous auxin at the time of excision to the rooting success of cuttings (Boerjan *et al.*, 1995; Nanda and Anand, 1970). Contrary to these findings, there is some evidence which suggests that there is no correlation between endogenous auxin levels and the rooting of cuttings (Jenkins and Shepherd, 1974). Other researchers have attempted to investigate the seasonal rooting patterns and associated auxin levels with varying degrees of success (Jenkins and Shepherd, 1974; Nanda and Anand, 1970). For example, Smith and Wareing (1972) reported a decline in auxin levels in the stem of *Populus* x *robusta* cuttings taken over the growing season and correlated this with the decline in the rooting ability of cuttings during this time.

There is surprisingly little research that has investigated the changes in endogenous auxin levels during ARF. This knowledge has largely been limited by the need for sensitive and reliable methods of PGR concentration estimation. However, In *Phaseolus aureus* hypocotyl cuttings a sharp 'peak' of free IAA was detected in the first 10 hours following excision (Blakesley *et al.*, 1991b). The concentration of IAA in the root zone then declined to a level below that observed at the time of excision. It appears that the change in IAA concentration is localised to the region of ARF. This evidence is supported by Blakesley *et al.* (1991b) who reported very little change in the concentration of IAA in the remainder of the hypocotyl during ARF. The transient rise in free IAA in the root zone was observed to occur before the first detectable sign of root initiation, but fell prior to subsequent cellular divisions. A similar finding was reported by Label *et al.* (1989) during the rooting of *Prunus avium* explants, where the growth of the root primordium occurred only after the concentration of IAA had declined. Although there is good evidence of a 'peak' in endogenous auxin being associated with the onset of adventitious root initiation, other evidence does appear to be poorly correlated to the timing of morphological processes involved with root initiation (Blakesley *et al.*, 1991a).

There has been an attempt to explain the rooting success of cuttings based on their ratio of free to conjugated IAA (IAA esters) in the rooting zone of cuttings (Blakesley *et al.*, 1991a). For example, the levels of free IAA in the rooting zone of *Cotinus coggygria* cuttings taken in spring were higher than those taken in summer, where the rooting success of spring cuttings was high and that of the summer cuttings was low (Blakesley *et al.*, 1991a). However, the level of conjugated IAA was significantly higher in the summer cuttings. Consequently the ratio of free IAA to conjugated IAA varied markedly at the two times of the year. Although the

observations of Blakesley *et al.* (1991a) may be somewhat convincing, others have demonstrated little or no relationship between the ratio of free and conjugated IAA and the rooting success of cuttings(Gaspar and Hofinger, 1988).

In conclusion, the bulk of the literature has related auxin to the rooting of cuttings based on the response of exogenous applications. Unfortunately, there have been relatively few studies which have examined thoroughly the changes in endogenous auxin during ARF. From the few reports which exist it appears that there is a transient 'peak' or 'pulse' in endogenous auxin which has been correlated to the root initiation phase of adventitious root growth. Once root initiation has occurred, the level of endogenous auxin falls to a level below that at the time of excision for the remainder of ARF. Knowledge of auxin metabolism during ARF is limited and is an area which certainly deserves more attention.

6.1.b Ethylene

6.1.b.1 Exogenous Applications

The PGR ethylene is a gaseous molecule which is primarily know for its senescence related effects, including abscission and fruit ripening (Mudge, 1988). Ethylene has generally been demonstrated to have promotive effects on the initiation and subsequent development of root systems (Mudge, 1988). There are however no reports which have specifically investigated the effects of ethylene on the rooting of *P. radiata* cuttings, or any *Pinus* species. The effects of ethylene on ARF in various plant species has been reviewed previously (Mudge, 1988). The effects of ethylene on ARF appear to be mostly promotory, like auxin, although it does appear to depend on the plant species being investigated. Contrary to this evidence, the application of 1-aminocyclopropane-1-carboxylic acid (ACC, a precursor to ethylene synthesis) to pea cuttings resulted in increased in ethylene production and a reduction in rooting success (Nordstrom and Eliasson, 1993).

6.1.b.2 Endogenous Levels

There are only a few reports which have investigated the change in ethylene production during the process of ARF. The change in ethylene production as a result of severance is postulated to be the first biochemical event relating to the induction of adventitious roots (Moncousin *et al.*, 1989). For example, in poplar shoots raised

in vitro a peak in ethylene production was observed to occur 18 hours prior to the peak in endogenous auxin concentration (Hausman, 1993). Hausman (1993) concluded that the early peak in ethylene may be the first biochemical event associated with the rooting of poplar shoots. This result would appear surprising because there are many reports which have demonstrated that auxin may actually stimulate ethylene production (Mudge, 1988); Section II.6.1.g). In conclusion, the involvement of ethylene in the process of ARF may be linked to auxin, however, the evidence at present appears quite poor.

6.1.c Cytokinins

6.1.c.1 Exogenous Applications

The application of the exogenous cytokinin kinetin to *P. radiata* hypocotyl cuttings prior to root initiation resulted in the inhibition of ARF (Smith and Thorpe, 1975a). However, if kinetin was applied after root initiation had occurred no inhibitory effects were observed. This suggests that the prime effect of kinetin is in the inhibition of physiological events preceding the formation of the meristematic locus (Smith and Thorpe, 1975a). The effect of cytokinin applications on ARF in other plant species has been reviewed previously (Van Staden and Harty, 1988). In general, it appears that the exogenous application of cytokinin to cuttings usually results in either a slight promotion of ARF over the control or clear inhibition of ARF. There are rare examples where the application of exogenous cytokinin has resulted in the promotion of ARF (Meredith *et al.*, 1970). However, the level of promotion is generally much less than that observed for auxin and ethylene (Van Staden and Harty, 1988).

6.1.c.2 Endogenous Levels

There have been no studies which have specifically examined changes in the concentration of endogenous cytokinin in *P. radiata* cuttings. The changes in endogenous cytokinin in relation to ARF have been reviewed previously (Van Staden and Harty, 1988). There are no studies which have monitored the endogenous concentration of cytokinin in the base on the cutting during ARF, probably because they are not considered as important as auxin or ethylene. There are a limited number of studies which have attempted to correlate the level of cytokinin in the plant tissue to the rooting ability of the cuttings, although poor

results were obtained (see review by Van Staden and Harty, 1988). In conclusion cytokinins are almost universally accepted to be inhibitors of ARF. It then appears surprising that little research has been conducted to monitor the levels of endogenous cytokinin during the process of ARF.

6.1.d Gibberellins

6.1.d.1 Exogenous Applications

The application of exogenous gibberellin (GA_3) to *P. radiata* hypocotyl cuttings within the first four days following excision resulted in the inhibition of root meristemoid formation and therefore ARF (Smith and Thorpe, 1975a). However, there was a marked stimulation of rooting success by GA_3 if it was supplied to the base of the hypocotyl cutting during days 4 to 6, which coincides with the first observable histological events of ARF (Smith and Thorpe, 1975a). If GA_3 was applied after day 6 then further inhibition of ARF occurred. The inhibitory effect of GA_3 after day 6 is thought to be caused by retardation of root primordium growth. In many other plant species GA_3 has generally been demonstrated to inhibit ARF (Haissig, 1972; Hansen, 1976; Hansen, 1988). Although Hansen (1976) did demonstrated that low concentrations (10^{-7}M) of GA_3 could actually stimulate the rooting of pea cuttings if stock plants were grown under low levels of irradiance (16 Wm^{-2}). In general, it appears that the exogenous application of gibberellin to cuttings will cause inhibition of ARF, however this depends on the time of application. The strongest inhibition is usually obtained when gibberellin is applied during the early stages of ARF (Hansen, 1988).

The use of synthetic antigibberellins (chlormequat chloride and paclobutrazol) have been demonstrated to generally promote the rooting of cuttings (Davis and Sankhla, 1988). Antigibberellins inhibit the synthesis of endogenous gibberellins and reduce the level of endogenous gibberellin. The reduced gibberellin levels in the cuttings is thought to stimulate rooting.

6.1.d.2 Endogenous Levels

There have been no studies which have investigated the changes in endogenous gibberellins during ARF. This is surprising considering that gibberellins are known to strongly inhibit the rooting process. Research by Smith and Thorpe (1975a)

indicated that gibberellin may be required at certain stages of adventitious root development. The problem with investigating changes in endogenous gibberellins in plant tissues is that there are so many different structures of natural occurring gibberellins. At present, there are approximately 95 different structures of naturally occurring gibberellins known, with new structures being discovered on a regular basis (J. Ross pers. comm., 1996).

6.1.e Abscisic Acid

6.1.e.1 Exogenous Applications

The influence of exogenously supplied abscisic acid (ABA) on the rooting of *P. radiata* cuttings has not yet been investigated. The effects of exogenously applied ABA on the ARF of cuttings has been reviewed elsewhere (Davis and Sankhla, 1988; Jarvis, 1986). In general, it appears that ABA opposes the action of gibberellins and stimulates adventitious rooting (Davis and Sankhla, 1988). However, ABA may also influence other physiological factors within the cutting, including stomatal appeture on leaves and net rates of photosynthesis, which could also influence ARF in cuttings (Davis and Sankhla, 1988). There are examples in the literature which also demonstrate that the application of ABA to cuttings may actually inhibit, or not effect the rooting success of those cuttings (Davis and Sankhla, 1988). In general, the promotive effects of applied ABA on ARF have been too small and inconsistent to be of any commercial value (Davis and Sankhla, 1988).

6.1.e.2 Endogenous Levels

There have been no studies which have specifically examined changes in the concentration of endogenous ABA in *P. radiata* cuttings during the process of ARF. However, (Wilson *et al.*, 1972 cited by Cameron and Rook, 1974) found a rooting inhibitor in *P. radiata* bud extract taken during spring when the rooting of stem cuttings is the lowest. Rhododendron cuttings were demonstrated to root the best during the times in the year when the endogenous ABA concentrations in the stem were the highest (Wu and Barnes, 1981) . However, Blakesley *et al.* (1991a) demonstrated that it was not possible to correlate the rooting ability of *Cotinus coggygria* cuttings with the endogenous levels of ABA alone. It was necessary to compare it with the level of free IAA present in the tissue at the same time. In conclusion, the knowledge of ABA metabolism during ARF in cuttings is poorly understood and should be considered further.

6.1.f Recently Discovered Plant Growth Regulators

Recently, many other compounds which exhibit PGR properties have been discovered (Baraldi *et al.*, 1995; Biondi *et al.*, 1990; Martin-Tanguy and Carre, 1993; Sankhla and Upadhyaya, 1988). These compounds do not appear similar in structure to other PGR's, but when applied exogenously, cuttings may exhibit a growth response which is similar to that observed with other PGR's. There are no specific examples of these 'recently discovered' PGR's being used specifically on *P. radiata* cuttings. These compounds include jasmonic acid, polyamines and papaverine, and have been demonstrated to influence many aspects of plant growth and development, including ARF (Sankhla and Upadhyaya, 1988).

6.1.g Interactions Between Plant Growth Regulators

Many researchers have demonstrated that PGR's may interact with each other to produce a growth response. Most of these theories arise because of the poor correlation between PGR concentration and plant growth response. Research has demonstrated that particular hormones appear to oppose each other in there action, eg GA and ABA (Davis and Sankhla, 1988). Because no PGR studies have been conducted using *P. radiata* cuttings, no reports of interactions between PGR's in relation to the rooting have been demonstrated. Perhaps the first evidence that interactions between different PGR's occurred came from *in vitro* studies which demonstrated that the ratio of auxin to cytokinin in the culture medium determined the type of growth the plant would display. For example if there was a high ratio of auxin to cytokinin the plant would produce roots. However, if there was a low ratio of auxin to cytokinin then the plant would continue shoot growth, and no roots would be produced (Bhojwani *et al.*, 1986; Bhojwani and Razdan, 1983). Although high auxin to cytokinin ratios may promote root formation in many *in vitro* systems, there are certainly many cases where this does not apply in the rooting of stem cuttings (Van Staden and Harty, 1988).

Other examples of interactions between other PGR's also exist. For example, the application of ABA has been demonstrated to partially overcome the gibberellin induced inhibition of ARF (Davis and Sankhla, 1988). Therefore, high ratios of ABA to GA would appear to be stimulatory to ARF. Blakesley *et al.* (1991a) demonstrated that the rooting success of *Cotinus coggygria* stem cuttings was high when the ratio of endogenous ABA to IAA was low. There have been many reports

which have demonstrated that an interaction between auxin and ethylene is involved in the rooting of cuttings (Hausman, 1993; Moncousin *et al.*, 1989; Riov and Yang, 1989). In pea cuttings Nordstrom and Eliasson (1993) demonstrated that ethylene inhibits the basipetal transport of endogenous auxin to the basal region of the cutting, thereby inhibiting ARF. In poplar shoots growth *in vitro* Hausman (1993) postulated that ethylene produced prior to the peak in endogenous auxin may have stimulated the auxin production. There are many other examples of plant growth regulators possibly interacting to cause a change in growth response. It highlights the fact that no single PGR is solely responsible for the process of ARF. In studies using exogenous applications of PGR's on *P. radiata* hypocotyl cuttings, Smith and Thorpe, (1975a) demonstrated the importance of different growth regulators at different stages/times of ARF.

6.2 Carbohydrates

The importance of carbohydrate metabolism in relation to ARF in cuttings has been reviewed previously (Jarvis, 1986; Veierskov, 1988). However, no specific investigation has examined the carbohydrate metabolism of *P. radiata* cuttings in relation to their rooting success. The growth and development of an adventitious root system is an energy demanding process. Cameron and Rook (1974) demonstrated that the rate of respiration in *P. radiata* cuttings was 40 percent higher during ARF than prior to severance. The initial carbohydrate content of the cutting must be sufficient to supply the cutting with energy reserves throughout the rooting period (Veierskov, 1988). The carbon building blocks which are used in the growth of the new adventitious root system are usually supplied by a carbohydrate source. Therefore carbohydrate levels and metabolism would appear crucial to the successful development of an adventitious root system.

6.2.a Stock Plant Carbohydrate Status and ARF

In a previous section of this thesis (Section II.2.2), stock plant growth conditions were reviewed as factors having a significant influence on the ARF process. A high carbohydrate content in the stock plant has generally been associated with a high rooting potential and increased root numbers, eg pea cuttings (Veierskov, 1988) However a high carbohydrate content has not always been associated with a high rooting potential (Veierskov, 1988). For example in *Pinus sylvestris* , Hansen *et al.* (1978) observed a negative correlation between

carbohydrate content of stock plants and the subsequent rooting of cuttings. A low stock plant carbohydrate content has been demonstrated to be responsible for the reduction of rooting potential in leafless hardwood cuttings and etiolated stock plants (Veierskov, 1988). This suggests that the carbohydrate content of stock plants has some effect on the rooting ability of cuttings, at least under some environmental conditions. In general, the literature suggests that the carbohydrate content of stock plants may not have a regulatory role in the rooting of cuttings, but it is apparent that the level of carbohydrate in the stock plant must be sufficient to support the growth and development of the new adventitious root system.

6.2.b Carbohydrate Metabolism During ARF

In general, it appears that soluble carbohydrates accumulate in the bases of cuttings until root emergence occurs (Haissig, 1982b; Jarvis, 1986; Veierskov *et al.*, 1982) . However, Haissig (1982b) and Veierskov *et al.* (1982) have reported that initially a decrease in the carbohydrate content of cuttings occurs during the first few days of propagation, which is rapidly followed by a net accumulation of carbohydrates until adventitious root emergence. The initial decrease in carbohydrate content was thought to be caused by reduced rates of net photosynthesis as a result of stomatal closure (Loach, 1988). Then once root initiation has occurred then the cutting appears to rapidly accumulate soluble sugars (Haissig, 1982b; Veierskov *et al.*, 1982). The formation of a root primordium may influence the carbohydrate source-sink relationships within the cutting to favour the accumulation in the basal region. The application of exogenous auxin has been demonstrated to increase the rate of soluble carbohydrate accumulation in the basal region of the cutting (Haissig, 1982b; Veierskov and Andersen, 1982). This suggested that another role of auxin in ARF may be to alter the carbohydrate source-sink relationships within the cutting to favour assimilate supply to the developing root system.

6.2.c Exogenous Applications of Carbohydrates

There have been a number of studies which have investigated the effects of applying exogenous sugars, alone, or in combination with an auxin, to cuttings in an attempt to enhance ARF (Eliasson, 1978; Haissig, 1986; Veierskov, 1988; Wiesman and Lavee, 1995). Eliasson (1978) applied a 1% sucrose solution to pea cuttings and doubled the mean numbers of roots produced by each cutting. This demonstrated

that the endogenous carbohydrate content may be a limiting factor during the rooting of pea cuttings. In olive cuttings the application of sucrose and IBA together significantly enhanced the rooting response over IBA alone (Wiesman and Lavee, 1995). ARF is usually suppressed where high concentrations of carbohydrates have been applied exogenously to cuttings (Veierskov, 1988). Unfortunately, there are a number of problems with applying sugars exogenously. Firstly, cuttings take up and translocate the exogenous carbohydrates from solutions primarily via the xylem, unlike endogenous carbohydrates which are primarily transported in the phloem (Haissig, 1986). Secondly, apart from their role as a carbon source, exogenously applied sugars can exert strong osmotic effects, possibly even toxic effects on plant cells (Veierskov, 1988). Generally, it appears that exogenously applied carbohydrates will promote rooting to a certain degree, however, not as much as applied auxin. The effects of applied carbohydrates is highly dependent on the concentration used, too high and toxic effects are observed.

6.3 Enzyme Activities

The activities of enzymes in the rooting zone provide a further insight into the metabolic activities which occur during the process of ARF (Bhattacharya, 1988). The requirement of oxygen during rooting has been adequately demonstrated by using uncouplers or inhibitors of oxidative phosphorylation, which inhibit ARF (Bhattacharya, 1988). There have been no studies which have examined the activity of various metabolic enzymes during the process of ARF in *P. radiata* cuttings. However, the activities of various enzymes have been examined and reviewed in many other plant species (Bhattacharya, 1988; Haissig, 1982a; Haissig, 1986). The are many different enzyme systems which operate simultaneously during ARF. However, the main enzymes thought to influence ARF are the oxidase and hydrolytic enzymes.

6.3.a Oxidases

The level of physiologically active IAA is stringently controlled by many mechanisms, including biosynthesis, conjugation and catabolism. The catabolism of endogenous IAA has been associated with the activity of the enzyme IAA-Oxidase (IAAox), which may be responsible for regulating the concentration of free-IAA during ARF (Bhattacharya, 1988). Bansal and Nanda (1981) demonstrated that the IAAox activity was the highest in cuttings which rooted well and the lowest in those

cuttings which rooted poorly. The increased IAAox activity has been associated with higher levels of endogenous auxin during ARF (Bhattacharya, 1988). Although high levels of IAAox have been associated with high levels of endogenous auxin, IAAox is actually responsible for the catabolism of IAA, and has no direct influence on the ARF process. In fact to maintain a high concentration of endogenous IAA one would expect the activity of IAAox to be maintained low, not high. There are reports of other oxidase enzyme systems, eg polyphenol oxidase (PPO), being associated with the seasonal rooting of M26 apple cuttings (Bassuk *et al.*, 1981).

6.3.b Hydrolytic Enzymes

The high level of soluble carbohydrates observed in the basal region of cuttings during the early stages of ARF has been associated with a high amylase activity (Bhattacharya, 1988). It has been demonstrated that there is a good correlation between the disappearance of starch and the increased activity of amylase (Bhattacharya, 1988). The seasonal variation in rooting response was correlated well with the amylase activity at different times in the season (Nanda and Anand, 1970). They reported high amylase activity during April-August when the rooting of cuttings were high, but decreased levels of activity in October when the rooting was poor (Nanda and Anand, 1970). However, there are other examples where the amylase activity did not correlate well with the rooting success of cuttings (Bhattacharya, 1988).

6.4 Nucleic Acid and Protein Metabolism

The influence of nucleic acid and protein metabolism has not been investigated in *P. radiata* cuttings. However, nucleic acid and protein metabolism during ARF has been reviewed previously (Haissig, 1986). In cuttings, the cells which divide to form the adventitious root system need to synthesise DNA, RNA and proteins. The need for nucleic acid synthesis during ARF has been demonstrated by the positive effects of applied purine and pyrimidine bases (Haissig, 1986). Bhattacharya and Nanda (1978) cited by (Haissig, 1986) demonstrated that, in the presence of IAA and sucrose, that the treatment of mung bean cuttings with adenine, guanine, cytosine and thymine stimulated rooting. Treatment of cuttings with auxin has been demonstrated to stimulate nucleic acid and protein metabolism during ARF (Haissig, 1974). Inhibitors of nucleic acid or protein metabolism have been demonstrated to generally inhibit ARF in cuttings (Haissig, 1986).

II.7 Summary and Rationale for Experimental Work

The influence of various environmental and stock plant related effects on the success of ARF in *P. radiata* are fairly well understood. However, the understanding of the underlying mechanisms controlling ARF in the cuttings is poorly understood. Two factors identified in the literature which appeared to influence the rooting success of cuttings the greatest are stock plant age and season of cutting collection. The rooting success of cuttings collected from older (mature) stock plants is much less than those collected from younger (juvenile) stock plants. Also, the time in the season when cuttings are excised from the stock plants appeared to be important in determining the rooting success of cuttings. Although genetic differences in rooting ability may exist, this study does not aim to examine them, only acknowledge and account for them by sampling cuttings from a large number of stock plants of seedling origin.

In Tasmania, there has been no experimental work conducted on the propagation of *Pinus radiata* by stem cuttings. Therefore, it would appear necessary to establish field trials which examine the influence of stock plant age and season of cutting collection on the rooting of *P. radiata* cuttings. It would be possible to conduct controlled environment studies to determine whether environmental factors or internal anatomical and/or physiological factors are responsible for the variation in rooting percentage. Also, the understanding of growth processes associated with ARF is essential in directing and focusing future research.

There appears to be limited knowledge of physiological and metabolic processes which occur during ARF in *P.radiata* cuttings. Most of the knowledge about many physiological and metabolic processes which occur during ARF appears to be associated with other plant species, and a need exists to further the knowledge base in these important areas. Most of the knowledge associated with the involvement of PGR's in ARF has been gained by exogenous application studies. Where natural changes in PGR's have been monitored during ARF, interesting results were observed. In *P. radiata*, the monitoring of PGR concentrations at the time of excision and during ARF may indicate if PGR's are involved in ARF. In the

literature, the metabolism of carbohydrates during ARF was considered to be an important area of research. However, in *P. radiata* no research has been conducted into the metabolism of carbohydrates during ARF. This is an area which warrants further investigation.

In a cutting, the natural pathway of water uptake (roots) have been removed. Although the existing root system has been removed, a cutting must still maintain adequate inter and intra-cellular water relations during ARF. The maintenance of adequate water relations during ARF would appear to be essential to the rooting success of cuttings. In *P. radiata*, little research has been conducted into the water relations of cuttings during ARF. This is an area which requires further investigation.

It is also important to correlated changes in biochemical or physiological events with anatomical and/or morphological events which occur during ARF. The time frame of certain cytological and morphological events should be assessed to better understand and interpret any biochemical and/or physiological changes occurring during different stages of ARF and development.

III. General Materials and Methods

III. General Materials and Methods

1. Cutting Collection

P. radiata cuttings with a stem length of 10cm and a basal diameter of 3-7mm were collected from stock plants of seedling origin using hand secateurs. Five cuttings per stock plant were collected from the tips of west facing second whorl branches. Cuttings were collected at or just prior to dawn and stored in cool styrofoam containers containing approximately 200ml of water prior to setting under field or phytotron conditions.

2. Propagation Conditions

2.1 Field Trials

Field propagated cuttings were set into 1.2m wide raised nursery beds at Meadowbank, which is located in the Derwent Valley of Southern Tasmania, Australia (Coordinates 42°38'S, 146°52'E, Elevation 50m). The Meadowbank soil is a floodplain and is classified as a deep brown clay loam (Dimmock, 1961). The yearly environmental data for Bushy Park (7km N.N.W. of Meadowbank) is shown in Appendix 8.

Cuttings were set 5cm deep into the 1.2m wide raised nursery beds at the recommended commercial spacing, six rows per bed and 7.5cm between cuttings in a row. A 'dibbler' was used to make the holes in the raised nursery beds at the correct spacing for setting. Cuttings were 'misted' for 3-5 minutes every hour with a timer controlled solenoid valve irrigation system. The time was increased to 5 minutes during the warmer summer months.

2.2 Phytotron Trials

Cuttings were propagated in a Biosystems® 8000 growth cabinet (phytotron) at the Horticultural Research Centre, University of Tasmania, Hobart, Australia. Conditions inside the phytotron were maintained at 20°C (+/- 0.5°C), 14 hours photoperiod (45μmol.m^{-2}.s^{-1} with fluorescent lights) and a relative humidity maintained at 90% (+/- 5%).

The cuttings were set in root trainer cells (PIM Plastics Pty. Ltd.; 5cm x 5cm x 12cm) at a depth of 5cm (half the length of the cutting) in the rooting medium. The rooting medium consisted of a 1:1:1 (v/v) mixture of peat, perlite and vermiculite containing Osmocote® (5g/L), Dolomite (5g/L) and Limil (0.5g/L). The rooting media was steam sterilised 24 hours prior to its use. The root trainer cells were placed into specialised holding racks (PIM Plastics Pty. Ltd.) which hold 20 root trainer cells. Supplementary watering was provided by automated drippers on capillary mats, plus daily watering by hand. Pathogens were controlled using Kocide® and Sumisclex® applied at alternating two week intervals.

3. Observation Methods

3.1 Rooting Percentage

The rooting percentage was determined at a predetermined date by carefully removing cuttings from the soil or rooting media and recording those cuttings with roots of any length and those without any roots. The rooting percentage was calculated by dividing the number of cuttings in the treatment with roots by the total number of cuttings in the treatment.

3.2 Stage of Root Growth

A semi-quantitative scale of root growth (SRG) was devised to assess adventitious root growth and development in cuttings. The scale ranged from 0 (no roots) to 6 (branched fibrous root system). The SRG was as follows (also see Plates 1-7);

Scale of Root Growth (SRG)	**Morphological Description**
0	no growth
1	callus present but no root initials
2	root initials visible on callus
3	roots 1-5mm in length
4	roots >5mm in length
5	branching evident on roots
6	fully fibrous root system

Plate 1 - A cutting at SRG=0, no growth (Bar=10mm).

Plate 2 - A cutting at SRG=1, callus present but no root initials (Bar=10mm).

Plate 3 - A cutting at SRG=2, root initials visible on callus (Bar=10mm).

Plate 4 - A cutting at SRG=3, roots 1-5mm in length (Bar=10mm).

Plate 5 - A cutting at SRG=4, roots greater than 5mm in length (Bar=10mm).

Plate 6 - A cutting at SRG=5, branching evident on roots (Bar=20mm).

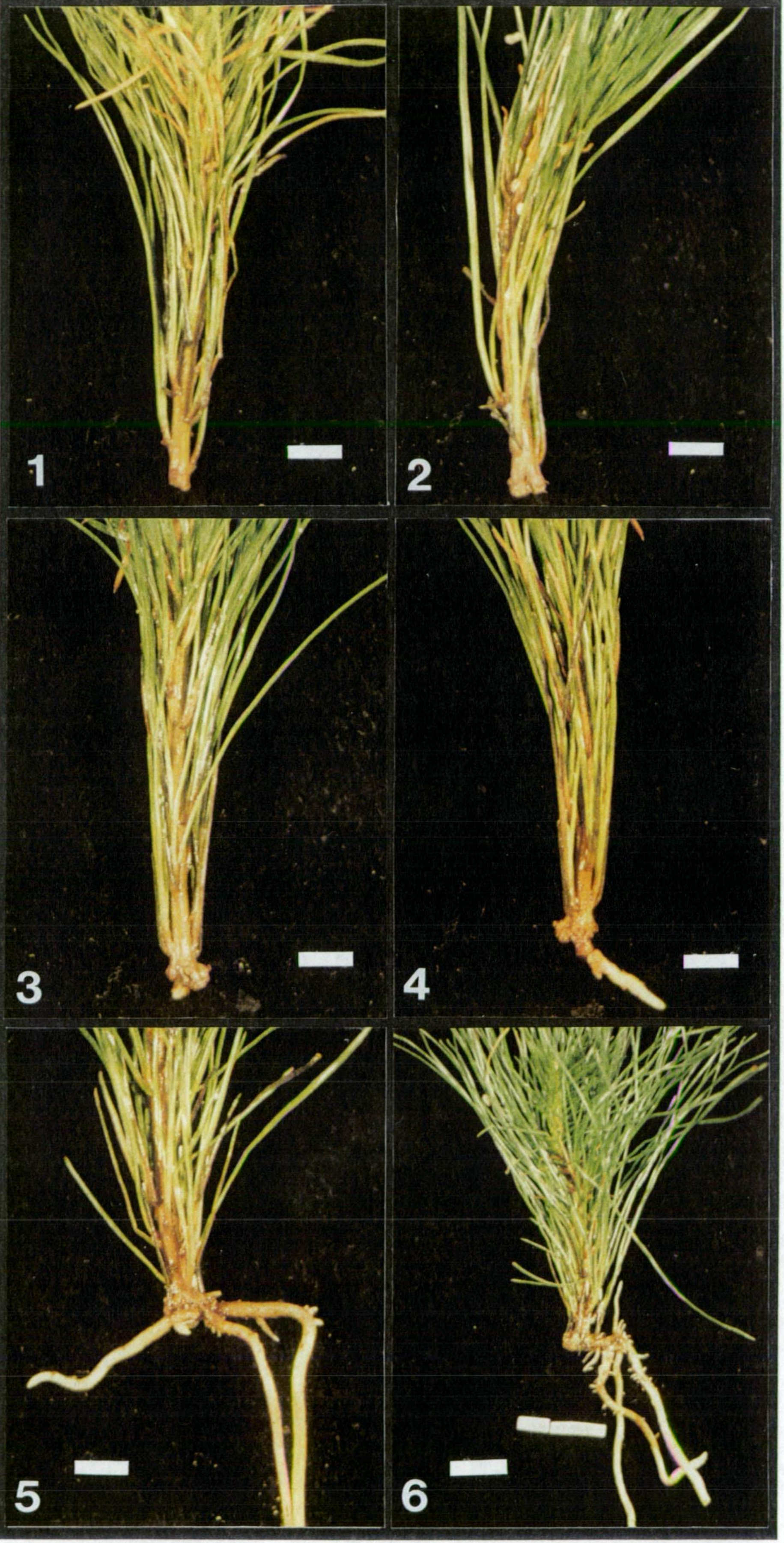

1
2
3
4
5
6

Plate 7 - A cutting at SRG=6, a fully fibrous root system (Bar=45mm). This photo only illustrates the stage of root growth, not necessarily the root distribution observed in other cuttings.

3.3 Callus and Top Growth

The callus diameter, shoot length, callus growth (CG) and top growth (TG) were all measured using vernier callipers. Callus growth was calculated by subtracting the original basal stem diameter from the callus diameter. Top growth was calculated by subtracting the initial cutting stem length (100mm) from the cutting stem length at the time of sampling.

4. Consumable Materials

4.1 Solvents and Reagents

4.1.a Solvents

Methanol	HPLC grade, obtained from Mallinckrodt (UN No. 1155)
Ethanol	AR grade, obtained from Rhone Poulenc Chemicals Pty. Ltd (UN No. 1170)
Ether	HPLC grade, obtained from Mallinckrodt (UN No. 1155)
Xylene	AR grade, obtained from BDH Chemicals Pty. Ltd. (UN No. 1307)

4.1.b Reagents

All chemicals and reagents were obtained from Sigma-Aldrich Pty. Ltd. unless stated otherwise.

5. Apparatus

Balances	Mettler Instruments, Switzerland: 4 decimal places, model AE200 Mettler Instruments, Switzerland: 2 decimal places, model PM4600
Distilled Water	Distilled in laboratory with LABGLASS unit, Brisbane, Australia
pH Meter	Radiometer, Copenhagen, type PHM 26

Centrifuge Beckman J2-21M/E; Beckman Instruments Australia Pty. Ltd.

Freeze Drier Dynavac High Vacuum Pty. Ltd., Melbourne, Australia Freeze drying unit, model FD 16

Pressure Chamber 1 litre chamber volume, made locally in Hobart

6. Tissue Sampling

6.1 Tissue Collection

Stem and leaf tissue required for plant growth regulator (PGR) or carbohydrate analysis were collected using hand secateurs from the tips of west facing first whorl branches. Care was taken to collect samples at approximately the same time of the day (dawn) in all trials to limit diurnal variation in PGR and carbohydrate concentrations. All samples collected were placed into labelled 6mL plastic screw-cap scintillation vials (Pico Prias, Canberra Packard Pty. Ltd.) and immediately placed into a dewar containing liquid nitrogen to prevent degradation and transferred back to the laboratory for freeze-drying.

6.2 Tissue Freeze-Drying and Storage

The vials containing the plant tissue collected from the field or phytotron were removed from the liquid nitrogen using forceps. The cap was removed and the vial containing the plant tissue transferred quickly to the freeze drier (Section III.5). The plant tissue was freeze-dried under a vacuum of 1.333^{-7}MPa at -80°C for a period of 48 hours. At this point, all tissue was found to be fully dehydrated, as determined by gravimetric analysis in initial studies. The vacuum was released and the lids replaced on vials and stored in sealed plastic bags at -20°C.

6.3 Tissue Grinding

Freeze-dried plant material was ground into a fine powder using a stainless steel mortar (15cm diameter) and a 19cm long teflon pestle. The freeze-dried plant tissue was placed into the mortar, snap frozen using liquid nitrogen and ground carefully with the teflon pestle. After grinding, the plant material was transferred back into the labelled 6ml vials using a small brush and stored at -20°C.

7. Radioimmunoassay Technique

7.1 Extraction and Purification of Plant Growth Regulators

The ground plant material (0.25g dry mass) was extracted with 10ml 80% methanol (MeOH) (containing 20mgL^{-1} butylated hydroxytoluene) by shaking overnight at 4°C. The extract was centrifuged in teflon centrifuge tubes at 20,000 rpm for 12 minutes and the pellet rinsed with a further 10ml 80% MeOH and recentrifuged. The supernatants were combined and the organic phase removed in a Speed-Vac concentrator (Savant, USA). Ammonium acetate (0.01M, 10ml) was added to the remaining aqueous phase and the extract purified using the method described by Roberts *et al.* (1991).

Briefly, the extract was purified through a series of connected columns consisting of polyvinylpolypyrrolidone (PVPP, Sigma P-6755; 5ml bed volume, with fines removed by decantation in distilled water), DEAE Sephadex (Sigma A-25-120; 3ml bed volume), and a SepPak C_{18} cartridge. The two former columns were conditioned with 20ml 1.0M ammonium acetate followed by 25ml 0.01M ammonium acetate, and the later with 5ml 100% MeOH and 5ml 0.01M ammonium acetate. The extract was passed through the columns, followed by an additional 30ml 0.01M ammonium acetate.

The SepPak cartridge containing the cytokinins was removed and rinsed with 5ml distilled water. The cytokinins were then eluted with 5ml 60% MeOH. The PVPP column was detached from the Sephadex column and a fresh SepPak C_{18} cartridge (rinsed with 5ml 100% MeOH, and 5ml 1% acetic acid) placed beneath the Sephadex column. The acidic PGR's were eluted from the Sephadex with 15ml 1.0M acetic acid. The SepPak cartridge containing the acidic PGR's was removed and rinsed with 5ml distilled water, and the acidic PGR's eluted from the SepPak with 5ml 80% MeOH. The 80% MeOH wash was found to elute all the gibberellin (GA), abscisic acid (ABA) and indole-3-acetic acid (IAA).

The extraction and purification procedure separated the free from the bound and conjugated forms of the acidic PGR's, but did not separate the cytokinin bases from their respective ribosides (Hofman, unpublished data).

The cytokinin and acidic PGR fractions were evaporated to dryness, redissolved in MeOH and an aliquot equivalent to 0.025g dry mass plant material dispensed in triplicate into polypropylene test tubes (12 x 55mm). All acidic PGR's were methylated with ethereal diazomethane and the organic solvents removed under vacuum. The samples were stored at -18°C until analysed.

The percentage recovery of each PGR was calculated by adding known amounts of tritiated PGR (in triplicate) to the plant material prior to overnight extraction. Following the normal extraction and purification procedure subsamples were taken in triplicate (each equivalent to 0.025g dry mass plant material) for counting. The percentage recovery for ABA, GA, IAA, ZR and DHZR was 97.8, 97.5, 77.5, 95.2 and 99.9 respectively.

7.2 Radioimmunoassay

The PGR's were quantified with previously developed and characterised radioimmunoassays (Cutting *et al.*, 1983; Cutting *et al.*, 1986; Hofman *et al.*, 1985; Hofman *et al.*, 1986 and Hofman, 1990). Standards were made in MeOH and the required amounts dispensed into polypropylene test tubes. The MeOH was removed and the standards and samples dissolved in 0.1ml of radiolabelled tracer (containing approximately 30, 000 dpm) in phosphate-buffered saline (PBS pH 7.4) containing 0.1% gelatine (150 Bloom Bovine-BDH Chemicals). [1,2(n)-^{3}H] gibberellin A_1, 3-[5(n)-^{3}H] indolylacetic acid and DL-*cis, trans-* [G-^{3}H] abscisic acid were purchased from Amersham International Pty Ltd, while tritiated Z, DHZ were produced by Dr N.J. Roberts. Antibody (0.1ml, diluted to provide 30-50% binding) and 0.25ml of 0.1% bovine serum (or phosphate-buffered saline for the ABA assay) were then added, the mixture vortexed, and incubated at 37 °C for 30 minutes. Ammonium sulphate (0.75ml, 90% saturated) was added to precipitate the bound fraction and the suspension was centrifuged for 15 minutes at 7, 000 rpm. The pellet was rinsed with 1ml 50% ammonium sulphate, re-centrifuged and the supernatant decanted. The pellet was re-dissolved in 0.25ml distilled water, 2.5ml scintillant (Packard Optifluor) added, the test tubes capped and shaken, and the radioactivity determined using a Beckman LS 5801 scintillation counter.

Polyclonal antibodies were used in this study and as such each assay was not specific to the PGR being examined. Each of the cytokinin antibodies displayed significant cross-reactions with corresponding ribosides. Thus the Z assays

determined the combined concentrations of zeatin and ribosylzeatin, the DHZ assay detected dihydrozeatin and dihydroribosylzeatin. Both the IAA and ABA antibodies showed no significant cross-reactions with other naturally occurring PGR's while the GA antibody was used to assay the combined concentrations of GA_1 and GA_3 with a lesser cross-reaction to GA_{20}.

7.3 Validation of Immunoassays

The need to validate all PGR immunoassays for each type of plant material sampled is described by Pengelly (1985). Two procedures for checking on the presence or absence of specific and non-specific inhibitors were used in this study. Stem segments taken 10cm below the growing apex were analysed for each hormone to be assayed, using internal standard and extract dilution techniques for validating the immunoassays. The internal standard technique involves adding a constant amount of plant material extract to each point of the standard curve, and counting this standard curve as an unknown using the previously described RIA procedure. The amount of PGR detected is then plotted against the amount of PGR added (ie. the standard curve), with slopes differing from additivity indicating the presence of an inhibitor(s) of PGR binding. Similarly, the extract dilution technique involves assaying a range of extract volumes or weights, and plotting extract volume or weight against the amount of PGR found. If the plot is linear and shows additivity, the extract is free of specific inhibitors (except for the possibility of inhibitors with the same affinity characteristics to the antibody as the desired PGR displays). Direct comparison of samples with analytical methods, such as GC-MS, would provide unequivocal evidence of chemical purity, but such methods were not available during this study.

The results obtained through the two validation steps discussed above indicated the absence of inhibitors in the extracts, but the possibility exists that a competitive inhibitor with the same affinity constant as the desired PGR may be present (Pengelly, 1985). The extract dilution curves showed additivity in levels of all PGR's between 0ng and 25ng of plant material extract.

8. Measurement of Plant Water Potential using the Pressure Chamber

The water potential of intact plants and cuttings were measured using the pressure chamber technique (Cleary & Zaerr, 1980; Scholander *et al.*, 1964;

Scholander *et al.*, 1965; Spomer, 1985; Turner, 1981). Individual needles were severed from an intact tree or from the mid-stem section of a cutting using a sharp scalpel blade (No. 10). The needles were rapidly transferred to the pressure chamber apparatus (Section III.5) and inserted into the neoprene seal so that the cut end of the needle projected outside the chamber. The pressure in the chamber was gradually increased using compressed air at a rate of 0.025MPa.s^{-1}(Turner, 1981). The pressure inside the chamber was recorded when the sap just returned to the severed ends of the xylem vessels (Turner, 1981). A stereo dissecting microscope was used to observe the sap exudation from the xylem vessels. The within sample variation in measurement, using the pressure chamber technique, was demonstrated to be +/- 0.025MPa.

9. Statistics and Computing

The percentage rooting data obtained from experiments did not require any transformation, because the range of percentages was mostly between 20 and 90% (Steel and Torrie, 1960). The analysis of variance (ANOVA) table was calculated using Systat® v5.2 for MacIntosh. Where the p value was significant, a least significant difference (LSD) was calculated at the 5% probability level and used to determine treatment differences. Other data requiring statistical analysis was analysed using Systat® v5.2.

All means and standard errors of the mean were calculated in Microsoft Excel v4.0, graphs were produced using Cricket Graph III and Microsoft Word v5.1 was the word processing package used in the compilation of this thesis.

IV. Results

IV.1 Effect of Parent Stock Plant Age, Time in Year of Cutting Collection and Cutting Size on the Growth and Rooting of *Pinus radiata* Cuttings

1.1 Introduction

Radiata pine (*Pinus radiata* D. Don) plantations have traditionally been established using seedlings as planting material. Only within the past decade has clonal propagation using rooted cuttings been adopted in large scale commercial operations (Ritchie, 1991). The major problem associated with the propagation of *P. radiata* by cuttings is the loss of rooting ability with plant ageing and the seasonal variation in the percentage of cuttings forming roots.

The rooting potential of *P. radiata* cuttings declines with increased age of the parent stock plant (Fielding, 1954; Fielding, 1969; Jacobs, 1939; Thulin and Faulds, 1968). Jacobs (1939) demonstrated that the rooting success of cuttings collected from trees less than five years old was significantly greater than cuttings from nine year old trees. Fielding (1954) reported data from field experiments in which success in rooting fell from 88.5% for cuttings collected from 3 year old stock plants to 68% at age 5, and 11% at age 26 years. However, Thulin and Faulds (1968) demonstrated a high rooting percentage in cuttings collected from 9 year old trees (100%) and a relatively small reduction in cuttings collected from 20 year old trees (70%). Fielding (1969) indicated that environmental differences may account for the inconsistencies in the reports. The decline in rooting percentage associated with increased age of the parent stock plant can be arrested by hedging. Hedged trees of seedling origin remained physiologically juvenile in comparison to unhedged trees. Cuttings removed from hedged trees root more readily than cuttings collected from unhedged trees of the same age (Fielding, 1954).

Most plant genera display an inherent seasonal trend in the rooting ability of cuttings collected from stock plants (Hartmann and Kester, 1983). The rooting success of *P. radiata* cuttings in Australia and New Zealand has been demonstrated to decline markedly between winter and spring (Fielding, 1954; Fielding, 1969; Jacobs, 1939). Fielding (1954) reported data from experiments in Canberra

(Australia) where rooting percentage of June (winter) set cuttings fell from 82% to 60% for September (early-spring) set cuttings, and 10% for November (mid-spring) set cuttings. Fielding (1954) concluded that the worst time in the year to collect cuttings was when the branchlets were growing rapidly in spring. However Menzies *et al.* (1988) demonstrated that it was possible to root small fascicle cuttings in a glasshouse throughout most of the year. Jenkins and Shepherd (1974) indicated that the concentration of IAA and ABA at time of excision may influence the rooting success of *P. radiata* cuttings at different times in the year. The rooting ability of *P. radiata* cuttings under Tasmanian conditions at different times in the year has not been investigated. While several researchers have demonstrated that a seasonal trend in rooting percentage exists with cuttings of *P. radiata,* no one has identified whether seasonal differences are due to environmental conditions experienced after excision in the nursery or due to a physiological and/or anatomical condition of the cutting at the time of excision.

The influence of cutting length and diameter on the percentage rooting of *P. radiata* cuttings was investigated previously by Fielding (1969). Fielding (1969) concluded that different shoot thickness and lengths *per se* had no significant effect on the percentage rooting, but a large effect on the nursery growth of cuttings. However, Martin *et al.* (1995) demonstrated that *P. radiata* cuttings 13cm in length rooted better than those which were 5cm in length. The diameter of the cutting did not appear to influence the rooting success (Martin *et al.*, 1995). Grace and Farrar (1945) cited by Girouard (1974) examined spruce (*Picea abies*) cuttings of three different lengths (4-8cm, 8-15cm and 15-24cm) and concluded that long cuttings form the greatest number of roots and shoots per rooted cutting, plus the most upright plants four years after rooting, but the 8-15cm cuttings had the highest percentage rooting. Conflicting reports about the effect of cutting length and diameter on the rooting of *P. radiata* stem cuttings highlights the need for further research. Also, the measurement of other growth parameters may provide further insights into the mechanism(s) controlling ARF.

The rooting potential of *P. radiata* cuttings is affected by both stock plant age and season of collection. A number of studies have demonstrated that these two factors have an important influence on the rooting percentage, however, their interaction has yet to be examined. The aim of the present study is to document the interaction of stock plant age and season of collection on the rooting ability of *P. radiata* cuttings grown under Tasmanian conditions. These results will form the

basis of a more detailed examination of the physiological processes occurring during adventitious root formation in *P. radiata.* The examination of cutting size characteristics would clear any ambiguity which exists in relation to the rooting of *P. radiata* cuttings and may provide an insight into the mechanism controlling ARF.

1.2 Materials and Methods

1.2.a Stock Plant Age and Timing of Collection Experiment

1.2.a.1 Experimental Design

In 1993, a trial was established to investigate the seasonality of rooting success and loss of rooting ability associated with plant ageing in cuttings of *P. radiata.* Samples of cuttings were removed at monthly intervals between June and November (30/6, 28/7, 25/8, 23/9, 20/10 and 23/11) from stock plants 1, 2, 4 or 8 years old located in close proximity to each other at Westerway and National Park, Tasmania (42°38S, 146°52E Elevation 50m). (Plates 8-11) Cuttings were either placed in soil or potting media under field or controlled environment (phytotron) conditions respectively. In the field, different aged cuttings were set in randomly preallocated positions utilising a split-plot design with four replicates of 80 cuttings per replicate. In the phytotron, aged 1 material was set randomly in preallocated positions with 4 replicates of 40 cuttings per replicate.

1.2.a.2 Cutting Material and Propagation Conditions

Five cuttings per stock plant were collected from the tips of west facing second whorl branches. Cuttings were collected at dawn and stored in cool styrofoam containers containing 200ml of water prior to setting under field or phytotron conditions (Section III.1). Cuttings were set under both field and phytotron conditions (Section III.2.1 and III.2.2 respectively).

1.2.a.3 Measurements

At time of cutting collection, basal stem samples were collected for PGR analysis (Section III.7). Each sample consisted of a 40mm stem segment which was collected from the base of randomly selected cuttings. A total of 10 replicates were collected from each different stock plant age and snap frozen in liquid nitrogen prior

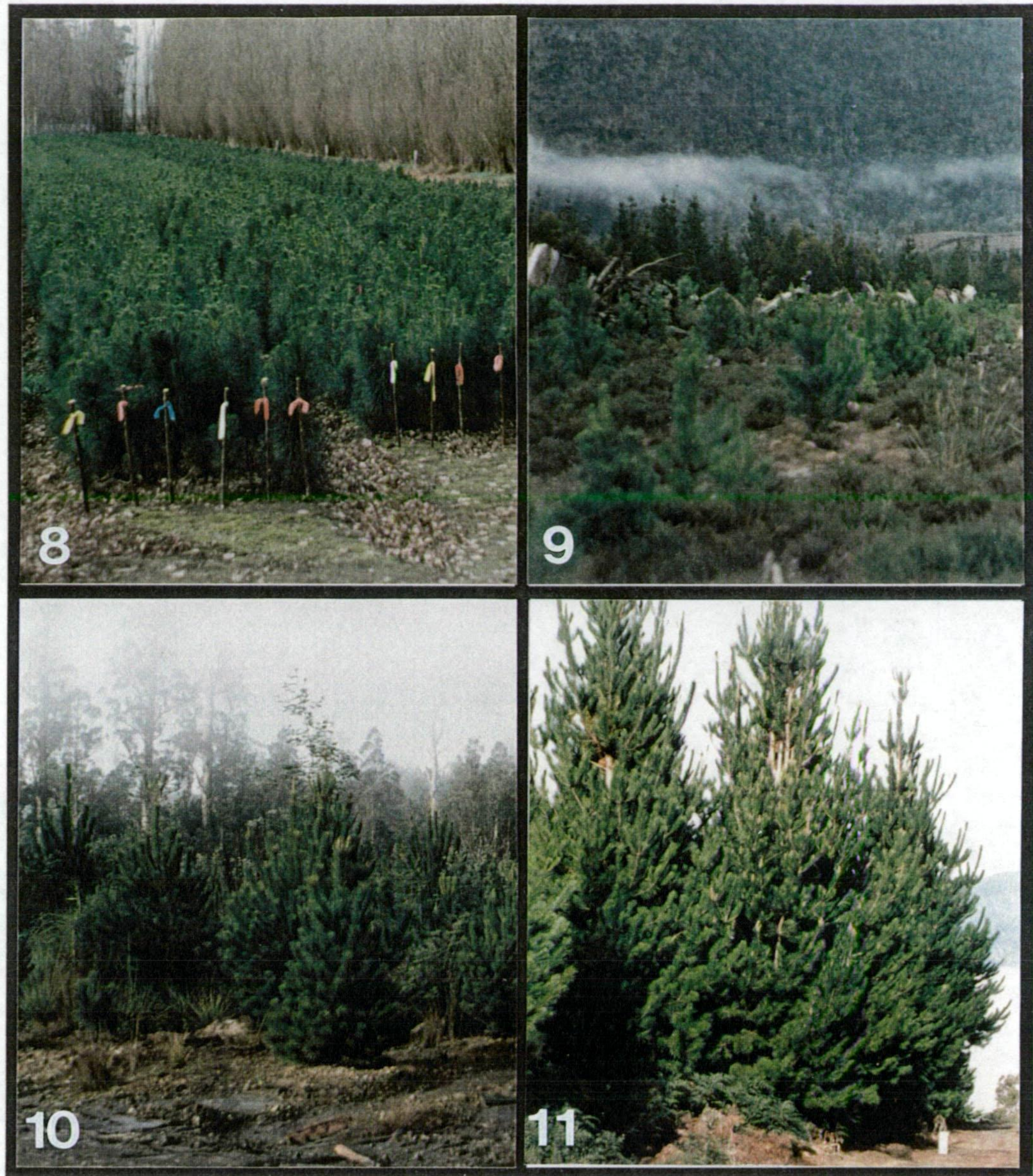


Plate 8 - One year old stock plants of located at Westerway (tree height=0.8-1.2m)

Plate 9 - Two year old stock plants located at Westerway (tree height=1.5-2.5m)

Plate 10 - Four year old stock plants located at National Park (tree height=3-4m)

Plate 11 - Eight year old stock plants located at National Park (tree height=9-15m)

to being freeze dried and stored at -20 °C until analysis (Section III.6). The concentration of indole-3-acetic acid (IAA), abscisic acid (ABA), zeatin riboside (ZR), dihydrozeatin riboside (DHZR) and gibberellin (GA) was determined using the radioimmunoassay technique (Section III.7).

At monthly intervals after setting, three random samples per replicate were collected for observational analysis. The scale of root growth (SRG), callus growth (CG) and top growth (TG) were recorded for each sample (Section III.3). The mean and standard error and LSD at t =0.05 were calculated for each sample date. The experiment was terminated on 9/3/94 and the rooting percentage determined. By this date, all cuttings which survived had formed a root system. Counts were made in every plot and averaged to give means and variances. Analysis of variance was conducted in Systat v5.2 and comparisons between means were made using least significant difference (LSD) test at t =0.05, calculated manually from the analysis of variance table.

1.2.b Length and Diameter Experiment

1.2.b.1 Experimental Design

An experiment was designed to investigate the effect of cutting stem length and basal stem diameter on the growth and rooting success of *P. radiata* cuttings in the field. A factorial experiment, with 3 replicates, was designed to examine three different cutting stem lengths (5cm, 10cm and 15cm) and three different cutting basal stem diameters (2-4mm, 4-6mm and 6-8mm). Cuttings were set in the field on 23 / 7 / 93 at Meadowbank, Tasmania (Section III.2.1). The experiment was terminated on the 9/3/94 and the rooting percentage, callus growth, top growth, root number, top growth dry weight and root dry weight recorded. Analysis of variance was conducted in Systat v5.2 and comparisons between means were made using least significant difference (LSD) test at t =0.05, calculated manually from the analysis of variance table.

1.3 Results

1.3.a Stock Plant Age and Timing of Collection Experiment

1.3.a.1 Effect on Rooting and Growth of Cuttings

The foliage characteristics differed markedly between cuttings collected from

the different aged stock plants. A high proportion of primary needles to secondary fascicle leaves were observed in cuttings removed from juvenile stock plants (age 1 and 2). In contrast to this primary needles were absent from the more mature foliage. In cuttings collected from 8 year old stock plants the secondary fascicle leaves were generally longer and thicker than those observed in cuttings collected from 4 year old stock plants.

The results presented indicate that both the age of the stock plant and the time of the year that cuttings were collected significantly affected the rooting potential of *P. radiata* cuttings under field conditions in Tasmania (Figure IV.1.1).

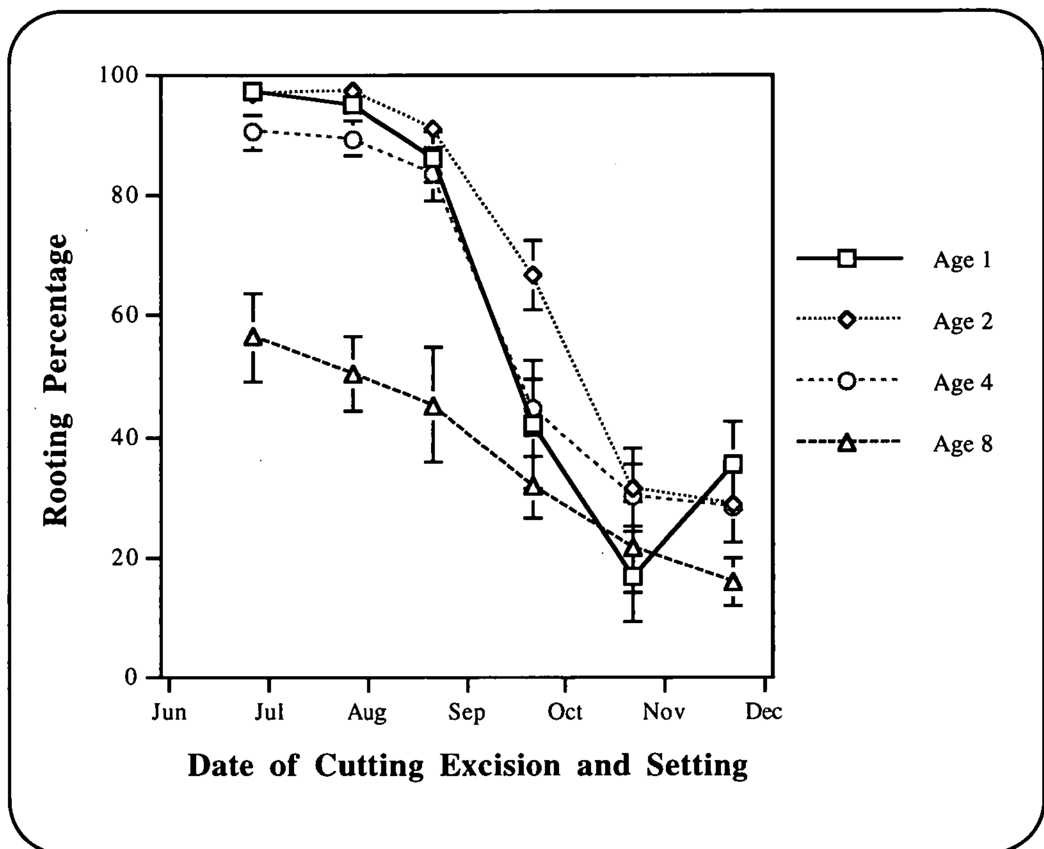


Figure IV.1.1 - The rooting percentage of field set cuttings collected from 1, 2, 4 and 8 year old stock plants at six different times in the year. The rooting percentage was recorded on the 9/3/94. The means and standard errors were calculated for (n=4) replicates. The $LSD_{0.05}$= 15.89 (data obtained from Appendix 1).

Overall, the ability of cuttings to form roots was observed to decline with increased age of the parent stock plant (Figure IV.1.1). The rooting success of cuttings collected from 8 year old stock plants between June and August was significantly (p=0.05) less than that of cuttings collected from 1, 2 or 4 year old stock plants at the same time. However in October and November no significant differences were observed in the rooting percentage of cuttings collected from stock plants of all ages. The variation observed in the rooting percentage at different times in the season varies significantly (p=0.05) with each age of cuttings sampled. This demonstrates the interactive effect of both stock plant age and time of setting upon the rooting percentage of *P. radiata* cuttings under Tasmanian field conditions.

A significant reduction in the rooting percentage of *P. radiata* cuttings was observed in cuttings collected from stock plants of all ages between June and November. A high rooting percentage (>80%) was observed in cuttings collected from 1, 2 and 4 year old stock plants between June and August, when stock plants are in a dormant state. The most significant (p=0.05) decline in rooting percentage was observed between August and October in cuttings collected from different aged stock plants. This reduction in rooting percentage was observed to occur at approximately the same time as the stock plant commenced shoot growth in spring (September). The magnitude of decline in rooting percentage differed depending on the age of the stock plants from which the cuttings were collected (Figure IV.1.1).

During the development of adventitious roots, the stage of root growth was recorded at monthly intervals after setting. Of those cuttings which survived to form an adventitious root system, cuttings collected from juvenile stock plants tended to initiate root growth earlier than cuttings collected from older stock plants (Figure IV.1.2.a). The rate of adventitious root development after initiation was generally higher in cuttings collected from younger stock plants compared to cuttings collected from older stock plants (Figure IV.1.2.a). A similar trend in initiation time and rate of adventitious root development was observed in cuttings set in July, August, September, October and November (see Appendices 3, 4, 5, 6, 7). In all cases, cuttings collected from younger stock plants initiated adventitious root growth earlier than cuttings collected from older stock plants.

Cuttings collected from 1 and 2 year old stock plants were observed to initiate callus growth significantly (p=0.05) earlier than cuttings collected from 8 year old stock plants (Figure IV.1.2.b). However once callus growth was initiated the growth

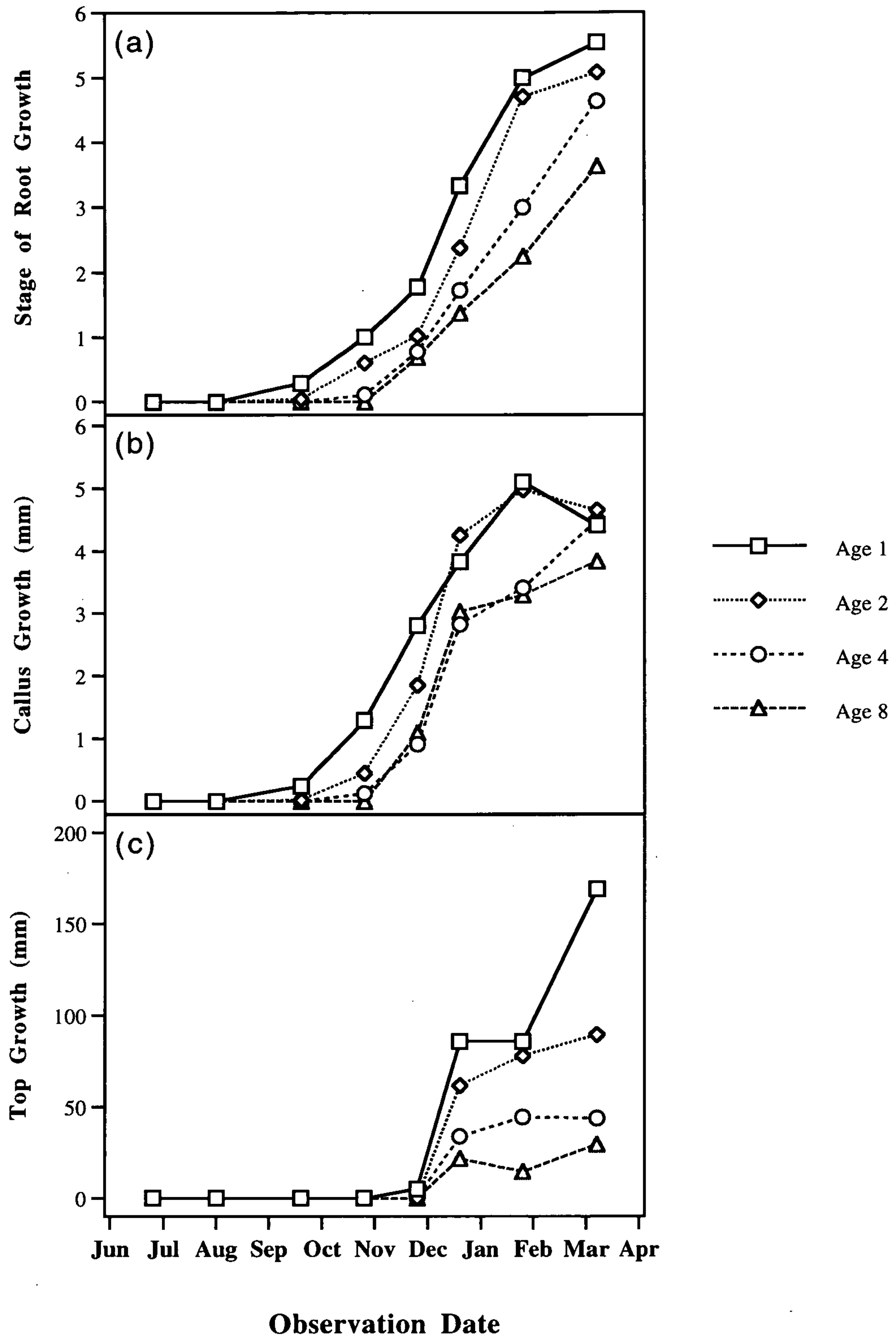


Figure IV.1.2 - (**a**) Average stage of adventitious root growth (0-6); (**b**) average amount of callus growth (mm) and (**c**) average amount of top growth (mm) of *P. radiata* cuttings collected from 1, 2, 4 and 8 year old stock plants, set on June 30th 1993 and monitored at monthly intervals after setting. Means calculated from (n=12) replicates (mean data, standard errors and LSD's included in Appendix 2).

rate appeared similar between cuttings collected from all ages of stock plants. A similar trend in initiation time of callus growth was observed in cuttings set in July, August, September, October and November (see Appendices 3, 4, 5, 6, 7). In all cases cuttings collected from 1 and 2 year old stock plants initiated callus growth earlier than cuttings collected from 8 year old stock plants.

Of those cuttings which formed adventitious roots, callus formation was observed to precede root development in cuttings collected from all ages of stock plants. In cuttings collected from 1, 2 and 4 year old stock plants callus was evenly distributed radially around the base of the cutting. In cuttings collected from 8 year old stock plants callus tissue tended to be unevenly distributed around the basal region of the cutting. No visible signs of callus growth were observed in the basal stem region of cuttings that did not root.

Cutting top growth was composed of new stem and leaf tissue which emerged from the apical region and initially was lighter in colour when compared with existing foliage. Top growth in cuttings occurred only after callus had formed in the basal region of the cutting (Figure IV.1.2.c). Of those cuttings that formed adventitious roots, the rate of top growth in cuttings collected from 1 and 2 year old stock plants was significantly ($p=0.05$) greater than that observed in cuttings collected from 4 and 8 year old stock plants (Figure IV.1.2.c). A similar trend was observed in cuttings set at all other times in the year (see Appendices 3, 4, 5, 6, 7). In all cases, cuttings collected from younger stock plants generally produced more top growth than cuttings collected from older stock plants.

1.3.a.2 Changes in Endogenous Plant Growth Regulators

Auxin

The concentration of IAA in the basal stem region of *P. radiata* cuttings increased significantly between June and December in cuttings collected from 1 and 2 year old stock plants (Figure IV.1.3.a). However, the concentration of IAA did not change significantly in cuttings collected from 4 and 8 year old stock plants at different times in the year (Figure IV.1.3.a). The poor relationship between the basal stem concentration of IAA and the rooting percentage indicated that the concentration of IAA, at the time of excision, may not be a major determinant or indicator of rooting ability in *P. radiata* cuttings.

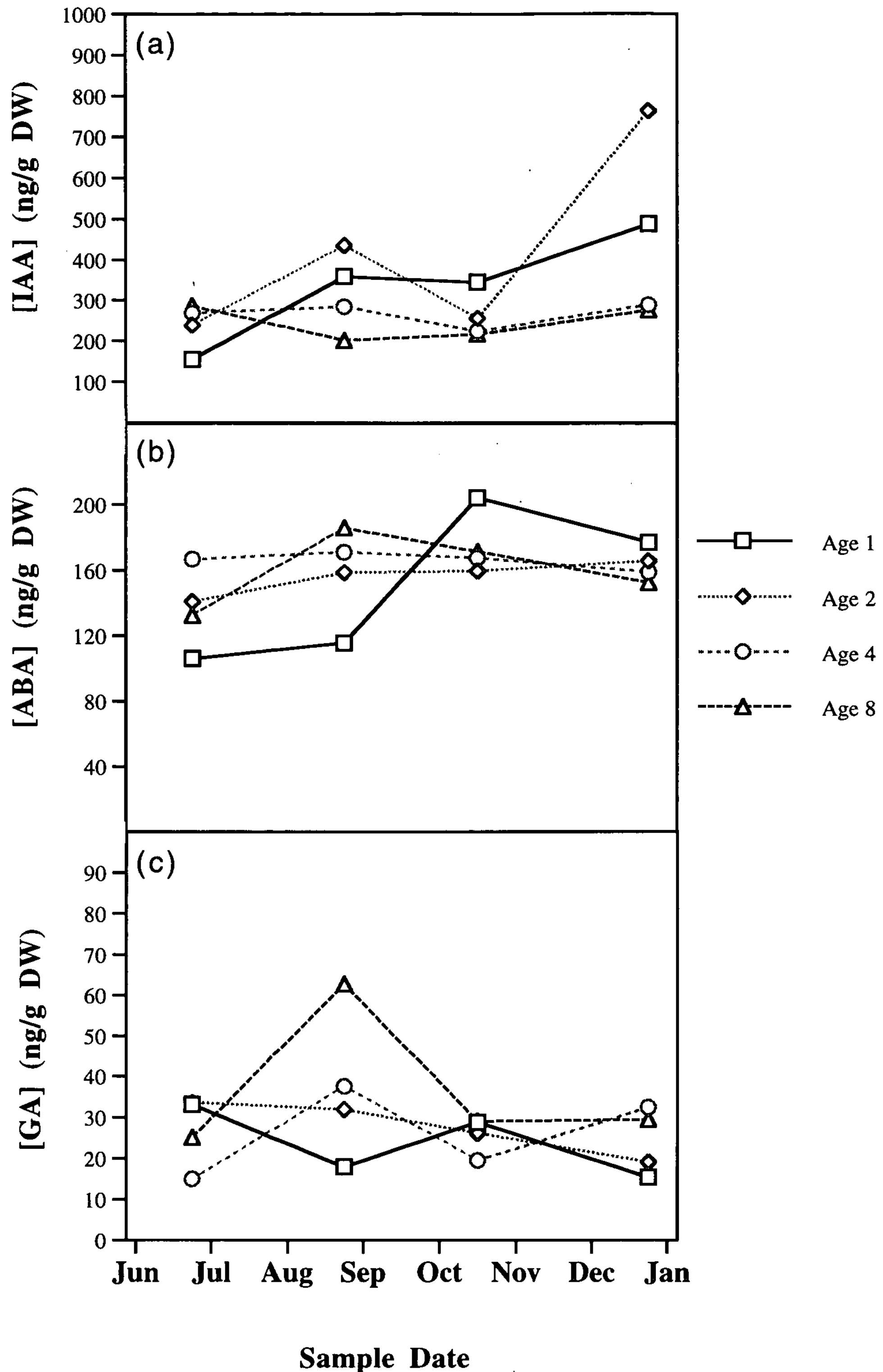


Figure IV.1.3 - The concentration of PGR's in the basal stem region of *P. radiata* cuttings collected from 1, 2, 4 and 8 year old stock plants at different times in the year; **(a)** IAA ($LSD_{0.05}$=112.99), **(b)** ABA ($LSD_{0.05}$=23.46) and **(c)** GA ($LSD_{0.05}$=8.68). Samples were collected at the time of cutting excision. The means were calculated from (n=10) replicates (mean data, standard errors and LSD's are included in Appendices 9 and 10).

Abscisic acid

In cuttings collected from 1 year old stock plants, the concentration of ABA in the basal stem region of *P. radiata* cuttings increased significantly from 106 ng/g DW in June to 204 ng/g DW in October before decreasing significantly to 177 ng/g DW in December (Figure IV.1.3.b). Based on these results, the concentration of ABA appeared to be inversely related to the rooting of cuttings which were collected from 1 year old stock plants. However, the change in ABA concentration, which was observed in cuttings collected from 2, 4 and 8 year old stock plants, did not appear to be related to the rooting of *P. radiata* cuttings (Figure IV.1.3.b). The poor relationship between the basal stem ABA concentration and the rooting percentage of cuttings collected from 2, 4 and 8 year old stock plants, indicated that the concentration of ABA at the time of cutting excision may not be a major determinant or indicator of rooting ability in *P. radiata* cuttings.

Gibberellins

In general, there was a poor relationship between the concentration of GA's in the basal stem region of cuttings at the time of excision and the final rooting percentage (Figure IV.1.3.c). This indicated that the concentration of GA at the time of excision may not be a major determinant or indicator of rooting ability in *P. radiata* cuttings.

Zeatin riboside

In cuttings collected from 1 year old stock plants, the concentration of ZR in the basal stem region of *P. radiata* cuttings decreased significantly from 45.45 ng/g DW in June to 11.67 ng/g DW in October before increasing significantly to 35.98 ng/g DW in December (Figure IV.1.4.a). Based on these results the concentration of ZR appeared to be related to the rooting percentage of cuttings. However, a poor relationship between the basal stem concentration of ZR and the rooting percentage was observed in cuttings collected from 2 and 4 year old stock plants (Figure IV.1.4.a). This poor relationship between the concentration of ZR and the rooting percentage indicated that the concentration of ZR at the time of cutting excision may not be a major determinant or indicator of rooting ability in *P. radiata* cuttings.

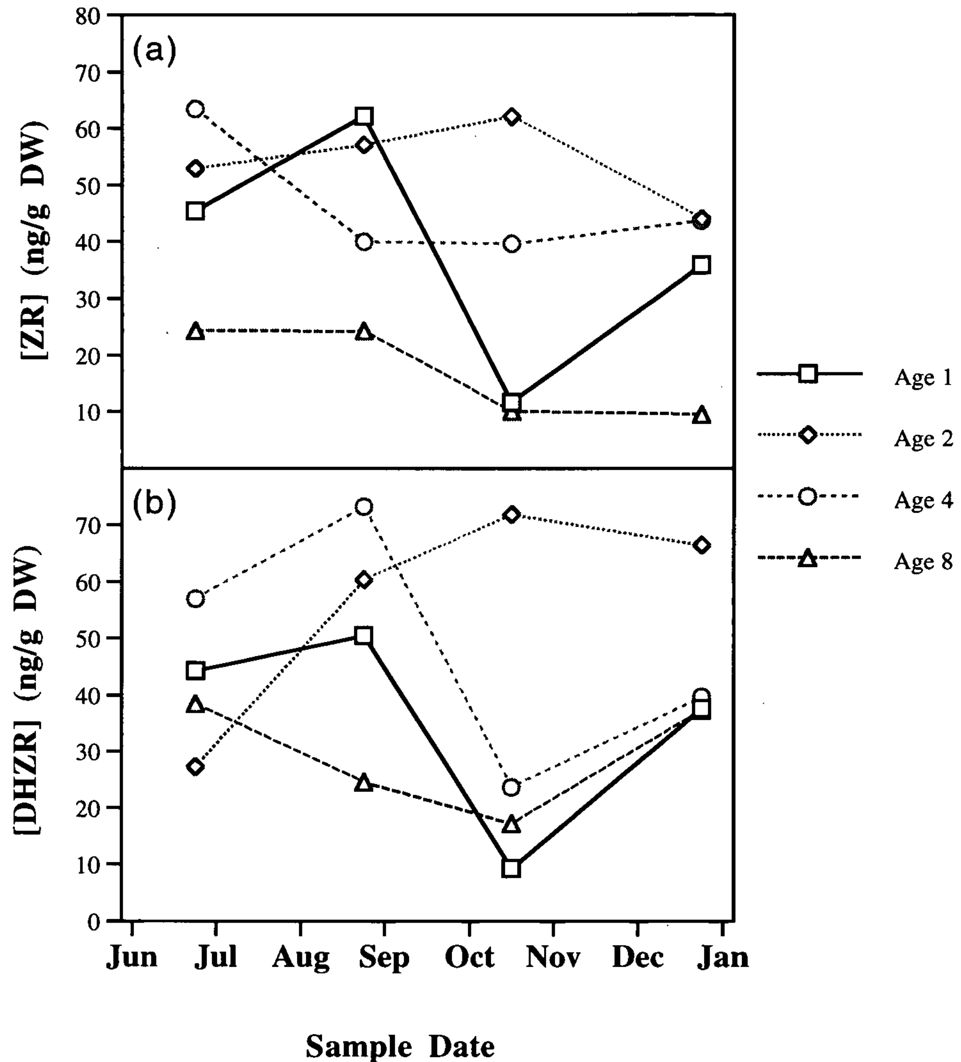


Figure IV.1.4 - The concentration of **(a)** ZR ($LSD_{0.05}$=17.00) and **(b)** DHZR ($LSD_{0.05}$=19.55) in the basal stem region of *P. radiata* cuttings collected from 1, 2, 4 and 8 year old stock plants at different times in the year. Samples were collected at the time of excision. The means were calculated from (n=10) replicates (mean data, standard errors and LSD's are included in Appendices 10 and 11).

Dihydrozeatin riboside

In cuttings collected from 1 year old stock plants, the concentration of DHZR in the basal stem region of *P. radiata* cuttings decreased significantly from 44.29 ng/g DW in June to 9.3 ng/g DW in October before increasing significantly to 37.65 ng/g DW in December (Figure IV.1.4.b). A similar trend was observed in cuttings collected from 4 and 8 year old stock plants (Figure IV.1.4.b). Based on these results, the concentration of DHZR appeared to be related to the rooting percentage of *P. radiata* cuttings. However, a poor relationship between the basal stem concentration of DHZR and the rooting percentage was observed in cuttings collected from 2 year old stock plants (Figure IV.1.4.b). This poor relationship between the concentration of ZR and the rooting percentage indicated that the concentration of ZR may not be a major determinant or indicator of rooting ability in *P. radiata* cuttings.

PGR Interactions

There did not appear to be any relationship between the ratio of different PGR's measured and the rooting of *P. radiata* cuttings. The only evidence of an interaction occurring between PGR's was observed in cuttings collected from one year old stock plants, where a high concentration of ABA and a low concentration of cytokinin (ZR and DHZR) was observed in October when the rooting percentage of cuttings was the lowest (Figures IV.1.3.b, IV.1.4.a and IV.1.4.b). However, inconsistencies in the PGR data for cuttings collected from 2, 4 and 8 year old stock plants lead to the general conclusion that the concentration of PGR's at the time of excision may not be a major determinant in the ARF of *P. radiata* cuttings.

1.3.b Controlled Environment Studies

1.3.b.1 Effect on Rooting and Growth of Cuttings

Cuttings collected from 1 year old stock plants and set under constant environmental conditions, in the phytotron, displayed a similar trend in rooting percentage as those cuttings collected from 1 year old stock plants and set under field conditions (Figure IV.1.5). Cuttings which were set in the phytotron initiated adventitious roots earlier and developed faster than those cuttings set under field conditions (comparison of data in Appendix 2 (a) and Appendix 12 (b)). In the

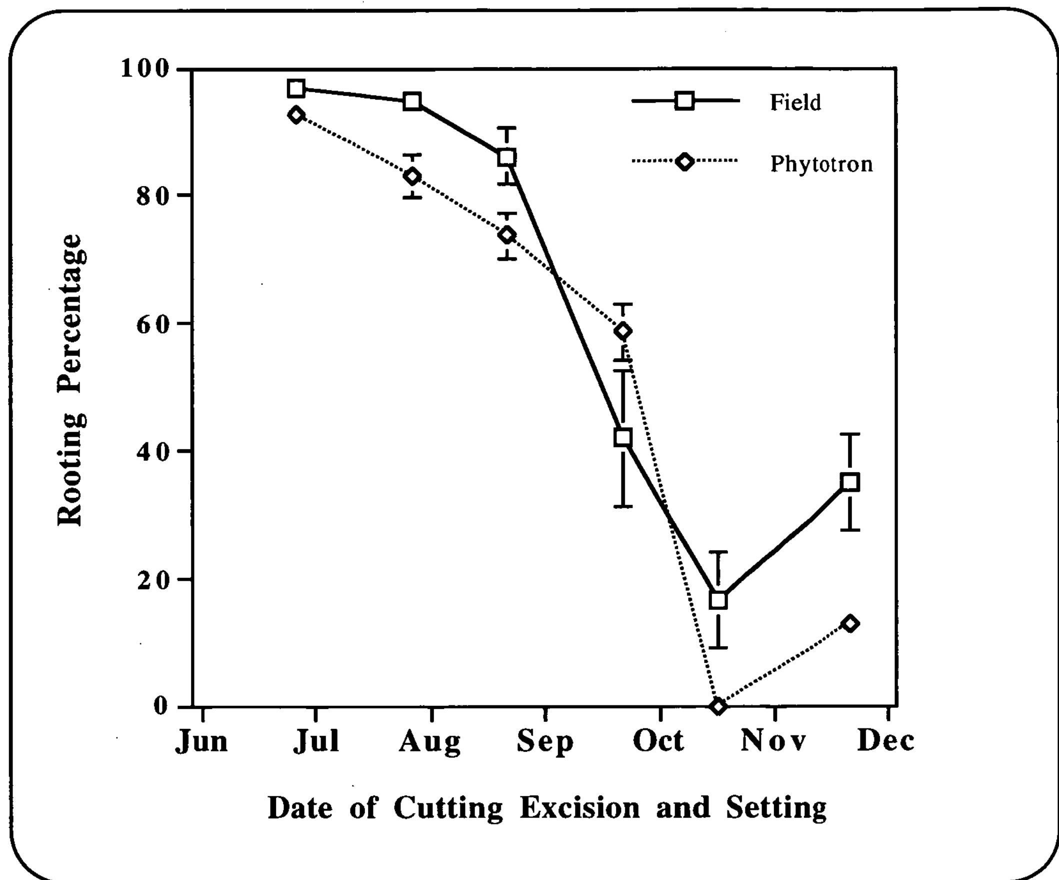


Figure IV.1.5 - A comparison of rooting percentage data for cuttings collected from 1 year old stock plants at different times in the year and set under both field and phytotron conditions. The rooting percentage was recorded on the 9/3/94. The means and standard errors were calculated for (n=4) replicates (data included in Appendices 1 and 12.a). No statistical comparison could be made between different environments because there was no replication of environments.

phytotron, cuttings reached SRG=1 (callus) by approximately 60-80 days after excision from the stock plant. Root initials were usually visible on the callus tissue between approximately 60-110 days after excision. The examination of cuttings which failed to survive in the phytotron revealed that no callus tissue was present in the basal region. A similar observation was noted in field set cuttings which failed to survive. It appeared that the development of callus tissue was an important first step in the development of adventitious roots in *P.radiata* cuttings.

The rooting ability of *P. radiata* cuttings appears only partially dependent on the environmental conditions experienced during the adventitious root formation period. The trend in rooting percentage of cuttings collected from 1 year old stock plants was similar under both field and phytotron conditions (Figure IV.1.5). No significant (p=0.05) change in rooting percentage was observed between June and August set cuttings under both field and phytotron conditions. In both the field and the phytotron, the most significant (p=0.05) reduction in rooting percentage occurs between August and October set cuttings. In both the field and the phytotron, the lowest rooting percentage was observed in October set cuttings.

1.3.c Effect of Cutting Length and Diameter

1.3.c.1 Effect on Rooting Percentage

The rooting percentage of *P. radiata* cuttings collected from 1 year old stock plants was significantly influenced by reducing the length of cuttings (Figure IV.1.6.a). The rooting percentage of 5cm cuttings (67.7%) was significantly less than cuttings which were 10 and 15cm in length (95.9 and 99.7% respectively). However, there was no significant difference in the rooting percentage of cuttings which were 10 and 15cm in length. The basal diameter of the cutting did not have a significant influence on the rooting percentage (Figure IV.6.a). This indicated that the length of the cutting is an important factor which may influence the survival of cuttings in the field.

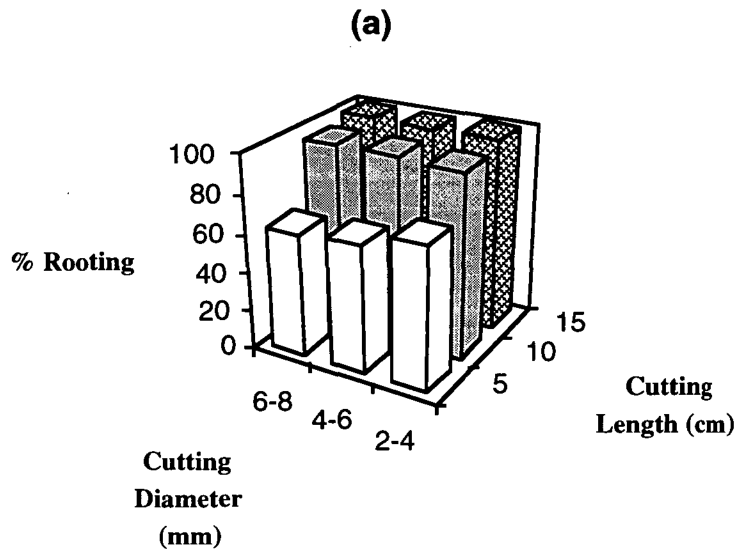
1.3.c.2 Effect on Cutting Growth

The amount of callus growth (callus diameter minus stem diameter) a cutting produced was significantly influenced by the length of the cutting (Figure IV.1.6.b). The callus growth of 5cm cuttings (3.84mm) was significantly less than that of both 10 and 15cm long cuttings (5.51 and 5.95mm respectively). However, there was no significant difference between 10 and 15cm in the amount of callus growth that was produced (Figure IV.1.6.b). The diameter of the cutting did not have a significant influence on the callus growth of the cuttings.

The amount of top growth a cutting produced was significantly influenced by the length of the cutting (Figure IV.1.6.c). The top growth of 5cm cuttings (11.61cm) was significantly less than that of both 10 and 15cm long cuttings (12.65

Figure IV.1.6 - The effect of cutting stem length and basal stem diameter on **(a)** mean percentage rooting ($LSD_{0.05}$=6.35), **(b)** mean callus growth (mm) ($LSD_{0.05}$=0.62) and **(c)** mean top growth (cm) ($LSD_{0.05}$=2.39) (means and standard errors included in Appendix 13).

(a)



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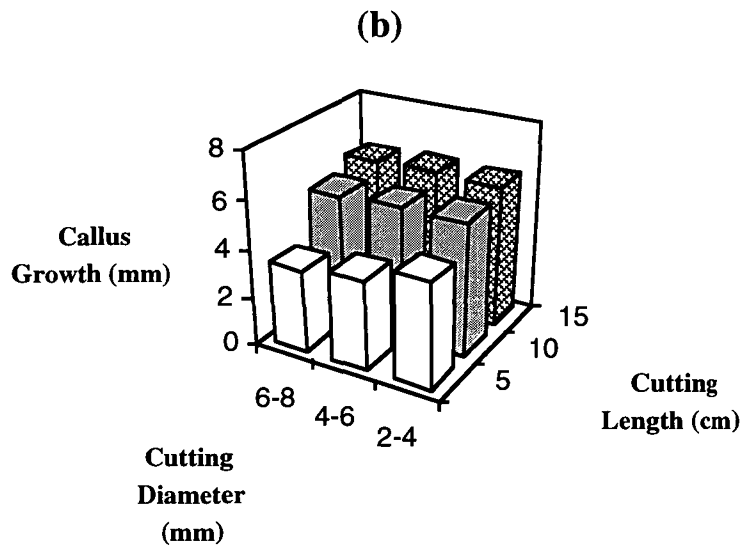
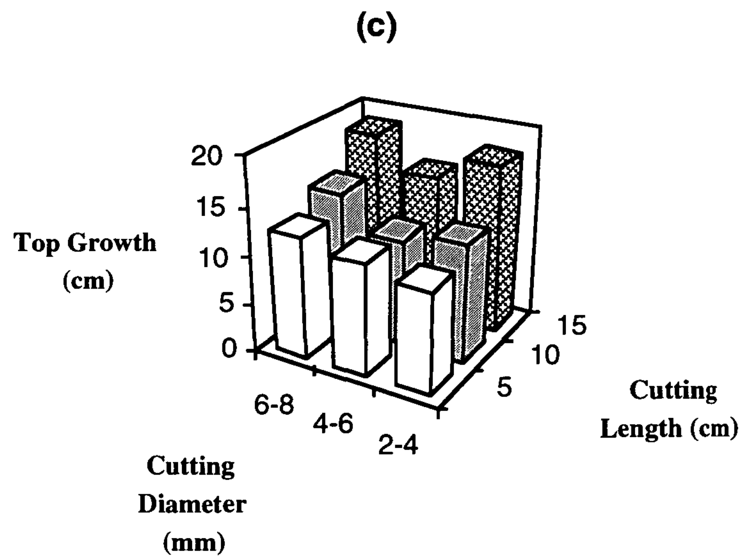


(c)



and 17.00cm respectively). However, there was no significant difference between 10 and 15cm in the amount of top growth that was produced (Figure IV.1.6.c). The diameter of the cutting did not have a significant influence on the top growth of the cuttings.

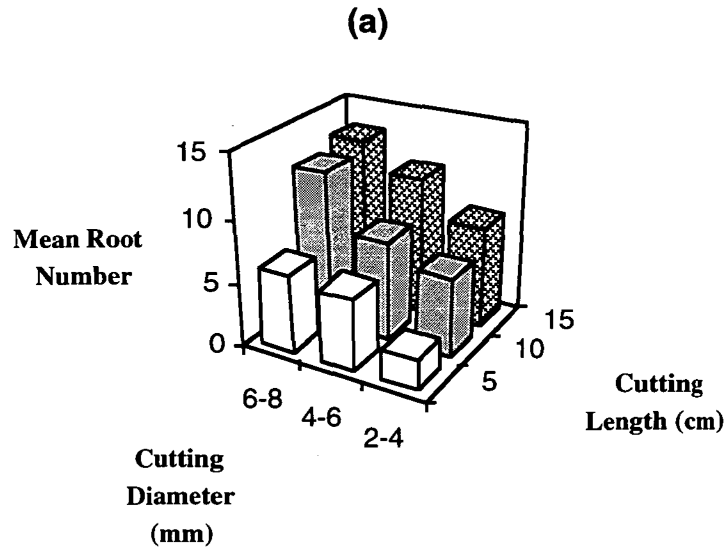
The number of roots which a cutting produced was significantly influenced by both the length and diameter of the cutting (Figure IV.1.7.a). Generally, the greater the length and diameter of the cutting the greater the number of roots which are produced (Figure IV.1.7.a). The mean number of roots produced in cuttings which were 5cm in length and 2-4mm in diameter was 2.4, whereas the mean number of roots produced in cuttings which were 15cm in length and 6-8mm in diameter was 13.0 (Figure IV.1.7.a). Therefore, the size of a cutting significantly influenced the number of roots produced by cuttings.

The dry weight of the adventitious root growth produced was significantly influenced by the length of the cutting (Figure IV.1.7.b). The 5, 10 and 15cm cuttings produced 0.55, 0.97 and 1.32g of root growth respectively. The diameter of the cutting did not significantly influence the amount of root dry weight produced ($p = 0.066$).

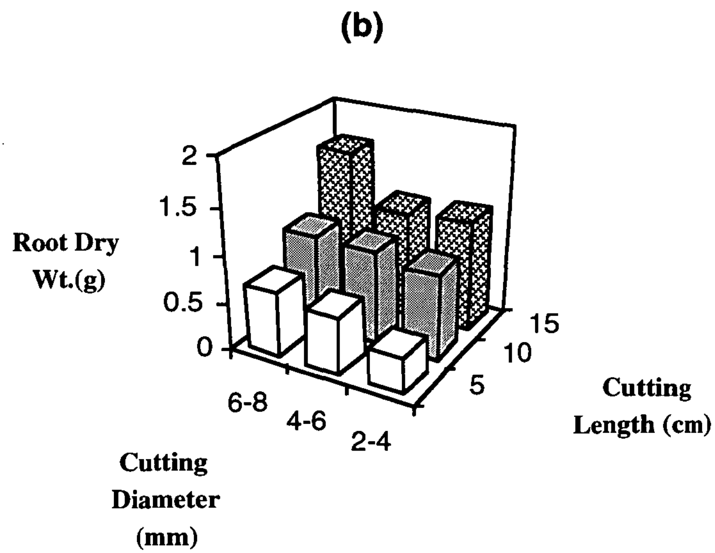
The top growth dry weight was significantly influenced by both the length and the diameter of the cutting (Figure IV.1.7.c). The 5, 10 and 15cm long cuttings produced 2.50, 4.21 and 5.85g of root growth respectively. The 2-4, 4-6 and 6-8mm diameter cuttings produced 3.16, 3.93 and 5.47g of top growth dry weight respectively.

Figure IV.1.7 - The effect of cutting stem length and basal stem diameter on **(a)** mean root number ($LSD_{0.05}$=1.70), **(b)** mean root dry weight (g) ($LSD_{0.05}$=0.26) and **(c)** mean top growth dry weight (g) ($LSD_{0.05}$=0.97) (means and standard errors included in Appendix 13).

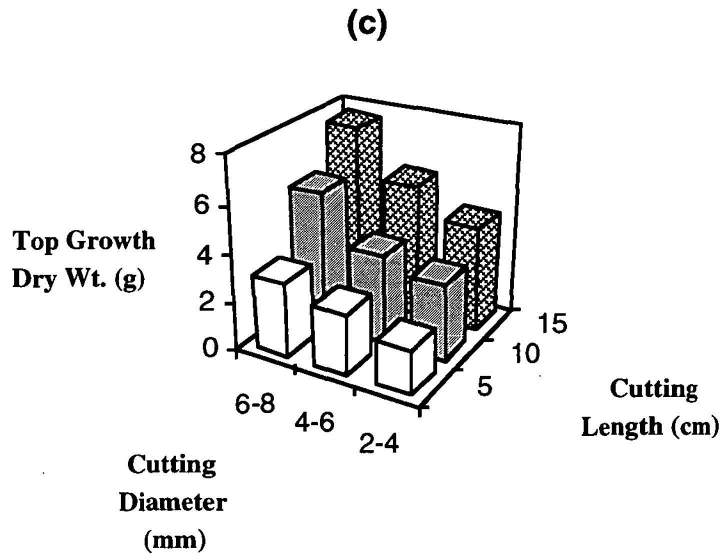
(a)



(b)



(c)



1.4 Discussion

A seasonal fluctuation in the ability of *P. radiata* cuttings collected from 1, 2, 4 and 8 year old stock plants to form adventitious roots was demonstrated under Tasmanian conditions, with a significant decline noted in October (spring). The seasonal variation in rooting percentage differed depending on the age of the stock plant. Although significant differences were observed in rooting percentages of cuttings collected from juvenile (1, 2 and 4 years old) and mature (8 year old) stock plants this only occurred between June and August (winter) when stock plants were relatively dormant. This result agrees with research which has demonstrated a reduction in rooting percentage associated with increased age of the stock plants from which cuttings are excised (Fielding, 1954; Fielding, 1969; Jacobs, 1939; Libby and Conkle, 1966; Menzies *et al.*, 1985; Mirov, 1944). At other times in the season (October and November) no significant difference was observed between cuttings collected from juvenile or mature stock plants. Therefore, when reporting differences in rooting percentage between cuttings collected from different aged stock plants one must consider the influence season of collection has on the rooting percentage.

Cuttings collected from juvenile stock plants initiated adventitious root growth significantly earlier than cuttings collected from mature stock plants at the same time in the year. Adventitious root growth occurred at a slightly faster rate in cuttings collected from juvenile stock plants, compared to cuttings collected from mature stock plants. This result is in agreement with Fielding (1969) and (Sweet, 1973) who reported that the early growth rate of cuttings collected from *P. radiata* seedlings tend to decline as the parent stock plant aged. The major differences observed in the growth rate of roots may be attributed mainly to the variation in initiation time. Once root growth was initiated, there were only small differences in the rate of adventitious root development between cuttings collected from different aged stock plants.

The growth of callus tissue in the basal region of the cutting appears to be important in terms of cutting survival. In cuttings which failed to root, callus tissue was absent in the basal region. Cameron and Thomson (1969) reported that in cuttings of *P. radiata* adventitious roots originate in the callus tissue that has formed at the base of the cutting. Hartmann and Kester (1983) stated that in most plant genera, the formation of callus and the formation of roots occur independent of each

other. The ability of cuttings of *P. radiata* to form callus tissue in the basal region of the cut stem appears to be an important step in the development an adventitious root system, and therefore a major determinant of rooting percentage. The major differences observed in the amount of callus growth cuttings from stock plants of different ages can be attributed to differences in initiation time and not the rate of growth. Cuttings collected from juvenile stock plants produced callus tissue which was more evenly distributed (radially) around the base of the cutting compared with cuttings collected from more mature stock plants. Callus growth in cuttings collected from 8 year old stock plants was more uneven with respect to its distribution around the basal region of the cutting. Menzies and Klomp (1988) reported problems in getting cuttings collected from older stock plants to form enough roots. This may be attributed to the poor callus development observed in cuttings collected from mature stock plants.

In cuttings collected from different aged stock plants, top growth was initiated only after callus growth had commenced. Top growth occurred at a faster rate in cuttings collected from juvenile stock plants than in cuttings collected from mature stock plants. This is in agreement with Fielding (1969) and Sweet (1973) who reported that the early growth rate of cuttings collected from *P. radiata* tended to decline with increased age of the stock plant.

The concentration of PGR's in the basal stem region of the cuttings did not appear to influence the final rooting percentage of *P. radiata* cuttings. Although the concentration of ABA, ZR and DHZR did relate well with the rooting of cuttings collected from one year old stock plants, this trend was not observed in cuttings collected from older stock plants. The poor relationship between PGR concentration and rooting success indicated that the concentration of PGR's, at the time of excision, may not be an important indicator of rooting ability in *P. radiata* cuttings.

The trend in rooting percentage of cuttings collected from 1 year old stock plants and set under constant environmental conditions (phytotron) at different times in the year was similar to that observed in field set cuttings. This similarity in the pattern of seasonal variation in rooting potential under both phytotron and field conditions indicated that some physiological and/or anatomical status of the stock plant at the time of cutting excision was a more significant determinant in the rooting process, than the environment in which the cuttings were set. Environmental influences experienced after setting did not appear to have a major influence on

adventitious root formation in *P. radiata* cuttings under Tasmanian field conditions, despite the pronounced difference in rooting environments.

The length of the cutting was demonstrated to influence the rooting success of cuttings collected from 1 year old stock plants during winter. The rooting percentage of 5cm cuttings was less than that of the longer 10 and 15cm cuttings. The longer cuttings also produced more callus growth, top growth, root numbers, root dry weight and top growth dry weight. Although the diameter of the cutting did not have a significant influence on the rooting percentage, it did have a significant influence on the number of roots produced by each cutting and the top growth dry weight. Therefore, the diameter of cuttings is a useful specification to ensure a quality root system is produced by the cutting. In a commercial *P. radiata* cutting nursery, the selection and excision of cutting material from the parent stock plant is an important process and one which will ultimately affect their subsequent rooting and growth characteristics.

IV.2 The Identification of Physiological and/or Anatomical Factors likely to Influence Rooting

2.1 Introduction

In section IV.1, it was reported that the rooting percentage of physiologically juvenile cuttings set at different times in the season under field and phytotron conditions was not significantly different. This indicated that some physiological and/or anatomical factor was a major determinant of the seasonal variation observed in the percentage rooting of *P. radiata* cuttings collected at different times in the year. A review of the literature indicated many areas of potential research. The main areas of research which were assessed as being potentially important, included ;

(a) anatomical studies
(b) plant growth regulator studies
(b) carbohydrate metabolism studies
(d) water relations studies

The aim of this experiment was to examine the changes which occur in these physiological and/or anatomical factors at different times in the season when the rooting potential of *P. radiata* cuttings was known to differ greatly. This may provide an insight into the mechanism(s) which limit the rooting of cuttings at different times in the season and between different aged cutting material.

2.2 Materials and Methods

Two experiments were designed, one which examined the changes in physiological and/or anatomical factors at different times in the season using juvenile cutting material. The other experiment examined the difference in physiological and/or anatomical factors which occurred between cuttings collected from different aged stock plants.

In the first experiment, cuttings were collected from two year old stock plants at four different times in the year when the rooting percentage was known to differ within cuttings collected from stock plants of the same age (3/8/94, 14/9/94, 26/10/94 and the 7/12/94). The cuttings were propagated in the phytotron under standard conditions and in the standard root trainer cells and rooting medium according to the technique described in Section III.2.2. In the second experiment, cuttings were collected from two and nine year old stock plants on the 3/8/94 when the rooting percentage was known to differ, at this time, between stock plants of different ages. In both experiments, 5 replicates of 100 cuttings per replicate were set in randomly pre-allocated positions within the phytotron.

At the time of cutting collection and at weekly intervals thereafter, 2 samples from each replicate were randomly selected for anatomical analysis (Section IV.3). Also, measurements were conducted on PGR and concentrations (Section IV.4), carbohydrate concentrations (Section IV.5) and cuttings water relations (Section IV.6). The rooting percentage was determined, according to the technique described in section III.3.1, after 16 weeks of propagation within the phytotron.

2.3 Results

In those cuttings collected from two year old stock plants on the 3/8/94 and the 14/9/94, visible callus tissue was first observed in weeks 8 and 9 after setting. The majority of the cuttings mortality occurred between weeks 4 and 9 after setting. The rooting percentage of cuttings collected from 2 year old stock plants on the 3/8/94 and the 14/9/94 was 91.0 and 76.0% respectively (Figure IV.2.1).

In those cuttings which were collected from two year old stock plants on the 26/10/94, signs of leaf chlorosis were observed in all cuttings 3 weeks after excision. In week 4, tissue necrosis and some secondary infection was observed in all cuttings. In those cuttings collected from two year old stock plants on the 7/12/94, signs of leaf chlorosis were observed in approximately 30% of cuttings in the fourth week following setting. The cuttings which were chlorotic in week 4 were entirely necrotic by week 5. However, further cuttings had become chlorotic during week 5. The chlorotic-necrotic cycle continued during weeks 5 through 9. The rooting percentage of cuttings collected from two year old stock plants on the 26/10/94 and the 7/12/94 was 0 and 14% respectively (Figure IV.2.1).

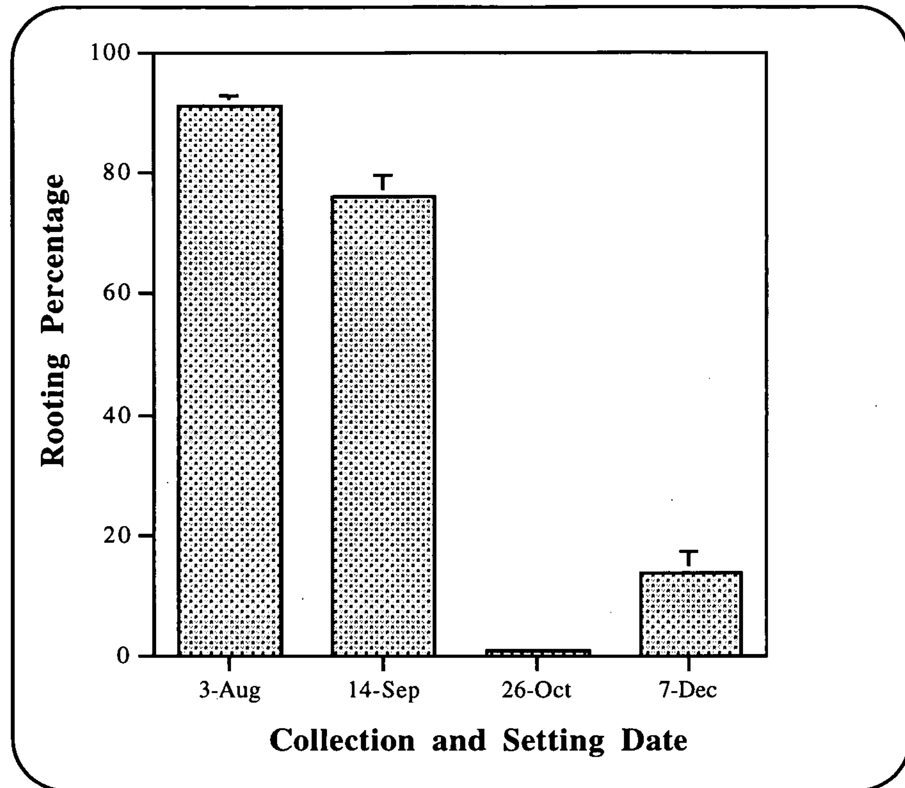


Figure IV.2.1 - The percentage rooting of cuttings collected from two year old stock plants at Westerway on the 3/8/94, 14/9/94, 26/10/94 and the 7/12/94 and set under standard conditions in the phytotron. The means and standard errors are displayed, the $LSD_{0.05}$=7.88 (data included in Appendix 14).

The rooting percentage of cuttings collected from two year old stock plants on the 3/8/94 was significantly higher than that observed at any other collection date (Figure IV.2.1). The greatest decline in rooting percentage was observed between the 14/9/94 and the 26/10/94. The rooting percentage increased significantly when cuttings were collected on the 7/12/94 in comparison to cuttings collected on the 26/10/94.

In those cuttings collected from nine year old stock plants on the 3/8/94, approximately 15-20% of the cuttings had developed signs of leaf chlorosis during the fourth week after setting. In the fifth week, the cuttings which were previously chlorotic had become entirely necrotic. However, during the fifth week further cuttings had developed signs of leaf chlorosis. This chlorotic-necrotic cycle continued between weeks 6-10. The rooting percentage of cuttings collected from

nine year old stock plants was significantly less than that observed in cuttings collected from two year old stock plants at the same time (Figure IV.2.2).

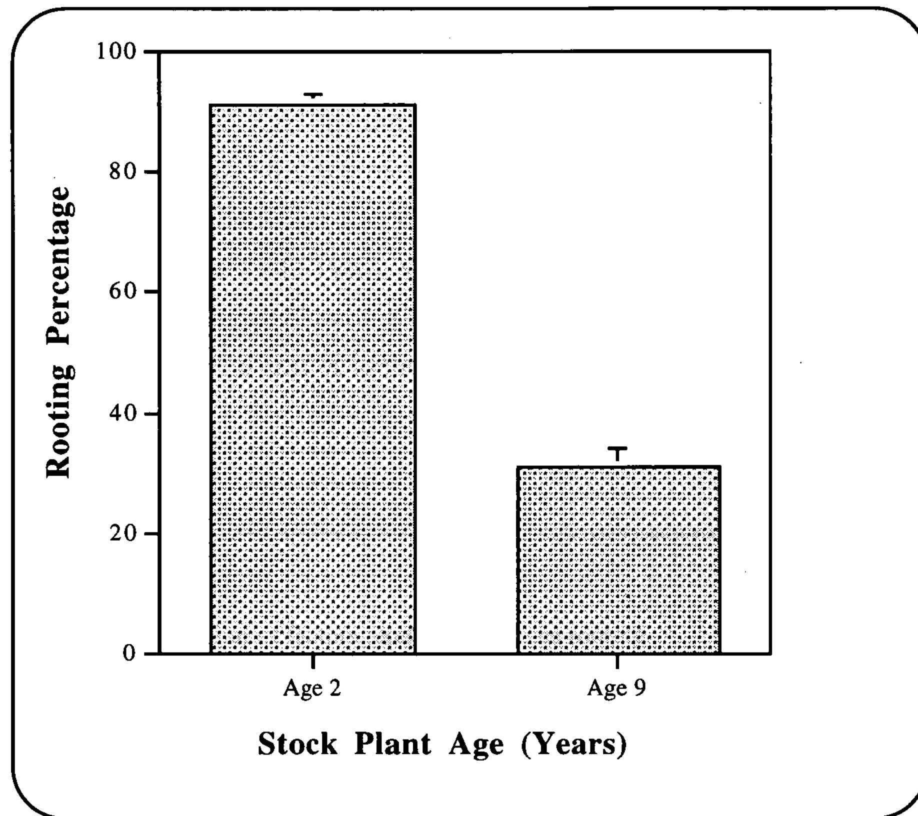


Figure IV.2.2 - The percentage rooting of cuttings collected from two and nine year old stock plants at Westerway on the 3/8/94 and set under standard conditions in the phytotron. The means and standard errors are displayed, the $LSD_{0.05}$=8.78 (data included in Appendix 14).

2.4 Discussion

In cuttings which were collected from two year old *P. radiata* stock plants, the rooting percentage of cuttings propagated in the phytotron was demonstrated to decline significantly between August and October. This result was observed previously in Section IV.1. of this thesis. Also, the rooting percentage of cuttings collected from nine year old stock plants was demonstrated to be lower than that observed in cuttings collected from two year old stock plants. In the following chapters (IV.3, IV.4, IV.5 and IV.6), various physiological and/or anatomical factors were examined in an attempt to elucidate the mechanism(s) involved in ARF of *P. radiata* cuttings, with the aim of identifying potential problems.

IV.3 Anatomical Changes occurring in *Pinus radiata* Cuttings during Adventitious Root Formation

3.1 Introduction

The anatomical changes which occur during adventitious root formation in *P. radiata* cuttings has previously been investigated by Cameron and Thomson (1969) and Smith and Thorpe (1975b). However, there has been no research which has examined the morphological and anatomical differences which may occur at different times in the year and between different aged cutting material. The timing of various anatomical events which occur during ARF was considered important to relate any biochemical and physiological changes which may occur at similar times.

The aim of the experiment was to examine the anatomical changes which occur during ARF at different times in the year, within the same age, and between different aged stock plants, collected at the same time, when the rooting percentage was known to differ. This may allow the microscopic determination of the early events associated with ARF and highlight potential anatomical problems associated with cutting production at times in the year when problems in production are observed.

3.2 Materials and Methods

3.2.a Experimental Design

Two experiments were designed to examine the anatomical changes which occur in *P. radiata* cuttings during the period of callus formation. In first experiment, cuttings were collected from two year old stock plants at four different times in the year (3/8/94, 14/9/94, 26/10/94 and the 7/12/94) and set in standard root trainer cells and rooting medium in the phytotron in accordance with procedures described in Section III.2.2. At the time of cutting collection and at weekly intervals for 11 weeks, 10 cuttings were randomly selected and placed into a FAA fixative (Appendix 15.a) until required for anatomical examination (Section IV.3.3.2.b). Three representative samples were subsequently selected and sectioned each week .

In the second experiment, cuttings were collected from 2 and 9 year old stock plants on the 3/8/94 and set in standard root trainer cells and rooting medium in the phytotron, in accordance with the procedures described in Section III.2.2. At the time of cutting collection and at weekly intervals for 11 weeks(only 9 weeks for cuttings collected from 9 year old stock plants), 10 cuttings were randomly selected and placed into a FAA fixative (Appendix 15.a) until required for anatomical examination (Section IV.3.3.2.b). Three representative samples were subsequently selected and sectioned each week .

3.2.b Fixation Procedure

The fixation, dehydration, embedding, sectioning and staining of samples was conducted as described by Jensen (1962). A 3cm stem sample was cut from the base of the cutting and the needles removed using hand secateurs. The sample was transferred into a 20ml glass screw-cap vial (Kimble) containing formalin acetic alcohol fixative (FAA; Appendix 15.a). The samples were stored in FAA at room temperature until they were dehydrated in an ethanol and xylene series prior to infiltration and embedding in paraffin (Section IV.3.3.2.c).

3.2.c Dehydration, Infiltration and Embedding Procedure

The samples were removed from FAA solution and trimmed using a No. 10 scalpel blade to a length of approximately 15mm and a quarter of the original stem diameter. The samples were then placed into a clean 20ml glass screw-cap vial (Kimble) and 15mL of 70% ethanol (EtOH) added. After 30 minutes, the vial was emptied and refilled with a further 15ml 70% EtOH. This process was repeated once more, then left over night. The 70% EtOH was removed and the samples rinsed with 95% EtOH. The samples were then left in 15 mL of 95% EtOH for 2 hours. The 95% EtOH was removed and the samples rinsed twice in 100% EtOH, before leaving in 15mL of 100% EtOH for 2 hours. The 100% EtOH was removed and the samples rinsed twice in EtOH:xylene (50:50 v/v), before leaving in 15mL of EtOH:xylene (50:50 v/v) for 1 hour. The EtOH:xylene (50:50 v/v) was removed and the samples rinsed twice with 100% xylene, before leaving samples in 15mL of 100% xylene for 1 hour. After an hour, half of the 100% xylene was removed and an equal amount of molten paraffin (Paraplast Plus, Monoject Scientific, St. Louis) was added. The paraffin and xylene was mixed and left heated between 60-62°C overnight. The samples were then transferred into a fresh 20ml screw-cap vial (Kimble) containing

100% molten paraffin and placed in a heated vacuum infiltration unit (Laboratory Equipment Pty. Ltd., Sydney) at 60-62°C, under a vacuum of approximately 7.4-8.8 kPa overnight. The paraffin was changed twice and then allowed to infiltrate under vacuum for a further 2 days. The samples were then embedded in paraffin blocks prior to sectioning (Section IV.3.3.2.d).

3.2.d Sectioning, Rehydration and Safranin-Fast Green Staining Procedure

The samples were sectioned using a base sledge microtome (Ernst Leitz Optical Works G.m.b.H., Wetzlar, Germany No. 1300). The thickness of the each section was 12μm. The sections were 'relaxed' in a heated (55°C) water bath and fixed to glass microscope slides using Mayer's adhesive (Appendix 15.b). The slides were allowed to air dry prior to rehydrating and safranin-fast green staining. The air dried slides containing the sections were placed into a slide rack and then into 100% xylene for 15 minutes. The rack was then transferred into EtOH:xylene (50:50 v/v) for 5 minutes, 100% EtOH for 5 minutes, 95% EtOH for 5 minutes, 70% EtOH for 5 minutes and 50% EtOH for 5 minutes. After the 50% EtOH, the slide rack was transferred into the safranin stain (Appendix 15.c) for 18 hours. The slides were then rinsed in distilled water for 20 seconds and then destained in acidified 70% ethanol (Appendix 15.d) for 5-10 seconds. The slides were then rinsed in 95% EtOH for 15 seconds and in 100% EtOH for 15 seconds, before being counterstained in fast green (Appendix 15.e) for 15-20 seconds. The slides were transferred to a differentiation solution (Appendix 15.f) for 15 minutes. This was repeated twice, for 15 minutes each. The slides were then transferred to 100% xylene for 15 minutes. This was repeated twice, for 15 minutes each. The slides were then removed from the xylene and allowed to partially dry before mounting permanently with Canada balsam and a cover slip.

3.2.e Microscopy and Photography

The 12μm sections were viewed using a Zeiss Stemi 2000-C stereomicroscope with light trasmitted from a fibre-optic Zeiss KL 1500 electronic unit below the plane of the slide. The sections were photographed using a Zeiss 35mm film cassette Mot mounted on the stereomicroscope with a Zeiss MC 100 phototube and separate Zeiss exposure control unit. A Kodak Ektar 25 colour negative film was used at 25 ASA and a reciprocity number of 3.

3.3 Results

In cuttings collected from two year old stock plants on the 3/8/94, a transverse section of the basal stem region at the time of excision revealed the normal arrangement of cells within a *Pinus radiata* stem (Plate 12). This consisted of large resin ducts located in the region between the phloem and the epidermis, and smaller resin ducts located in the xylem. At this stage, there were no visible signs of ARF. In week 4 after excision, the first signs of callus growth were observed (Plate 13). This callus growth (parenchyma tissue) appeared to originate near the cambial region. A similar finding was reported by Cameron and Thomson (1969). In week 8, more callus tissue was visible (Plate 14). At this stage, callus growth was also visible on a macroscopic scale. By week 11, tracheids were observed within the callus tissue (Plate 15). Although there did not appear to be much cellular organisation within the callus tissue, the tracheids within the callus were connected to the existing vascular system (Plate 16). The rooting percentage of cuttings collected from two year old stock plants on the 3/8/94 was 91%.

In cuttings collected from two year old stock plants on the 14/9/94, a transverse section of the basal stem region at the time of excision revealed a similar organisation of cells to that observed in cuttings collected from two year old stock plants on the 3/8/94 (Plate 17). In week 4 after excision, the first signs of callus growth (parenchyma tissue) were observed near the cambial region (Plate 18). This was similar to that observed previously in cuttings collected from two year old stock plants on the 3/8/94. By week 8, a larger amount of callus tissue was visible (Plate 19). At this stage the callus growth was visible on a macroscopic scale. By week 11, tracheids were observed within the callus tissue (Plates 20 and 21). Similar anatomical changes were observed in cuttings collected from two year old stock plants on the 3/8/94. The rooting percentage of cuttings collected from two year old stock plants on the 14/9/94 was 76%.

In cuttings collected from two year old stock plants on the 26/10/94, a transverse section at the time of excision revealed a similar organisation of cells in the stem region as that observed on the 3/8/94 and the 14/9/94 (Plate 22). However, the epidermal cells appeared much thinner than those observed in cuttings collected on the 3/8/94 and the 14/9/94 (Plates 12 and 17 respectively). Also, there was less secondary thickening of tissues. In the week prior to cutting necrosis (week 3), no signs of callus development were observed (Plate 23).

In cuttings collected from two year old stock plants on the 7/12/94, a transverse section at the time of excision revealed a similar organisation of cells as that observed in cuttings collected from two year old stock plants on the 26/10/94 (Plate 24). Again, the epidermal cell layer was much thinner compared to those cuttings collected on the 3/8/94 and the 14/9/94. In week 4, no callus growth was observed in the cambial region (Plate 25). In the week preceding cutting necrosis (week 6), there was still no sign of callus growth (Plate 26).

In cuttings collected from nine year old stock plants on the 3/8/94, a transverse section of the basal stem region at the time of excision revealed a normal organisation of cells within the stem (Plate 27). However, the resin ducts located in the region between the phloem and the epidermis appeared larger than those observed in cuttings collected from two year old stock plants on the same date (Plate 12). Although the resin ducts were larger in cuttings collected from nine year old stock plants, they did not appear to occupy space near the cambial region where the callus growth initiates. This indicated that the larger resin ducts observed in cuttings collected from nine year old stock plants are not acting as a barrier to ARF. In week 4, no change was observed in comparison to that at the time of excision (Plate 28). In week 9, and in those cuttings which survived, callus parenchyma observed in the cambial region (Plate 29). This indicated callus growth initiated later in cuttings collected from nine year old stock plants than in cuttings collected from two year old stock plants on the same date. This supports the findings in Section IV.1, where the initiation of adventitious roots was thought to occur at a later date than in cuttings collected from more juvenile stock plants.

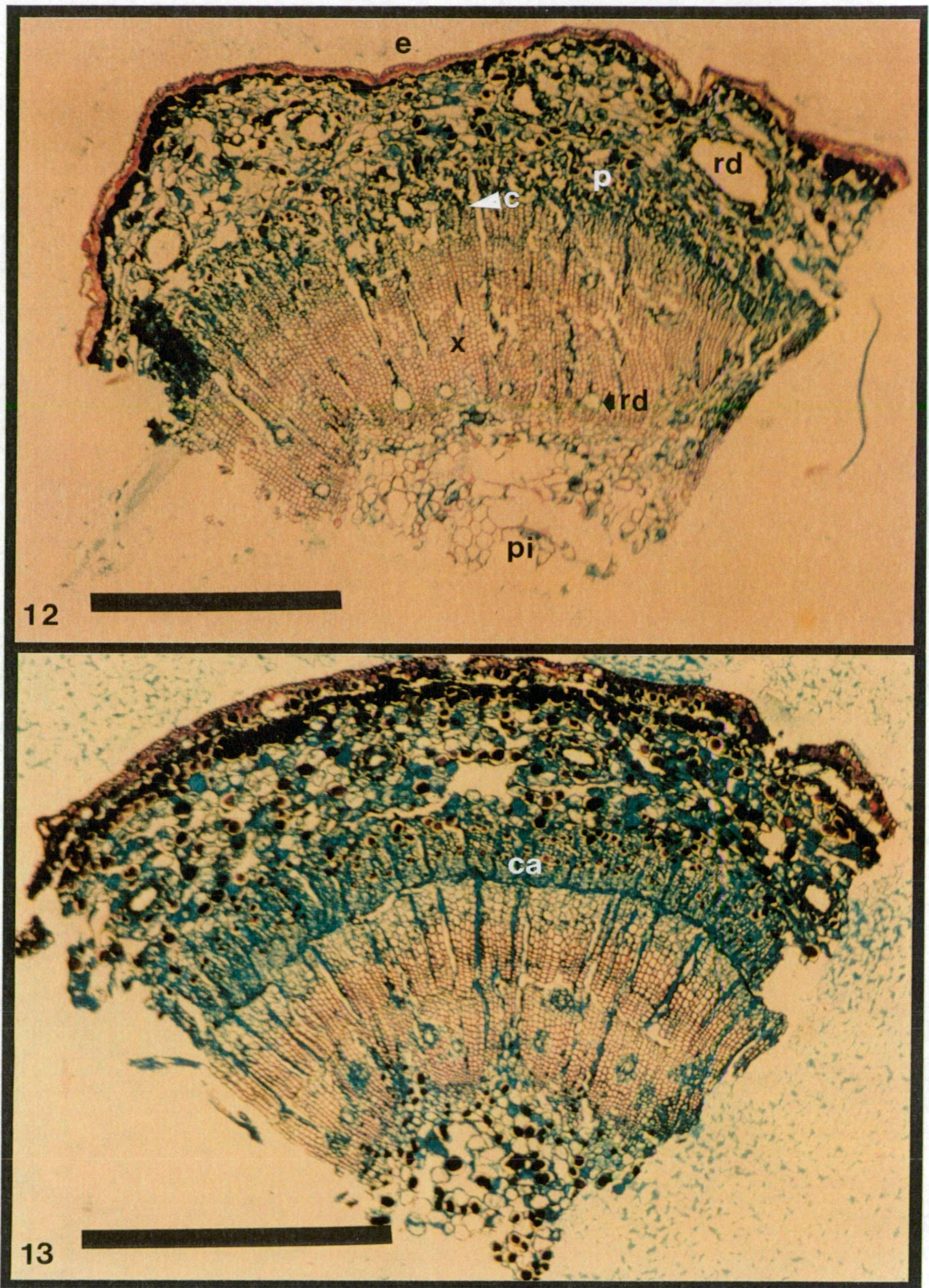


Plate 12 - Collection Date=3/8/94; Age 2; Week 0 (time of excision); Transverse section of basal stem region; e=epidermis, rd=resin duct, p=phloem, c=cambium, x=xylem, pi=pith (Bar=1mm).

Plate 13 - Collection Date 3/8/94; Age 2; Week 4 (after setting);Transverse section of basal stem region; ca=callus (Bar=1mm).

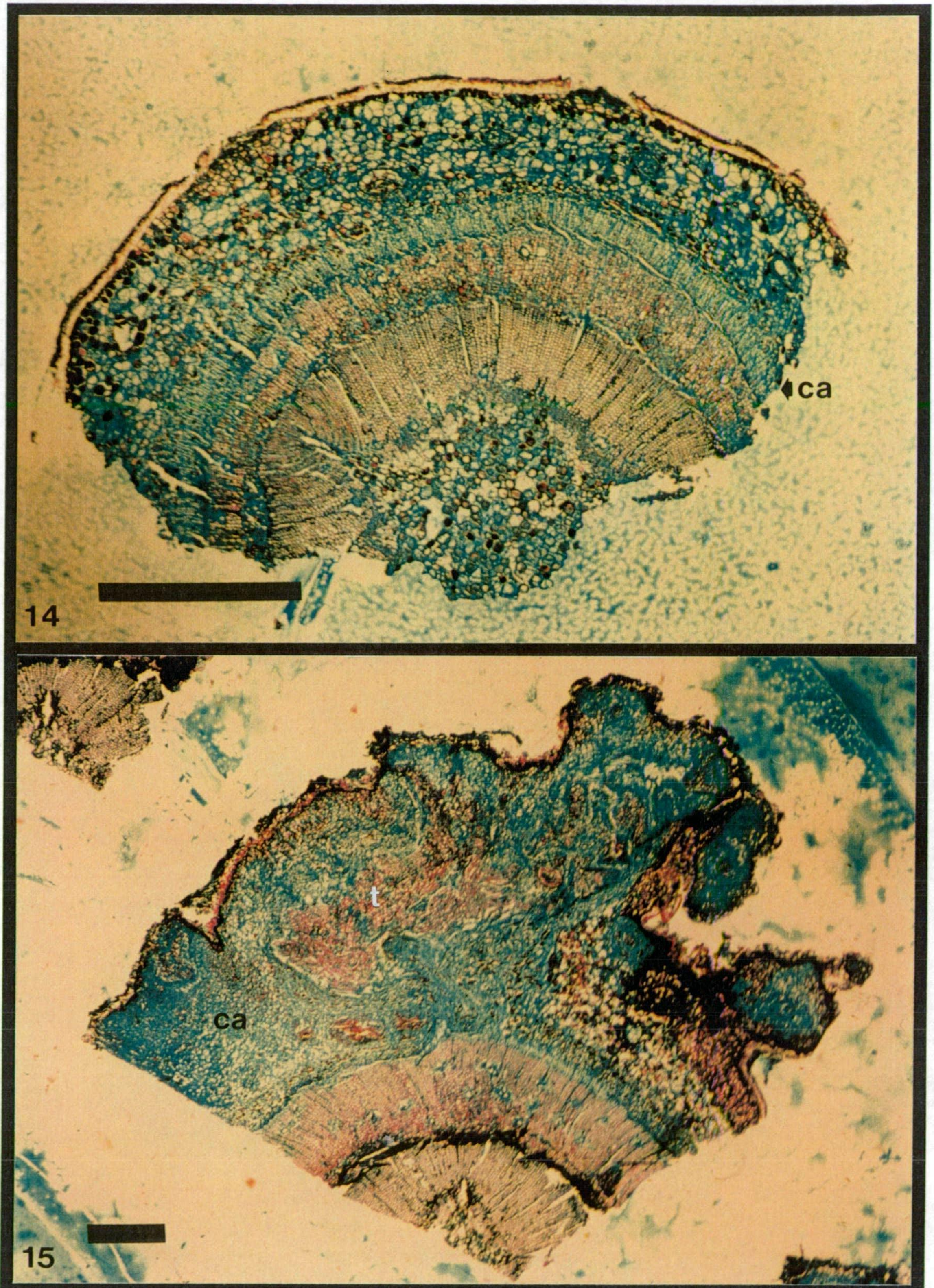


Plate 14 - Collection Date 3/8/94; Age 2; Week 8 (after setting);Transverse section of basal stem region; ca=callus (Bar=1mm).

Plate 15 - Collection Date 3/8/94; Age 2; Week 11 (after setting);Transverse section of basal stem region; ca=callus, t=tracheids (Bar=1mm).

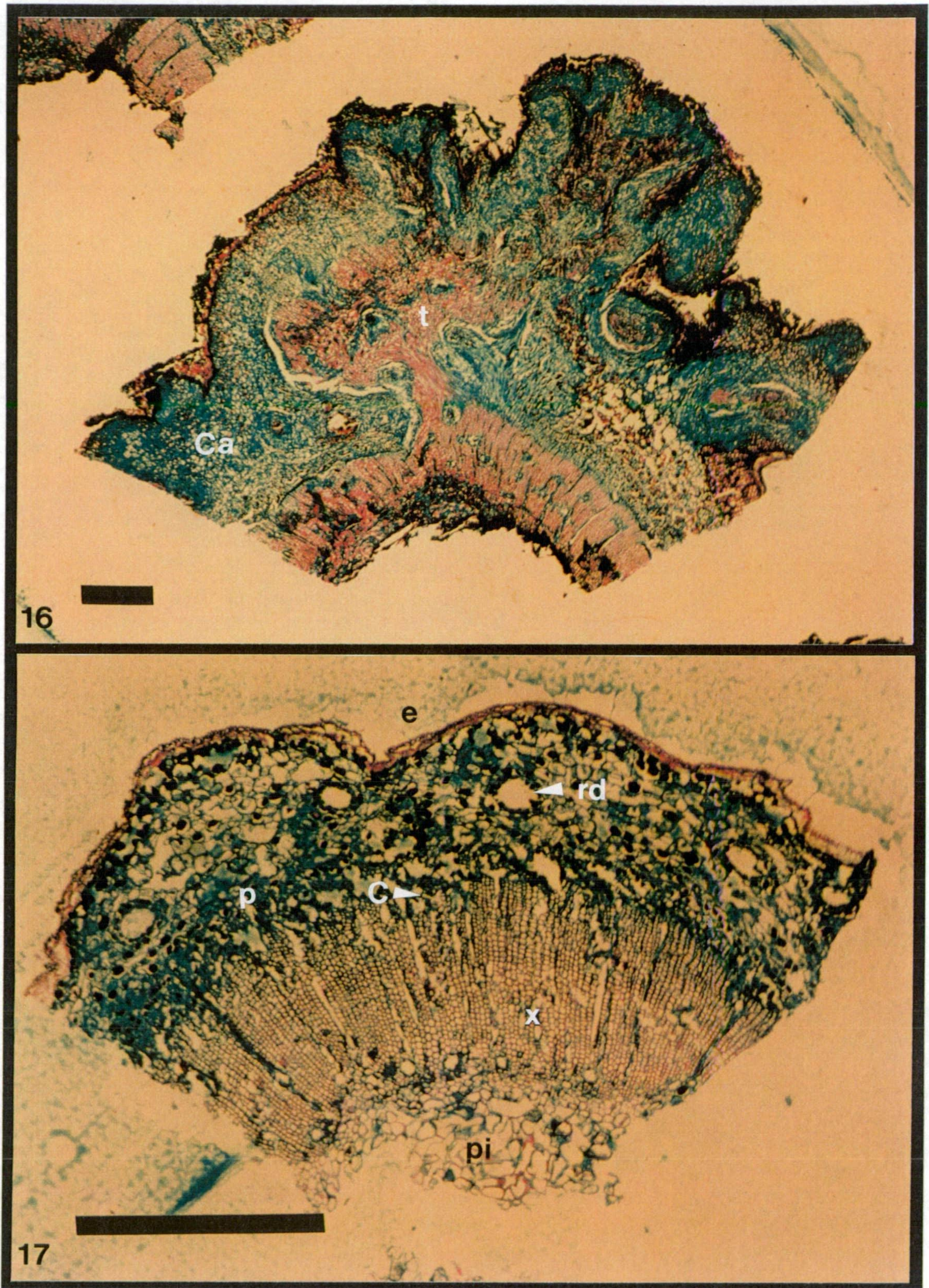
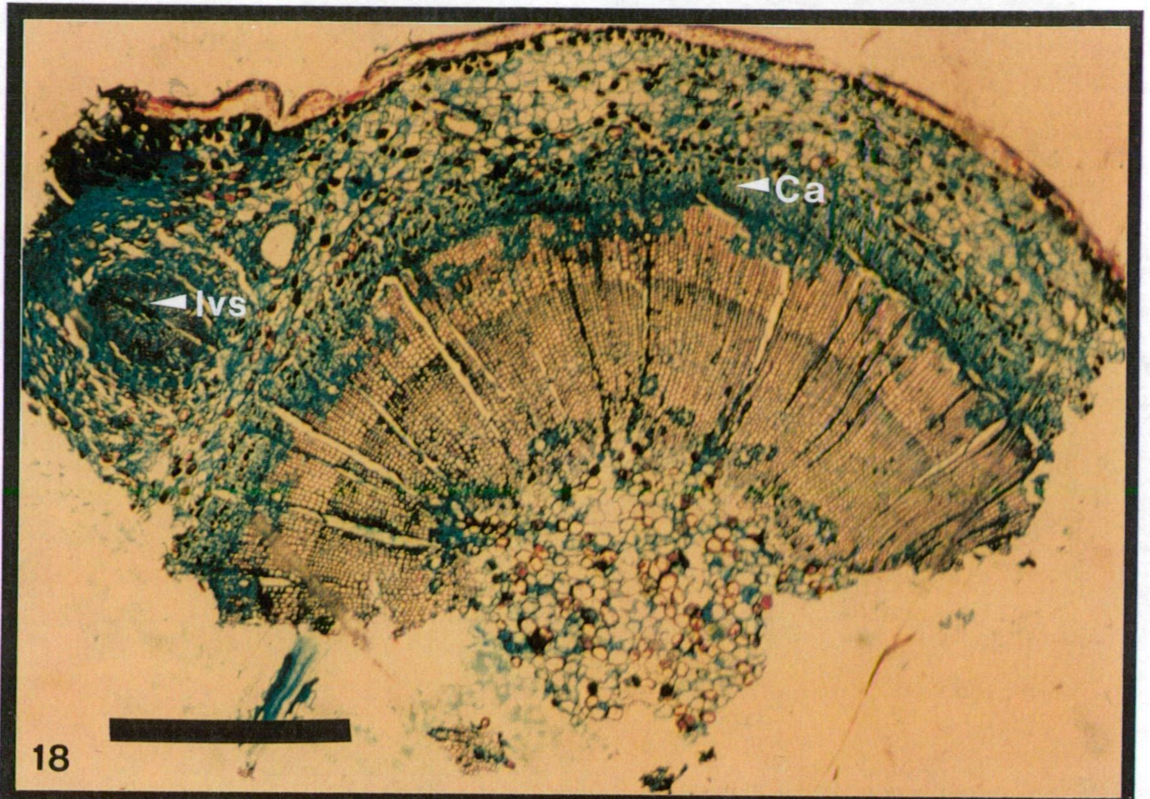


Plate 16 - Collection Date 3/8/94; Age 2; Week 11 (after setting);Transverse section of basal stem region; ca=callus, t=tracheids (Bar=1mm).

Plate 17 - Collection Date 14/9/94; Age 2; Week 0 (time of excision);Transverse section of basal stem region; e=epidermis, rd=resin duct, p=phloem, c=cambium, x=xylem, pi=pith (Bar=1mm).



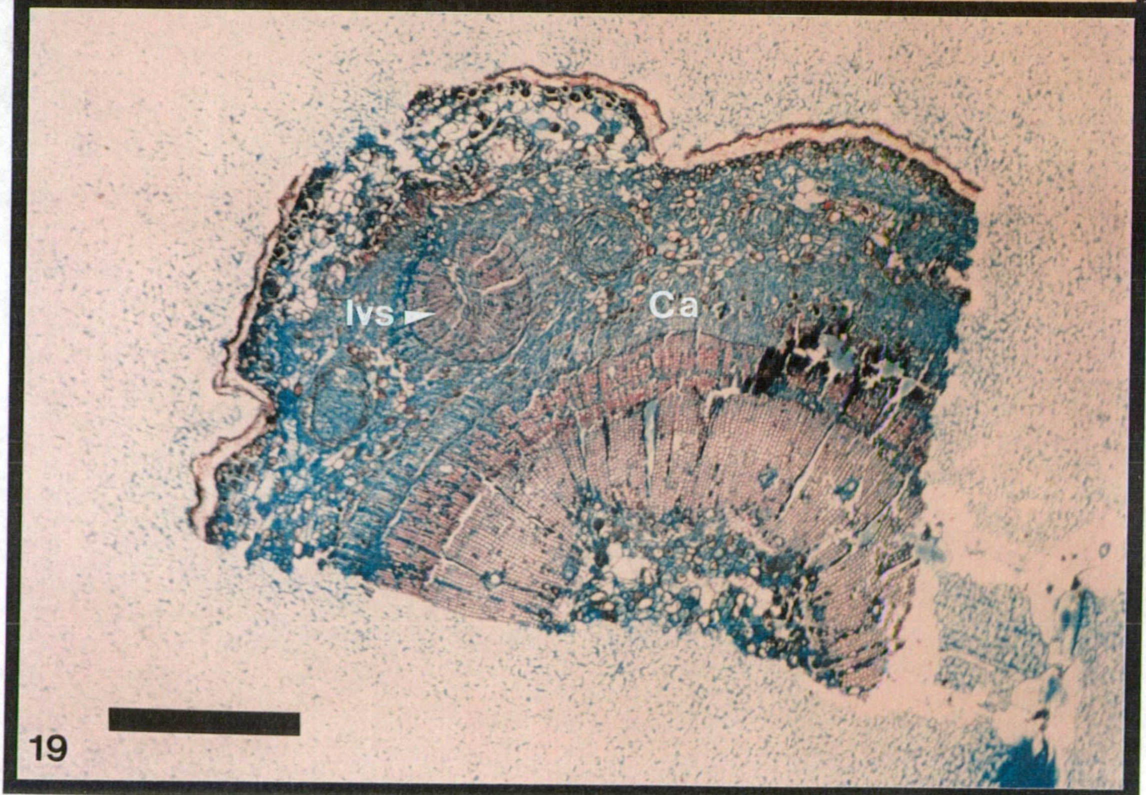


Plate 18 - Collection Date 14/9/94; Age 2; Week 4 (after setting);Transverse section of basal stem region; ca=callus, lvs=leaf vascular system (Bar=1mm).

Plate 19 - Collection Date 14/9/94; Age 2; Week 8 (after setting);Transverse section of basal stem region; ca=callus, lvs=leaf vascular system (Bar=1mm).

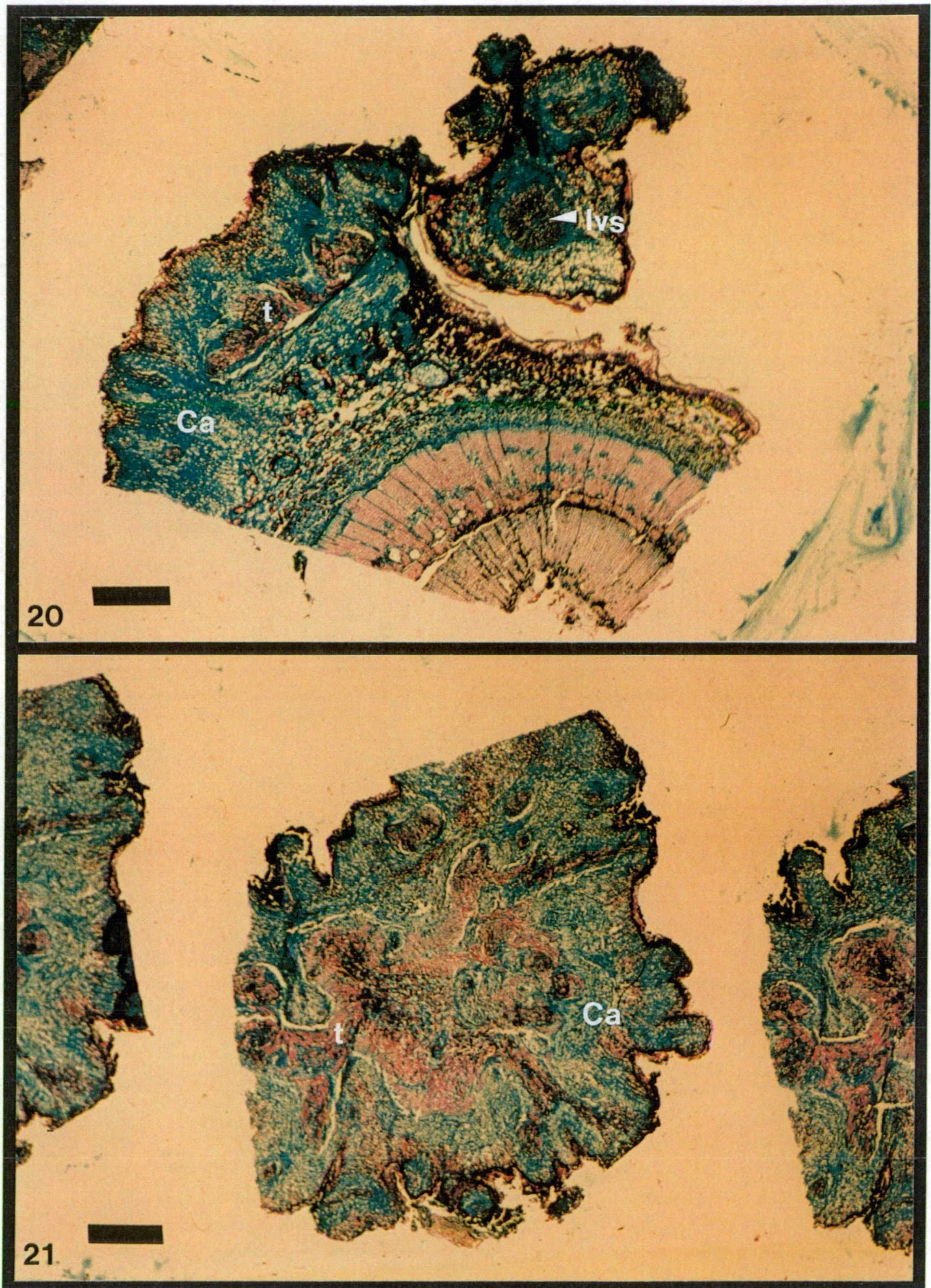


Plate 20 - Collection Date 14/9/94; Age 2; Week 11 (after setting);Transverse section of basal stem region; ca=callus, t=tracheids, lvs=leaf vascular system (Bar=1mm).

Plate 21 - Collection Date 14/9/94; Age 2; Week 11 (after setting);Transverse section of basal stem region; ca=callus, t=tracheids (Bar=1mm).

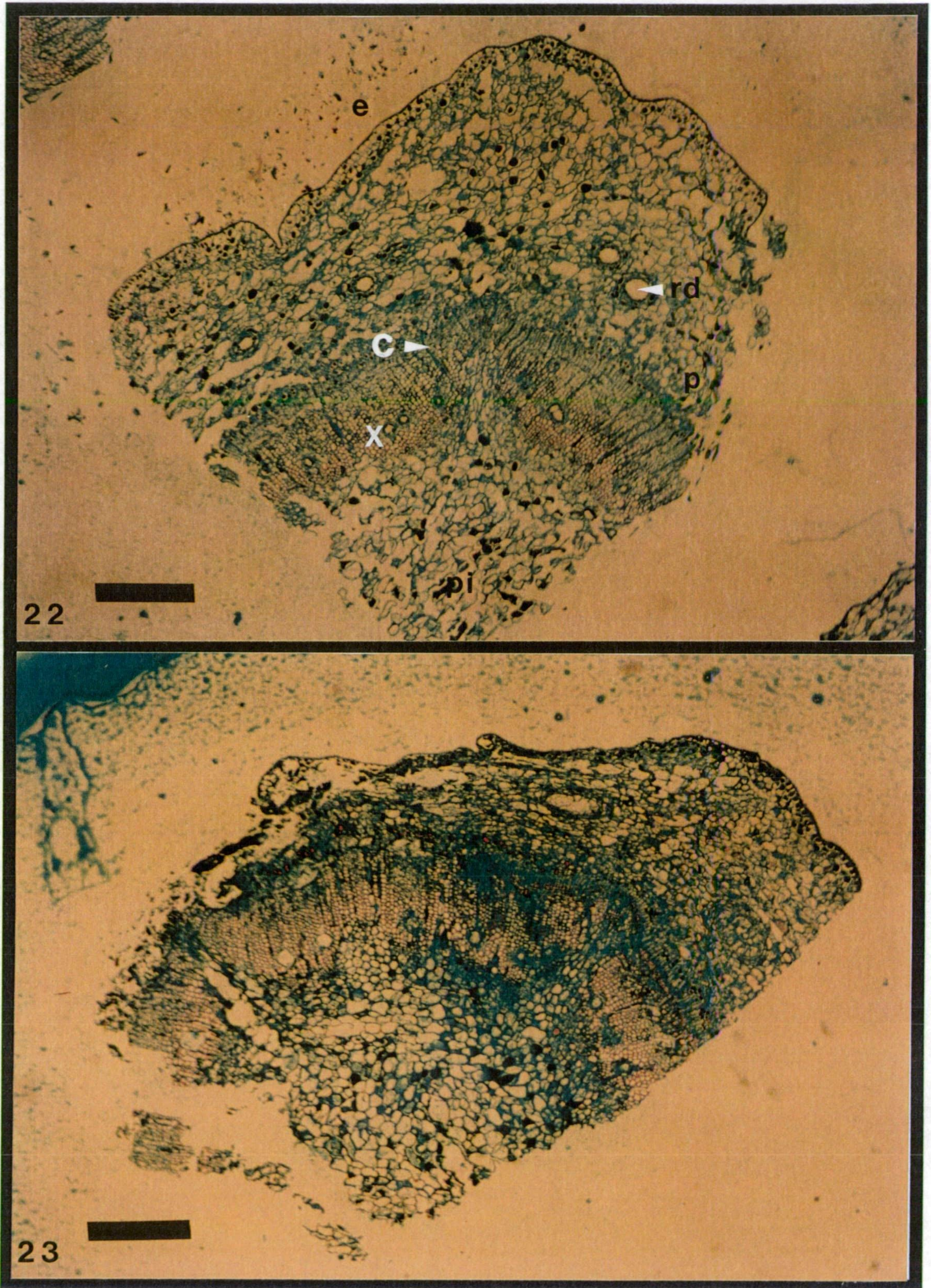


Plate 22 - Collection Date 26/10/94; Age 2; Week 0 (time of excision);Transverse section of basal stem region; e=epidermis, rd=resin duct, p=phloem, c=cambium, x=xylem, pi=pith (Bar=1mm).

Plate 23 - Collection Date 26/10/94; Age 2; Week 3 (after setting);Transverse section of basal stem region (Bar=1mm).

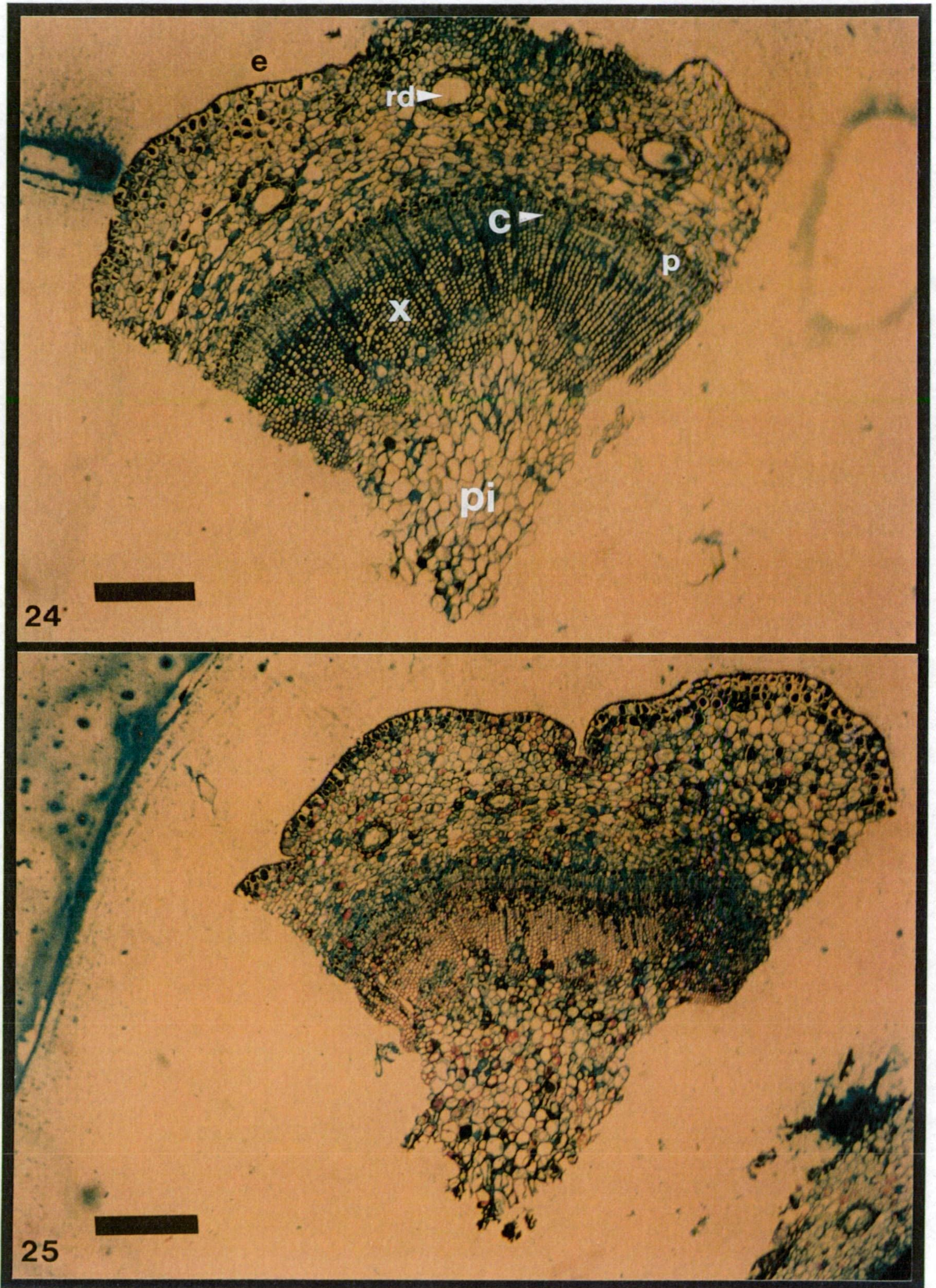


Plate 24 - Collection Date 7/12/94; Age 2; Week 0 (time of excision);Transverse section of basal stem region; e=epidermis, rd=resin duct, p=phloem, c=cambium, x=xylem, pi=pith (Bar=1mm).

Plate 25 - Collection Date 7/12/94; Age 2; Week 4 (after setting);Transverse section of basal stem region (Bar=1mm).

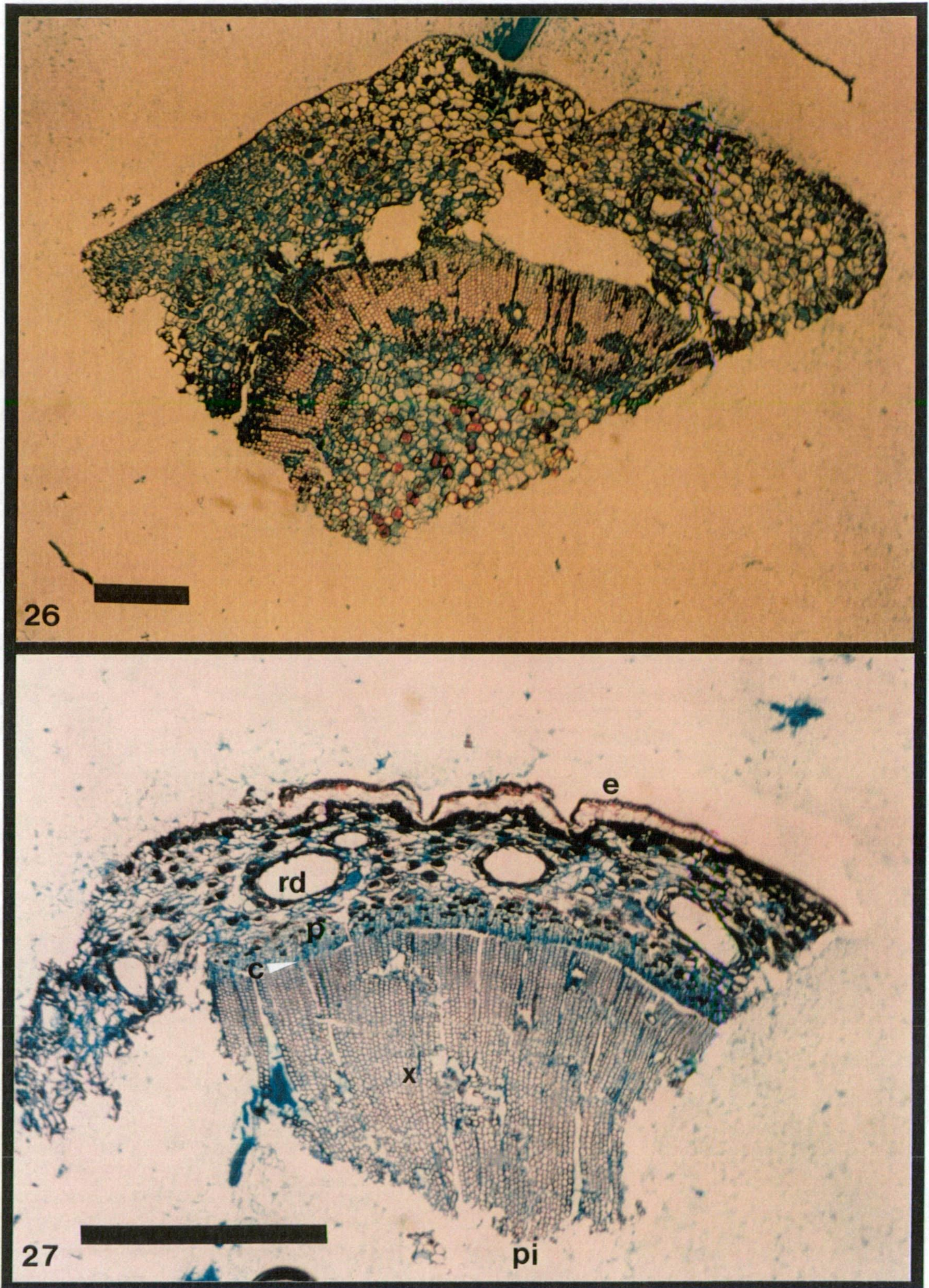


Plate 26 - Collection Date 7/12/94; Age 2; Week 6 (after setting);Transverse section of basal stem region (Bar=1mm).

Plate 27 - Collection Date 3/8/94; Age 9; Week 0 (time of excision);Transverse section of basal stem region; e=epidermis, rd=resin duct, p=phloem, c=cambium, x=xylem, pi=pith (Bar=1mm).

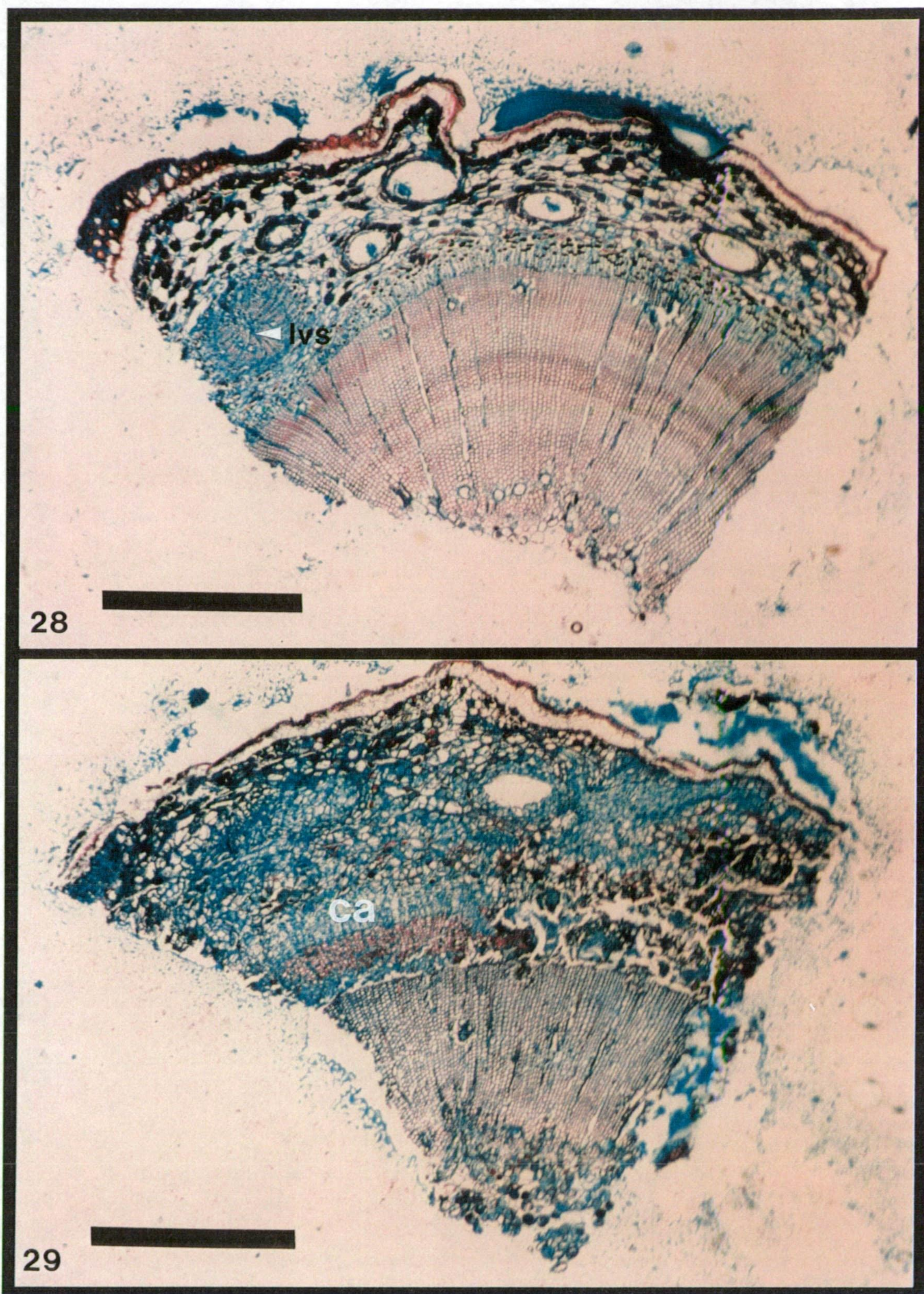


Plate 28 - Collection Date 3/8/94; Age 9; Week 4 (after setting);Transverse section of basal stem region; lvs=leaf vascular system (Bar=1mm).

Plate 29 - Collection Date 3/8/94; Age 9; Week 9 (after setting);Transverse section of basal stem region; ca=callus (Bar=1mm).

3.4 Discussion

In cuttings which were collected from two year old stock plants on the 3/8/94 and the 14/9/94, which exhibited a high rooting percentage, the first anatomical signs of callus initiation was observed 4 weeks after excision. This involved the appearance of callus parenchyma which appeared to originate from the cambial region. This finding was similar to that reported by Cameron and Thomson (1969). The callus growth continued between weeks 4 and 8, where callus became visible on a macroscopic scale. By week 11, callus xylem (tracheids) were observed within the callus tissue. This indicated that root initiation occurred sometime between week 10 and 11 after excision. The callus xylem was connected to the existing stem xylem by a thin branch of tracheids.

In those cuttings which were collected from two year old stock plants on the 26/10/94 and the 7/12/94, which exhibited a low rooting percentage, no anatomical signs of callus growth were observed in the weeks prior to cutting necrosis. These cuttings generally had a thinner epidermal layer and less secondary thickening in comparison to cuttings collected from two year old stock plants on the 3/8/94 and 14/9/94. Also, the resin ducts did not appear to act as a barrier to ARF.

In comparing the anatomical differences in cuttings collected from two and nine year old stock plants on the 3/8/94, the cuttings collected from nine year old stock plants appeared to have larger resin ducts than those observed in cuttings collected from two year old stock plants. Although cuttings collected from nine year old stock plants possessed larger resin ducts, these resin ducts did not appear to act as a barrier ARF. In cuttings collected from two year old stock plants, the first signs of callus were observed in week 4 after excision. However, in cuttings collected from nine year old stock plants, no callus growth was observed 4 weeks after excision. In fact, callus tissue was not observed until approximately week 9. This suggested that callus initiation occurred at a latter date in cuttings collected from mature stock plants compared with cuttings collected from juvenile stock plants. This supported the findings of previous studies (Section IV.1), where cuttings collected from mature stock plants initiated callus growth at a latter date than those collected from juvenile stock plants.

IV.4 Plant Growth Regulator Metabolism

4.1 Introduction

An initial investigation indicated that some anatomical and/or physiological factor, present in cuttings at the time of excision, was mainly responsible for the seasonal variation observed with the percentage rooting of *P. radiata* cuttings (Section IV.1). Plant growth regulators (PGR's) have been demonstrated to influence the rooting success of cuttings in many plant genera (Hartmann and Kester, 1983; Gaspar and Hofinger, 1988; Hansen, 1988; Mudge, 1988; Van Staden and Harty, 1988). The literature review revealed that most of PGR research, with respect to ARF in cuttings, focussed on the exogenous applications of PGR's and their subsequent growth effects. However, there have been relatively few reports that have examined the change in endogenous PGR concentrations during ARF. This was surprising considering that exogenous applications of PGR's, particularly auxin, were demonstrated to have large effects on the rooting success of cuttings. Blakesley *et al.* (1991b) observed a transient rise in the concentration of free-IAA, in the root zone of *Phaseolus aureus* hypocotyl cuttings, just prior to root initiation. After root initiation, the concentration of auxin in the root zone declined to a level below that observed at the time of excision.

Although most of the PGR research into ARF has focussed on auxin, because of its specific root promoting properties, other PGR's are thought to play a crucial role in the rooting success of cuttings. Wu and Barnes (1981) demonstrated that Rhododendron cuttings rooted the best at times in the year when the endogenous concentration of ABA in the stem was the highest. The endogenous concentration of cytokinins may also be important in controlling ARF in cuttings. Cytokinins are generally considered as inhibitors of ARF in many plant species (Van Staden and Harty, 1988).

The aim of this experiment was to examine the difference in PGR concentration at the time of excision and the changes which occur during ARF using populations of cuttings which are known to differ significantly in their rooting percentages. The monitoring of endogenous PGR concentrations during ARF may provide an insight into potential treatments which may be applied to promote the rooting of *P. radiata* cuttings in the future.

4.2 Materials and Methods

4.2.a Experimental Design

Two experiments were designed to investigate changes in PGR concentration in *P. radiata* cuttings during ARF. In the first experiment, cuttings were collected from two year old stock plants, at Westerway, Tasmania, at four different times in the year when the rooting percentage was known to differ within the same age (3rd of August, 14th of September, 26th of October and 7th of December: Section IV.1). The cuttings were collected at dawn, transported to the phytotron and set in standard root trainer cells and rooting media and propagated under standard conditions (Section III.2.2). The rooting percentage was determined after 16 weeks. In the second experiment, cuttings were collected from two year old stock plants, at Westerway, and nine year old stock plants, at National Park, at a time in the year when the rooting percentage of those cuttings was known to differ (August 3rd: Section IV.1). The cuttings were collected at dawn, transported to the phytotron and set in standard root trainer cells and rooting media and propagated under standard conditions (Section III.2.2). In both experiment, the rooting percentage of cuttings was determined after 16 weeks, as described in Section III.3.1.

At the time of cutting collection and at weekly intervals thereafter, basal stem samples were collected from cuttings for the determination of tissue PGR concentration. Each sample consisted of five 1cm basal stem segments collected from different cuttings which were bulked together and snap frozen in liquid nitrogen prior to being freeze dried and stored at -20°C until analysis, in accordance with the procedures detailed in Section III.6. Each week, a total of five replicates of each bulked sample were collected and the concentration of indole-3-acetic acid (IAA), abscisic acid (ABA), zeatin riboside (ZR) and dihydrozeatin riboside (DHZR) determined using the radioimmunoassay (RIA) technique(Section III.7). The concentration of PGR's were expressed in units of nanograms of PGR per gram of freeze-dried plant tissue (ng/g DW). This accounted for differences in the percentage dry weight (%DW) of different tissue types.

The mean and standard error of 5 replicates were calculated for all PGR's assayed. In the first experiment, a LSD (at t =0.05) was calculated for each PGR, within each collection date, to compare the change in PGR concentration which occurred at different weeks after setting. In the second experiment, a LSD (at t

=0.05) was calculated for each PGR, within each week after setting, to compare the differences in PGR concentration which occurred between cuttings collected from two and nine year old stock plants. A LSD (at t =0.05) was also calculated for each PGR to compare the change in PGR concentration which occurred at different weeks after setting. An LSD was only calculated if the ANOVA was significant at the p=0.05 level.

4.3 Results

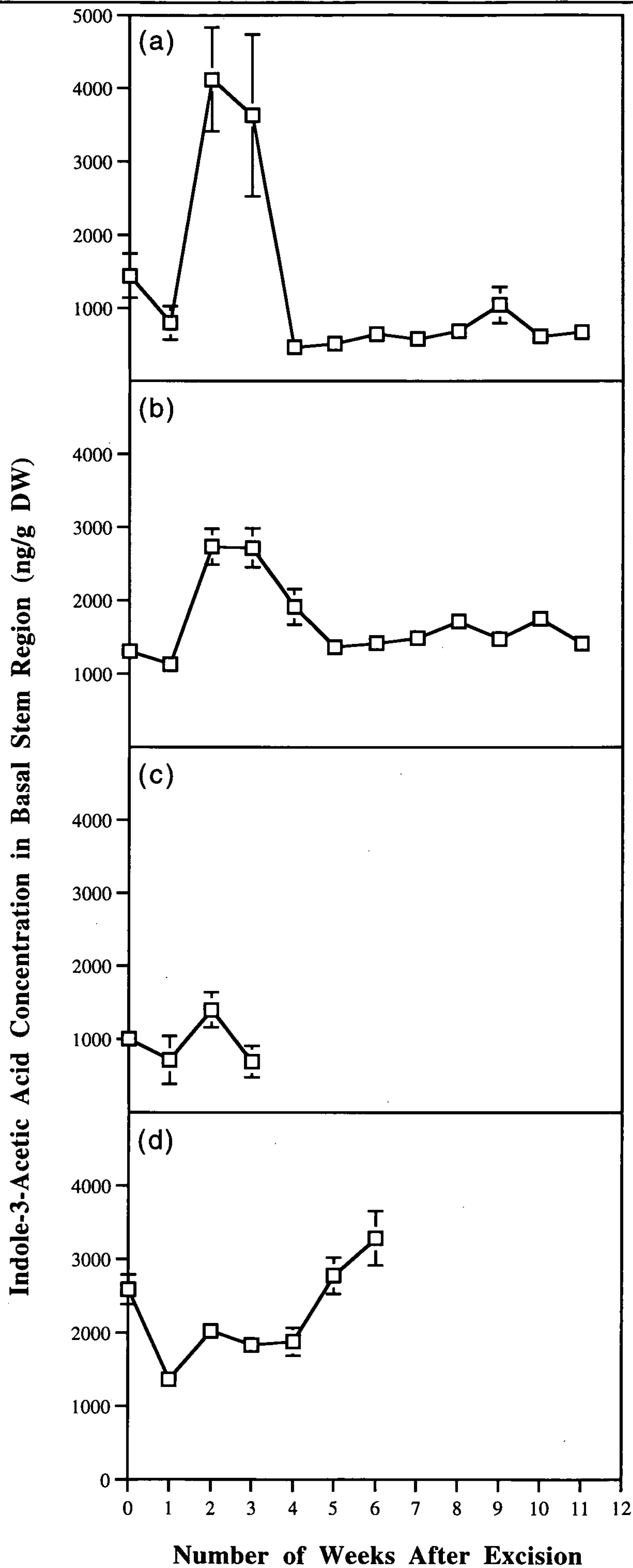
4.3.a Effect of Cutting Collection at Different Times in the Season

4.3.a.1 Auxin

In those cuttings which were collected from two year old stock plants on the 3/8/94, the concentration of auxin in the basal stem region increased significantly from 797.8 ng/g DW in week 1 to 4121.2 ng/g DW in the second week following excision (Figure IV.4.1.a). The concentration of auxin in the basal stem region did not change significantly between week 2 and 3. However, the concentration of endogenous auxin in the basal stem region decreased significantly from 3632.4 ng/g DW in week 3 to 461.6 ng/g DW in week 4, the later being a similar concentration to that observed at the time of excision. After week 4, the concentration of auxin in the basal stem region of the cuttings did not change significantly during the remainder of the callus formation period. The rooting percentage of cuttings collected on the 3/8/94 was 91%, which was consistent with earlier findings (Section IV.1.3.b.1).

In those cuttings which were collected from two year old stock plants on the 14/9/94, the concentration of auxin in the basal stem region increased significantly from 1132.2 ng/g DW in week 1 to 2734.8 ng/g DW in the second week following excision (Figure IV.4.1.b). The concentration of auxin in the basal stem region did not change significantly between week 2 and 3. However, the concentration of endogenous auxin in the basal stem region decreased significantly from 2719.6 ng/g DW in week 3 to 1364.0 ng/g DW in week 5, at which point no further change was observed in the concentration of endogenous auxin for the remainder of the callus formation period. The rooting percentage of cuttings collected on the 14/9/94 was 76%, which was consistent with earlier findings (Section IV.1.3.b.1).

Figure IV.4.1 - Change in the mean concentration of endogenous indole-3-acetic acid (IAA) and associated standard error, in the basal stem region of *P. radiata* cuttings during the callus formation period. The cuttings were collected from two year old stock plants at Westerway at four different times in the season; (a) cuttings collected 3/8/94 ($LSD_{0.05}$=1148.2), (b) cuttings collected 14/9/94 ($LSD_{0.05}$=468.5), (c) cuttings collected 26/10/94 (no significant difference) and (d) cuttings collected 7/12/94 ($LSD_{0.05}$=597.7) (data included in Appendix 16).



(a)
(b)
(c)
(d)
5000
4000
3000
2000
1000
0
0 1 2 3 4 5 6 7 8 9 10 11 12
Indole-3-Acetic Acid Concentration in Basal Stem Region (ng/g DW)
Number of Weeks After Excision

In those cuttings which were collected from two year old stock plants on the 26/10/94, no significant (p=0.160) change in the concentration of endogenous auxin was observed in the basal stem region during the 3 week period prior to cutting necrosis (Figure IV.4.1.c).

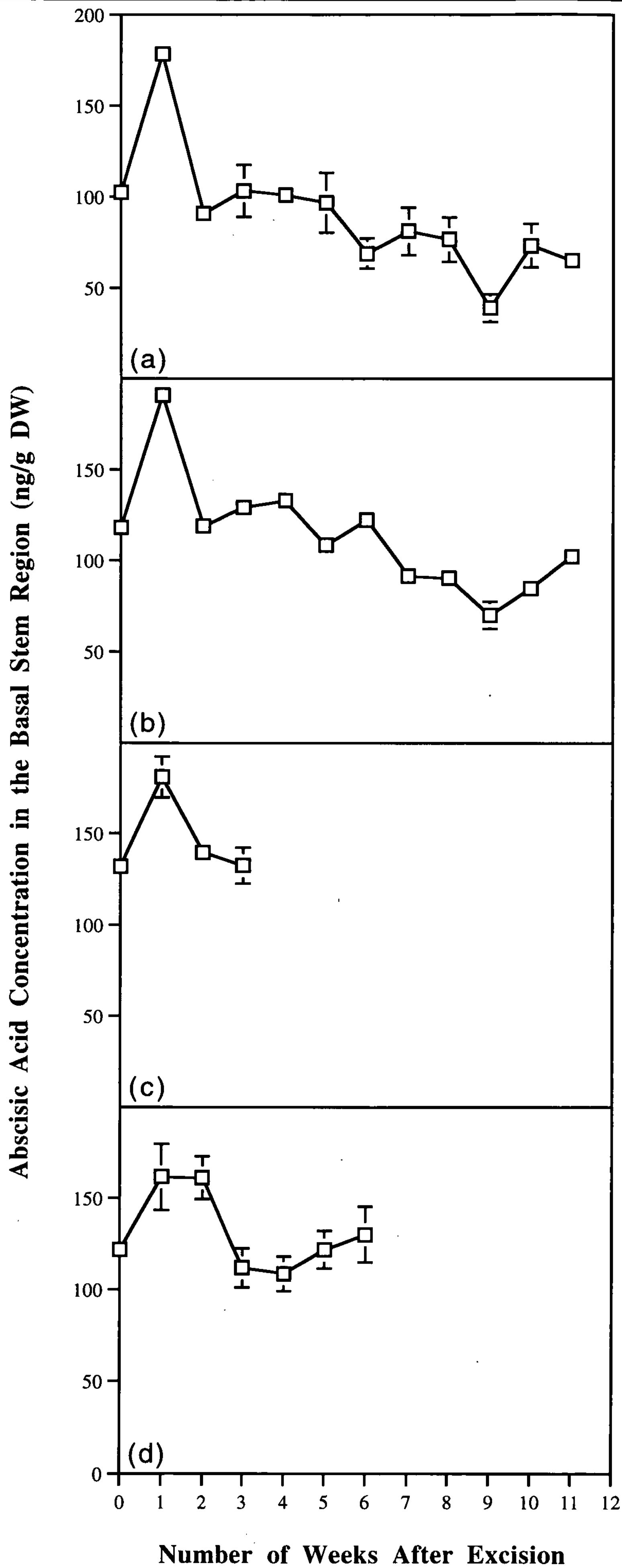
In those cuttings which were collected from two year old stock plants on the 7/12/94, the concentration of auxin in the basal stem region decreased significantly from 2589.9 ng/g DW at the time of excision to 1361.3 ng/g DW after the first week of propagation (Figure IV.4.1.d). The concentration of endogenous auxin in the basal stem region increased significantly from 1361.3 ng/g DW in week 1 to 2027.3 ng/g DW in week 2, which was still significantly lower than that concentration of auxin observed at the time of cutting excision. The concentration of auxin in the basal stem region of the cuttings did not change significantly between week 2 and 4, but increased significantly from 1882.1 ng/g DW in week 4 to 3285.6 ng/g DW in week 6, which was significantly higher than that observed at the time of excision. The rooting percentage of cuttings collected on the 7/12/94 was 11%, which was consistent with earlier findings (Section IV.1.3.b.1).

Therefore, in those cuttings which exhibited a high rooting percentage, a 'peak' in endogenous auxin was observed in the basal stem region during weeks 2 and 3 following setting. After week 3, the concentration of endogenous auxin in the basal stem region decreased rapidly to a concentration similar to that observed at the time of cutting excision, where it remained approximately the same throughout the remainder of the callus formation period. In those cuttings which exhibited a low rooting percentage, the above 'peak' in endogenous auxin was not observed during the callus formation period.

4.3..a.2 Abscisic Acid

In those cuttings which were collected from two year old stock plants on the 3/8/94, the concentration of abscisic acid (ABA) in the basal stem region increased significantly from 102.3 ng/g DW at the time of cutting excision to 178.4 ng/g DW after the first week (Figure IV.4.2.a). The concentration of endogenous ABA in the basal stem region decreased significantly from 178.4 ng/g DW in week 1 to 90.9 ng/g DW in week 2, which was a similar concentration to that observed at the time of excision. The concentration of ABA in the basal stem region did not change significantly between week 2 and 8. However, the concentration of ABA decreased

Figure IV.4.2 - Change in the mean concentration of endogenous abscisic acid (ABA) and associated standard error, in the basal stem region of *P. radiata* cuttings during the callus formation period. The cuttings were collected from two year old stock plants at Westerway at four different times in the season; (a) cuttings collected 3/8/94 ($LSD_{0.05}$=27.7), (b) cuttings collected 14/9/94 ($LSD_{0.05}$=14.4), (c) cuttings collected 26/10/94 ($LSD_{0.05}$=24.0) and (d) cuttings collected 7/12/94 ($LSD_{0.05}$=35.0) (data was obtained from Appendix 17).


Abscisic Acid Concentration in the Basal Stem Region (ng/g DW)
200
150
100
50
(a)
150
100
50
(b)
150
100
50
(c)
150
100
50
0
(d)
0 1 2 3 4 5 6 7 8 9 10 11 12
Number of Weeks After Excision

significantly from 76.7 ng/g DW in week 8 to 39.0 ng/g DW in week 9. The concentration of ABA increased significantly from 39.0 ng/g DW in week 9 to 73.1 ng/g DW in week 10, at which point no further change in ABA concentration was observed for the remainder of the callus formation period.

In those cuttings which were collected from two year old stock plants on the 14/9/94, the concentration of ABA in the basal stem region increased significantly from 118.3 ng/g DW at the time of excision to 190.8 ng/g DW in week 1 (Figure IV.4.2.b). The concentration of endogenous ABA then decreased significantly from 190.8 ng/g DW in week 1 to 119.1 ng/g DW in week 2, which was a similar concentration to that observed at the time of excision. The concentration of ABA in the basal stem region decreased gradually from 119.1 ng/g DW in week 2 to 90.6 ng/g DW in week 8 before decreasing significantly to 70.2 ng/g DW in week 9. The concentration of ABA then increased significantly from 70.2 ng/g DW in week 9 to 102.5 ng/g DW in week 11.

In cuttings which were collected from two year old stock plants on the 26/10/94, the concentration of ABA in the basal stem region increased significantly from 132.0 ng/g DW at the time of excision to 181.2 ng/g DW after the first week (Figure IV.4.2.c). The concentration of endogenous ABA in the basal stem region decreased significantly from 181.2 ng/g DW in week 1 to 139.6 ng/g DW in week 2. An ABA concentration of 132.5 ng/g DW was observed in the week prior to cutting necrosis, which was similar to that observed at the time of cutting excision.

In those cuttings which were collected from two year old stock plants on the 7/12/94, the concentration of ABA in the basal stem region increased significantly from 121.9 ng/g DW at the time of excision to 161.6 ng/g DW in week 1 (Figure IV.4.2.d). The concentration of endogenous ABA in the basal stem region remained the approximately the same between weeks 1 and 2 before decreasing significantly from 161.1 ng/g DW in week 2 to 111.9 ng/g DW in week 3. The concentration of ABA did not change significantly between week 3 and 6, which was the last measurement recorded prior to cutting necrosis.

Therefore, in all cuttings collected on different dates the concentration of ABA increased significantly in first week following excision. In the following week, the concentration of ABA generally decreased to a concentration which was similar to that observed at the time of excision. In those cuttings which exhibited a high

rooting percentage, the concentration of ABA remained relatively unchanged after week 3, except for the significantly lower reading observed during week 9 after excision. In those cuttings which did not root or rooted poorly, the concentration of ABA the week prior to cutting necrosis was similar to that ABA concentration observed at the time of excision.

4.3.a.3 Zeatin Riboside

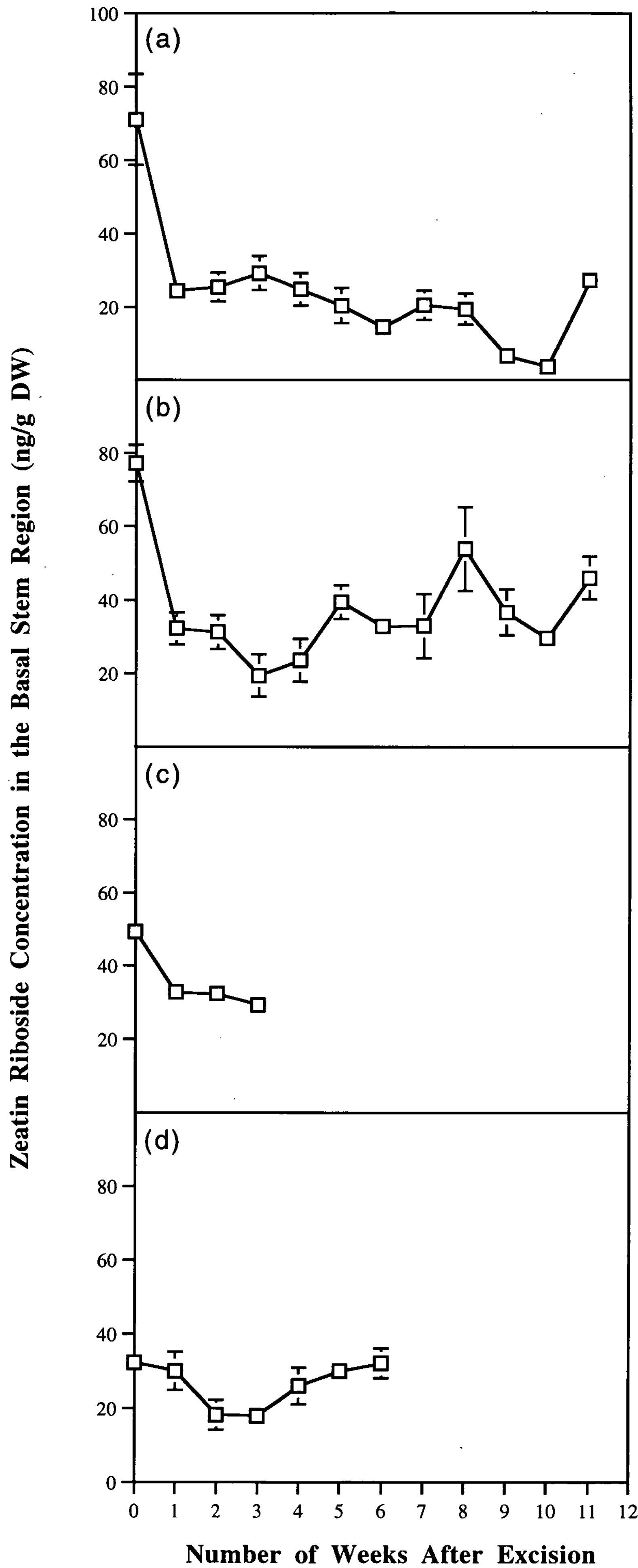
In those cuttings which were collected from two year old stock plants on the 3/8/94, the concentration of zeatin riboside (ZR) in the basal stem region decreased significantly from 71.1 ng/g DW at the time of excision to 24.4 ng/g DW in week 1 (Figure IV.4.3.a). The concentration of endogenous ZR in the basal stem region remained generally constant between weeks 1 and 8 before decreasing significantly from 19.6 ng/g DW in week 8 to 3.7 ng/g DW in week 10. The concentration of ZR then increased significantly from 3.7 ng/g DW in week 10 to 27.1 ng/g DW in week 11.

In those cuttings which were collected from two year old stock plants on the 14/9/94, the concentration of ZR in the basal stem region decreased significantly from 77.2 ng/g DW at the time of excision to 32.3 ng/g DW after the first week (Figure IV.4.3.b). The concentration of endogenous ZR in the basal stem region remained relatively constant between weeks 1 and 3 before increasing significantly from 19.4 ng/g DW in week 3 to 39.5 ng/g DW in week 5. The concentration of ZR then remained relatively constant between weeks 5 to 7 before increasing significantly from 33.0 ng/g DW in week 7 to 54.0 ng/g DW in week 8. The concentration of ZR decreased significantly from 54.0 ng/g DW in week 8 to 29.6 ng/g DW in week 10, before increasing significantly to 46.0 ng/g DW in week 11.

In cuttings which were collected from two year old stock plants on the 26/10/94, the concentration of ZR in the basal stem region decreased significantly from 49.3 ng/g DW at the time of excision to 32.8 ng/g DW after the first week (Figure IV.4.3.c). The concentration of endogenous ZR in the basal stem region remained relatively constant between weeks 1 and 3, prior to cutting necrosis being observed in week 4 .

In cuttings which were collected from two year old stock plants on the 7/12/94, the concentration of ZR in the basal stem region did not change significantly in the

Figure IV.4.3 - Change in the mean concentration of endogenous zeatin riboside (ZR) and associated standard error, in the basal stem region of *P. radiata* cuttings during the callus formation period. The cuttings were collected from two year old stock plants at Westerway at four different times in the season; (a) cuttings collected 3/8/94 ($LSD_{0.05}$=13.7), (b) cuttings collected 14/9/94 ($LSD_{0.05}$=17.2, (c) cuttings collected 26/10/94 ($LSD_{0.05}$=12.8) and (d) cuttings collected 7/12/94 ($LSD_{0.05}$=11.4) (data was obtained from Appendix 18).


(a)
(b)
(c)
(d)
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10 11 12
Zeatin Riboside Concentration in the Basal Stem Region (ng/g DW)
Number of Weeks After Excision

first week following excision (Figure IV.4.3.d). However, the concentration of ZR decreased significantly from 30.1 ng/g DW in week 1 to 18.2 ng/g DW in week 2 The concentration of endogenous ZR in the basal stem region remained relatively constant between weeks 2 and 3 before increasing significantly from 17.9 ng/g DW in week 3 to 30.0 ng/g DW in week 5. The concentration of ZR remained relatively constant between weeks 5 to 6, prior to cutting necrosis being observed in week 7.

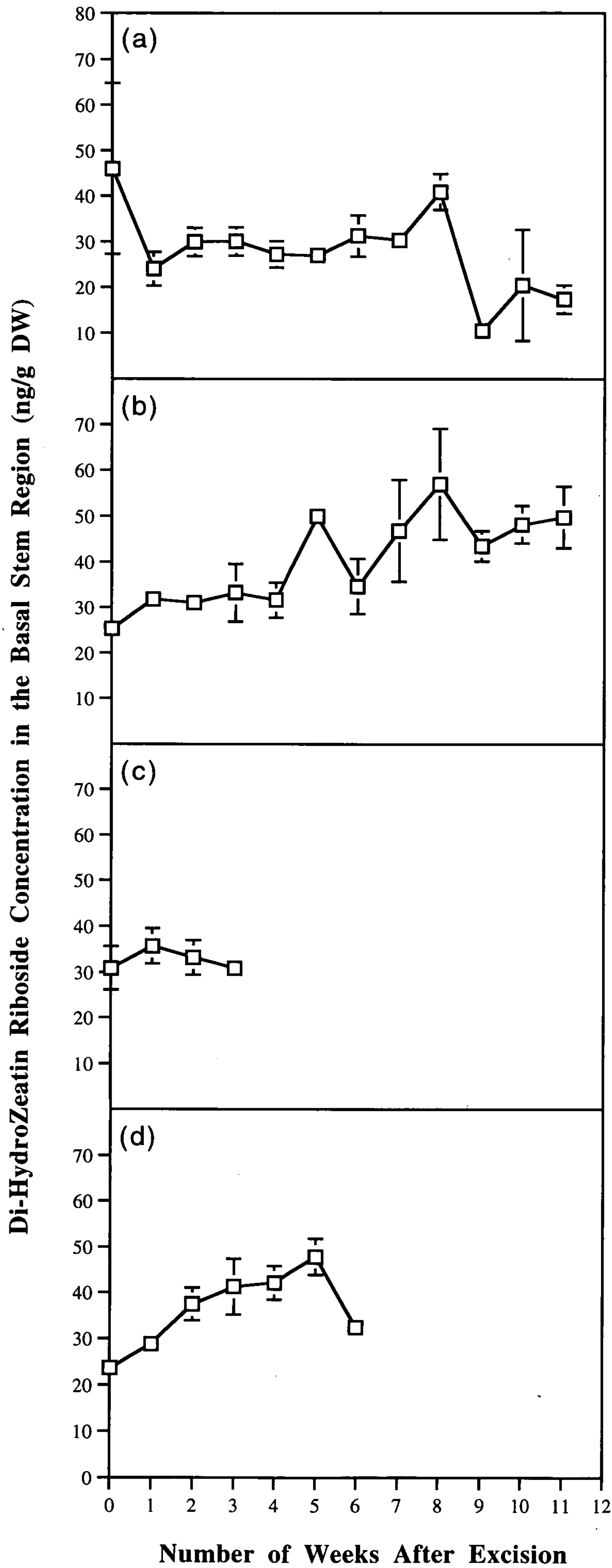
In summary, a higher concentration of ZR was observed at the time of excision in cuttings collected on the 3/8/94 and 14/9/94, compared to cuttings collected on the 26/10/94 and the 7/12/94. The concentration ZR decreased within the first week following excision, where the most pronounced decrease in ZR was observed in cuttings which displayed a high rooting percentage. In those cuttings which displayed a low rooting percentage, prior to cutting necrosis there were no significant differences in the concentration of ZR in comparison to those cuttings which displayed a high rooting percentage. In those cuttings which displayed a high rooting percentage, the concentration of ZR in the basal region of the cutting decreased significantly between week 8 and 10.

4.3.a.4 Dihydrozeatin Riboside

In those cuttings which were collected from two year old stock plants on the 3/8/94, the concentration of dihydrozeatin riboside (DHZR) in the basal stem region decreased significantly from 46.0 ng/g DW at the time of excision to 24.1 ng/g DW by week 1 (Figure IV.4.4.a). The concentration of endogenous DHZR in the basal stem region remained relatively constant between weeks 1 and 8 before decreasing significantly from 40.9 ng/g DW in week 8 to 10.5 ng/g DW in week 9. After week 9, no further change was observed in the concentration of DHZR during the remainder of the callus formation period.

In those cuttings which were collected from two year old stock plants on the 14/9/94, the concentration of DHZR in the basal stem region did not change significantly during the first 4 weeks after excision (Figure IV.4.4.b). The concentration of DHZR increased significantly from 31.6 ng/g DW in week 4 to 49.9 ng/g DW at week 5. However, the concentration of DHZR in the basal stem region did not change significantly during the remainder of the callus formation period.

Figure IV.4.4 - Change in the mean concentration of endogenous dihydrozeatin riboside (DHZR) and associated standard error, in the basal stem region of *P. radiata* cuttings during the callus formation period. The cuttings were collected from two year old stock plants at Westerway at four different times in the season; (a) cuttings collected 3/8/94 ($LSD_{0.05}$=20.0), (b) cuttings collected 14/9/94 ($LSD_{0.05}$=17.3), (c) cuttings collected 26/10/94 (no significant difference) and (d) cuttings collected 7/12/94 ($LSD_{0.05}$=10.3) (data was obtained from Appendix 19).


(a)
(b)
(c)
(d)
Di-HydroZeatin Riboside Concentration in the Basal Stem Region (ng/g DW)
Number of Weeks After Excision
0
10
20
30
40
50
60
70
80
1
2
3
4
5
6
7
8
9
10
11
12

In cuttings which were collected from two year old stock plants on the 26/10/94, no significant change in the concentration of DHZR was observed between the time of excision to the week prior to cutting necrosis (Figure IV.4.4.c).

In cuttings which were collected from two year old stock plants on the 7/12/94, the concentration of DHZR in the basal stem region increased significantly from 23.6 ng/g DW at the time of excision to 47.8 ng/g DW in week 5 (Figure IV.4.4.d). However, the concentration of endogenous DHZR in the basal stem region decreased significantly from 47.8 ng/g DW in week 5 to 32.4 ng/g DW in the week prior to cutting necrosis .

It was difficult to draw many conclusions from the DHZR results because cuttings, which were collected from 2 year old stock plants on the 3/8/94 and the 14/9/94 when the rooting percentage was high, exhibited markedly different trends in the concentration of DHZR during the callus formation period. The only similarity between the two cutting collection dates was the fact that the DHZR concentration in the basal stem region did decrease between week 8 and 9 after excision. In those cuttings which exhibited a low rooting percentage, the concentration of DHZR in the week prior to necrosis displayed no apparent difference in comparison to the DHZR concentration observed in cuttings which exhibited a high rooting percentage.

4.3.b Effect of Parent Stock Plant Age

The rooting percentage of cuttings which were collected from two and nine year old stock plants on the 3/8/94, after 16 weeks of growth in the phytotron, was 91% and 30% respectively. In cuttings which were collected from two year old stock plants, visible callus tissue (SRG=1) was observed on the base of cuttings at approximately week 8 or 9 after setting. All cutting mortality was observed in the weeks prior to callus formation. In cuttings which were collected from nine year old stock plants on the 3/8/94, leaf chlorosis was observed in approximately 20% of cuttings 4 weeks after setting. The cuttings which displayed signs of leaf chlorosis in week 4 were entirely necrotic 5 weeks after excision. However, during week 5 more cuttings appeared chlorotic and the cycle continued during weeks 5 to 9 until all cuttings allocated for PGR sampling had become necrotic, at which point no further samples were collected for PGR analysis. The samples collected during week 9 after setting displayed no visible signs of adventitious root development.

4.3.b.1 Auxin

The concentration of IAA in the basal stem region of cuttings collected from nine year old stock plants did not change significantly during the 9 week period following excision (Figure IV.4.5).

The concentration of IAA in cuttings collected from two year old stock plants was significantly higher than that observed in cuttings collected from nine year old stock plants only during week 2 and 3 after setting (Figure IV.4.5). At all other times during the period of callus formation no significant difference was observed in the concentration of IAA in the basal stem region of cuttings collected from two and nine year old stock plants.

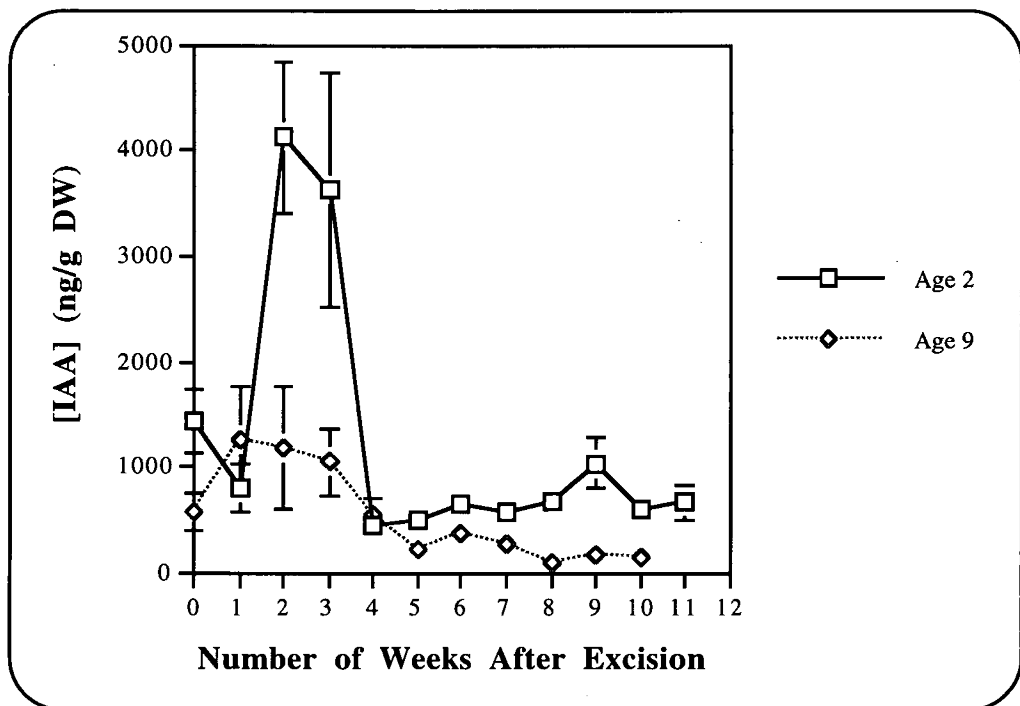


Figure IV.4.5 - Change in the mean concentration of endogenous indole-3-acetic acid (IAA), and associated standard error, in the basal stem region of *P. radiata* cuttings during the callus formation period. The cuttings were collected from two and nine year old stock plants, at Westerway and National Park respectively, on the 3/8/94 (interaction between age and week $LSD_{0.05}$=980.8) (data included in Appendix 16).

4.3..b.2 Abscisic Acid

The concentration of ABA in the basal stem region of cuttings collected from nine year old stock plants increased significantly during the first week after setting (Figure IV.4.6). However, the concentration of ABA decreased significantly from 129.7 ng/g DW in week 1 to 70.5 ng/g DW in week 2 before increasing significantly again to 114.5 ng/g DW in week 3. The concentration of ABA did not change significantly between weeks 3 and 4, but decreased significantly from 103.1 ng/g DW in week 4 to 78.5 ng/g DW in week 5. The concentration of ABA decreased gradually, but significantly, from 78.5 ng/g DW in week 5 to 55.3 ng/g DW in week 10.

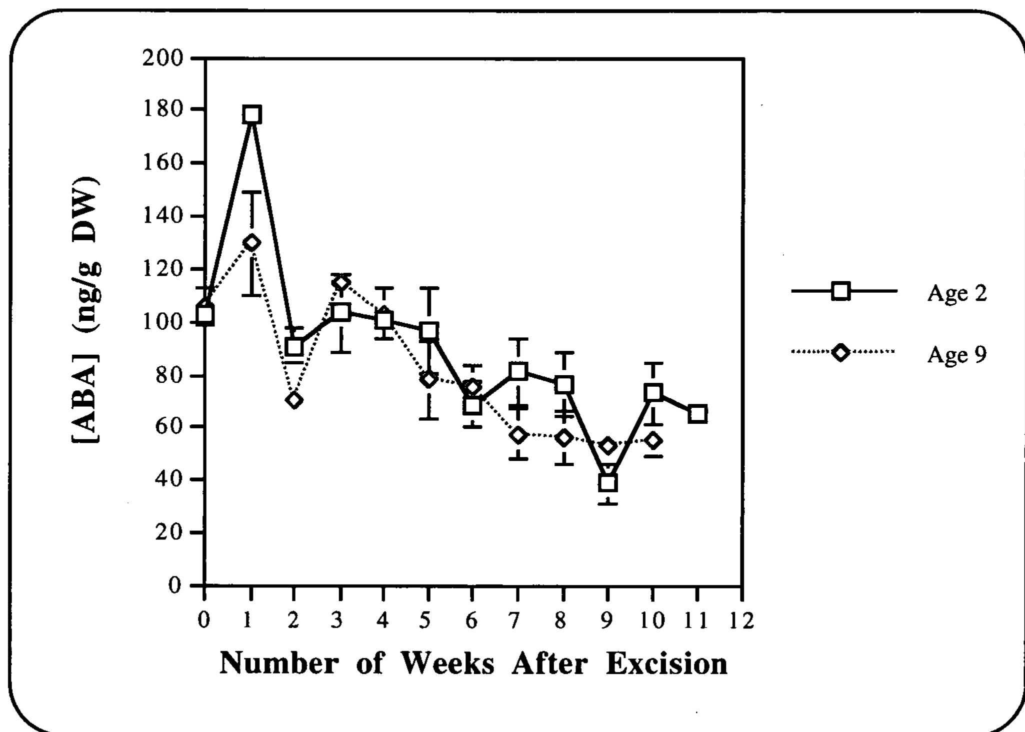


Figure IV.4.6 - Change in the mean concentration of endogenous abscisic acid (ABA), and associated standard error, in the basal stem region of *P. radiata* cuttings during the callus formation period. The cuttings were collected from two and nine year old stock plants, at Westerway and National Park respectively, on the 3/8/94 (difference between weeks $LSD_{0.05}$=20.0; difference between age $LSD_{0.05}$=21.16) (data included in Appendix 17).

The concentration of ABA in cuttings collected from nine year old stock plants was significantly higher than that observed in cuttings collected from nine year old stock plants in the first week after excision only (Figure IV.4.6). The concentration of ABA in cuttings collected from nine year old stock plants did not differ significantly from the concentration of ABA measured in cuttings collected from two year old stock plants at any other time after excision.

4.3.b.3 Zeatin Riboside

The concentration of ZR in the basal stem region of cuttings collected from nine year old stock plants did not change significantly during the first five weeks after excision (Figure IV.4.7). Although a significant difference was observed in the concentration of ZR between week 2 (18.2 ng/g DW) and week 6 (6.6 ng/g DW), generally no significant changes in the concentration of ZR were observed during the callus formation period.

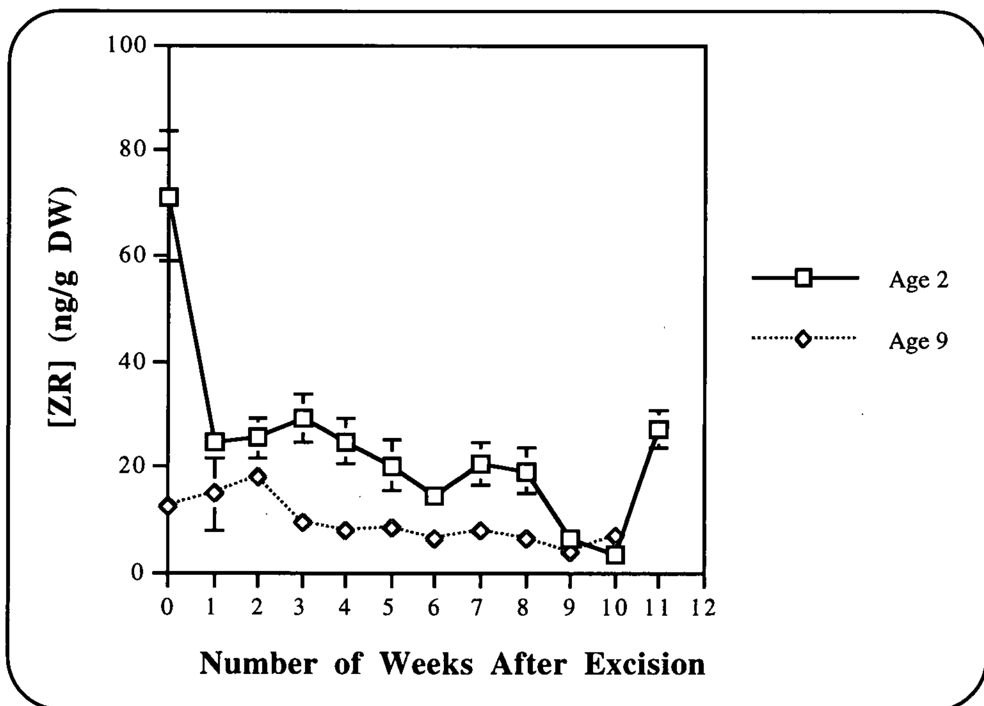


Figure IV.4.7 - Change in the mean concentration of endogenous zeatin riboside (ZR), and associated standard error, in the basal stem region of *P. radiata* cuttings during the callus formation period. The cuttings were collected from two and nine year old stock plants, at Westerway and National Park respectively, on the 3/8/94 (interaction between age and week $LSD_{0.05}$=11.2) (data included in Appendix 18).

The concentration of ZR in cuttings collected from 2 year old stock plants was significantly higher than that observed in cuttings collected from nine year old stock plants at the time of excision, 71.1 and 12.5 ng/g DW respectively (Figure IV.4.7). The concentration of ZR in the basal stem region did not differ significantly between the cuttings collected from two and nine year old stock plants during weeks 1, 2, 6, 9 and 10 after excision . However, during weeks 3, 4, 5, 7 and 8 after excision the concentration of ZR in the basal stem region was significantly higher in cuttings collected from two year old stock plants compared with the concentration of ZR observed in cuttings collected from nine year old stock plants.

4.3.b.4 Dihydrozeatin Riboside

The concentration of DHZR in the basal stem region of cuttings collected from nine year old stock plants did not change significantly during the callus formation period (Figure IV.4.8).

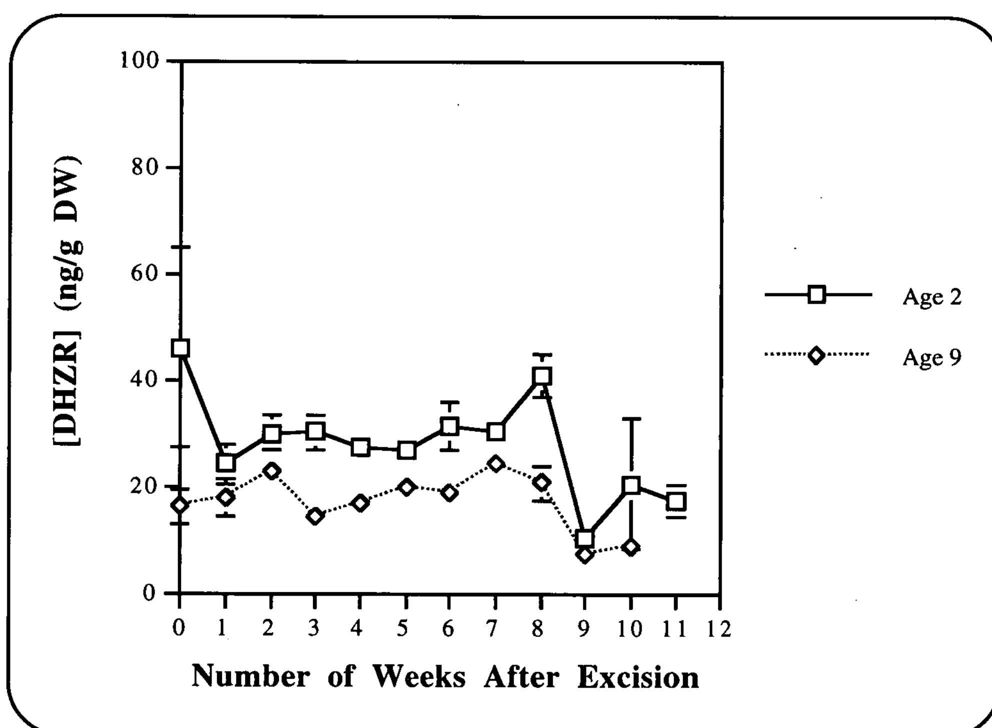


Figure IV.4.8 - Change in the mean concentration of endogenous dihydrozeatin riboside (DHZR), and associated standard error, in the basal stem region of *P. radiata* cuttings during the callus formation period. The cuttings were collected from two and nine year old stock plants, located at Westerway and National Park respectively, on the 3/8/94 (difference between weeks $LSD_{0.05}$=15.38; difference between age $LSD_{0.05}$=10.87) (data included in Appendix 19).

The concentration of DHZR in cuttings collected from nine year old stock plants was significantly higher than that observed in cuttings collected from nine year old stock plants at the time of excision and at week 1, 2, 6 and 9 after excision (Figure IV.4.8). At all other times after excision, the concentration of DHZR did not differ significantly between cuttings collected from both two and nine year old stock plants.

4.4 Discussion

In those cuttings which were collected from two year old stock plants on the 3/8/94 and the 14/9/94 where the rooting percentage was 91 and 76% respectively, a 'peak' in endogenous IAA was observed in weeks 2 and 3 after excision. This was not observed in cuttings collected from two year old stock plants on the 26/10/94 and the 7/12/94 or in cuttings collected from nine year old stock plants on the 3/8/94, where the rooting percentage of cuttings was low. The concentration of auxin during ARF was considered to be important to the rooting success of *P. radiata* cuttings. A 'peak' of endogenous auxin has been reported previously as being the first biochemical event occurring prior to adventitious root initiation in *Phaseolus aureus* hypocotyl cuttings (Blakesley *et. al.*, 1991b). In a previous section of this thesis (Section IV.3), anatomical studies revealed that visible signs of ARF were only observed after week 5. This indicated that the 'peak' in endogenous IAA may be an important factor which may be associated with the ARF of *P. radiata* cuttings. For this reason, the concentration of IAA in *P. radiata* cuttings will be investigated in future studies.

The concentration of ABA in the basal stem region of cuttings collected from two and nine year old stock plants at different times in the year displayed no pronounced differences. A 'peak' in the concentration of ABA was generally observed in the first week following excision. This 'peak' in endogenous ABA concentration may be associated with a wound response, which has been reported in many other plant species (Leopold and Kriedmann, 1985). The similar concentration of ABA, in cuttings which differed greatly in their rooting percentage, indicated that ABA may not be associated with the ARF of *P. radiata* cuttings. In those cuttings which were collected from two year old stock plants on the 3/8/94 and the 14/9/94 where the rooting percentage was 91 and 76% respectively, a significantly lower ABA concentration was observed in the basal stem region was in week 9 after excision. This may be related to the appearance of tracheid nests within the callus tissue, which was observed in week 11 (Section IV.3).

The concentration of ZR at the time of excision was higher in cuttings which were collected from 2 year old stock plants on the 3/8/94 and the 14/9/94, compared to cuttings collected on the 26/10/94 and the 7/12/94. After excision, the concentration of ZR decreased significantly in all cuttings collected from two year old stock plants. The decrease in concentration may reflect either an increased catabolism of cytokinins or decreased synthesis possibly due to the removal of the site of cytokinin biosynthesis, which may be the existing root system (Van Staden and Harty, 1988). The similar concentrations of ZR, in cuttings which differed greatly in their rooting percentage, indicated that ZR may not be associated with the ARF of *P. radiata* cuttings. The concentration of ZR in the basal region of cuttings collected from nine year old stock plants was generally lower than that observed in the basal region of cuttings collected from two year old stock plants. In general, the concentration of ZR in cuttings collected from nine year old stock plants did not change significantly throughout the callus formation period In those cuttings which were collected from 2 year old stock plants on the 3/8/94 and the 14/9/94 where the rooting percentage was 91 and 76% respectively, a significantly lower ZR concentration in the basal stem region was observed in week 9 and 10 after excision. A similar finding was demonstrated with ABA. This may be related to the appearance of tracheid nests within the callus tissue, which was observed in week 11 (Section IV.3).

It was difficult to draw many conclusions from the DHZR results, based on the findings of this experiment. In those cuttings which were collected from two year old stock plants on the 3/8/94, the change in the concentration of DHZR was similar to that observed in the concentration change of ZR. However, the concentration of DHZR in cuttings collected from two year old stock plants on the 14/9/94 was markedly different, even though the rooting percentage was high in both collection dates. Although the concentration of DHZR differed markedly, the rooting percentage of cuttings remained similar. This indicated that DHZR may not be associated with the ARF of *P. radiata* cuttings. The concentration of DHZR in the basal region of cuttings collected from nine year old stock plants did not change significantly throughout the callus formation period. Also, the concentration of DHZR in cuttings collected from nine year old stock plants was generally lower than that observed in cuttings collected from two year old stock plants.

The ratio of different PGR's were examined in detail, however, no significant relationship between the ratios of PGR's and the rooting percentage were observed in this study.

In conclusion, the concentration of PGR's measured at the time of excision did not relate well to the rooting ability of cuttings collected at different times in the year and from different aged stock plants. However, significant changes in PGR concentrations were observed during the callus formation period. This indicated that the changes in concentration which occur during ARF may be more important than the concentration of PGR measured at the time of excision. The rooting percentage of *P. radiata* cuttings may be somewhat dependent upon the concentration of the PGR auxin in the root formation zone. The concentration of auxin in *P. radiata* cuttings will be investigated in later experiments.

IV.5 Carbohydrate Metabolism

5.1 Introduction

The concentration of carbohydrates in *P. radiata* cuttings was suggested as one physiological factor which may influence ARF. In a review which considered the relationship between carbohydrate content and subsequent ARF, Veierskov (1988) stated that the initial carbohydrate content of a cutting must be sufficient to supply the cutting with energy reserves throughout the rooting period. However, Hansen *et al.* (1978) demonstrated that a high carbohydrate level may not be associated with an increased rooting success of cuttings . The ease of translocation and metabolism enable carbohydrates to be used as the main source of carbon in the growth of a new adventitious root system. Therefore, the initial level of carbohydrate in the cutting and the metabolism of carbohydrates during ARF would appear crucial to the successful development of an adventitious root system. In *P. radiata* cuttings, Cameron and Rook (1974) demonstrated that the rate of respiration increased during ARF, indicating also that the rate of carbohydrate metabolism may have increased during ARF. Therefore, the successful development of an adventitious root system would appear to be dependant on the carbohydrate metabolism during ARF.

The aim of this experiment was to examine differences in carbohydrate content of cuttings and changes in concentration which may occur during ARF using a population of cuttings which was known to have a high rooting percentage and compare these with changes in carbohydrate concentration in a population of cuttings where the rooting percentage is known to be low. The comparison of carbohydrate concentrations between populations of high and low rooting percentage will provide valuable data to assist in an understanding of ARF in *P. radiata*.

5.2 Materials and Methods

5.2.a Experimental Design

Two experiments were designed to investigate the change in carbohydrate concentration of *P. radiata* cuttings during ARF. In the first experiment, cuttings were collected from two year old stock plants, at Westerway, Tasmania, at four different times (six week intervals) in the year when the rooting percentage was known to differ within the same age (3rd of August, 14th of September, 26th of October and 7th of December: Section IV.1). The cuttings were collected at dawn, transported to the phytotron and set in standard root trainer cells and rooting media and propagated under standard conditions (Section III.2.2). In the second experiment, cuttings were collected from two year old stock plants, at Westerway, and nine year old stock plants, at National Park, at a time in the year when the rooting percentage of cuttings was known to differ between different ages (August 3rd: Section IV.1). The cuttings were collected at dawn, transported to the phytotron and set in standard root trainer cells and rooting media and propagated under standard conditions (Section III.2.2).

At the time of cutting collection and at weekly intervals thereafter, basal stem and basal leaf samples were collected for the determination of tissue carbohydrate concentration. In basal stem samples, each sample unit consisted of five 1cm basal stem segments collected from different cuttings which were combined and snap frozen in liquid nitrogen prior to being freeze dried and stored at -20°C until analysis (Section III.6). Each week, a total of five replicates of each sample unit were collected for the determination of carbohydrate concentration. In basal leaf samples, each sample unit consisted of the leaf material which was carefully removed from the five 1cm basal stem sample and combined and snap frozen in liquid nitrogen prior to being freeze dried and stored at -20°C until analysis (Section III.6). The concentration of starch in the basal leaf tissue was determined at the time of cutting severance and in the first week following excision.

5.2.b Soluble Carbohydrate Assay

The quantitative analysis of soluble carbohydrate levels in *Pinus radiata* tissue using an automated gas liquid chromatographic (GLC) procedure has been described previously (Cranswick and Zabkiewicz, 1979; Cranswick *et al.*, 1987). Soluble

carbohydrate levels have also been quantified in *Pinus sylvestris* L., *Pinus taeda* L. and other woody species using a gas chromatographic (GC) procedure (Sundberg *et. al.*, 1993; Tschaplinski *et. al.*, 1993; Tschaplinski *et. al.*, 1995). A gas chromatography linked to a mass spectrometer (GC-MS) technique was used to quantify and identify trimethylsilyl (TMS) derivatives of soluble sugar extracts from *P. radiata* tissue.

The collection and storage of tissue prior to carbohydrate analysis has been described previously (Section III.6). Freeze dried and ground plant tissue (0.2g) was weighed into a 20ml glass scintillation vial with a screw cap lid (Kimble) and arabinose standard (1ml of 1mg/ml), trehalose standard (1ml of 1mg/ml) and 60% ethanol (18ml) added. The vials were then incubated in a shaking water bath at 60°C for 8 hours. After incubation the organic phase was removed in a Speed-Vac concentrator (Savant, USA) and the remaining aqueous phase washed with diethyl ether (2 x 5ml). After removal of the ether phase the aqueous extract was filtered (Whatman No. 1, 12.5cm circles) into a 10ml volumetric flask, rinsing the filter paper several times using distilled water. The filter paper containing the insoluble plant material was retained for starch analysis (Section IV.5.2.d). The extract in the volumetric flask was made up to 10mls using distilled water, capped and homogenised. An aliquot (5µL) was removed and placed into a 150µL glass GC insert which was held inside a normal GC vial and freeze dried. Samples were stored in a desiccator prior to GC-MS analysis.

The freeze-dried soluble carbohydrate extract was silylated with 20µL of Tri-Sil 'Z'® (Pierce Chemical Co., Rockford, Illinois) capped and incubated at 60°C for 10 minutes and placed in a Hewlett Packard 7673A series autosampler to await GC injection. 1µL was injected into a Hewlett Packard 5890A series gas chromatograph linked to a Hewlett Packard 5970 series Mass Selective Detector. The GC was fitted with a HP-1 column, (cross-linked methyl silicon gum) 25m length x 0.32mm internal diameter x 0.52µm film thickness. The head pressure was 15psi, with column a flow rate of 5mL/min at 60°C, splitless flow and helium carrier gas. The oven temperature was increased from 50°C to 150°C at a rate of 30°C/minute and from 150°C to 300°C at a rate of 15°C/minute where it remained at 300°C for 4 minutes. The injector temperature was 260°C and the detector temperature was 290°C. Compounds were detected in selected ion monitoring (SIM) mode (characteristic ions of carbohydrates, 204-arabinose and glucose, 318-pinitol, sequoitol, myo-inositol and pinpollitol, 361-sucrose and trehalose and 437-fructose)

with dwell time of 40 milliseconds per ion. The solvent delay was 6.5 minutes. All peaks eluted between 6.5 and 16.5 minutes were integrated with Hewlett Packard ChemStation software. Peaks were initially identified by GC-MS. The quantities (mg/g dry weight) of components in the sample was calculated from the weight of the extract and the peak area relative to a known amount of internal standard (ratio analysis). Arabinose was used as the monosaccharide internal standard and trehalose was used as the disaccharide internal standard (ie. double internal standard method).

5.2.c Validation of Soluble Carbohydrate Technique

An initial investigation indicated that TMS derivatives of disaccharide carbohydrates degraded into undetectable compounds at a slow rate. The TMS derivatives of monosaccharide carbohydrates were observed to be stable with respect to time. Because a difference in stability was observed between monosaccharide and disaccharide carbohydrates TMS derivatives, a double internal standard technique was employed. The ratio of extract monosaccharide peak area to the monosaccharide internal standard (arabinose) peak area was used to quantify the concentration of monosaccharide (glucose, fructose, pinitol, myo-inositol, sequoitol and pinpollitol) carbohydrate in the extract. The ratio of extract disaccharide (sucrose) peak area to the disaccharide internal standard (trehalose) peak area was used to quantify the concentration of disaccharide (sucrose) carbohydrate in the extract. The decomposition of disaccharide TMS derivatives trehalose and sucrose, relative to the monosaccharide carbohydrates in the extract, occurred at approximately the same rate. The rate of disaccharide TMS derivative decomposition was demonstrated to be moisture sensitive, therefore GC vials were capped tightly to minimise decomposition prior to GC injection.

A linear relationship between carbohydrate concentration and peak area was observed in aqueous extract samples from *P. radiata* using the GC-MS method of analysis. The percentage recovery of each soluble carbohydrate was calculated from standard additions (Table IV.5.1).

Carbohydrate Name	Percentage Recovery
sucrose	80.0
glucose	77.9
fructose	78.3
myo-inositol	75.8

Table IV.5.1 - Percentage recovery of soluble carbohydrates from *P. radiata* tissue.

5.2.d Starch Assay

Starch levels were assayed enzymatically (total starch assay kit, Megazyme Pty. Ltd., Australia). The procedure used was as follows; 100mg of dry ground tissue retained from the soluble sugar assay (Section IV.5.2.a) was weighed into a 20ml glass centrifuge tube with a 10.0ml volumetric marking. To ensure complete removal of all residual soluble sugars in the sample 5ml of 80% aqueous ethanol (v/v) was added and incubated at 80-85°C for 5 minutes. Contents were vortexed and a further 5ml of 80% aqueous ethanol added. The tube was centrifuged at 3000rpm for 10 minutes and the supernatant discarded. The pellet was resuspended in 10ml of 80% aqueous ethanol, vortexed, recentrifuged at 3000rpm for 10 minutes and the supernatant again discarded. 3ml of thermostable α-amylase (EC 3.2.1.1; 300 units; Megazyme Australia Pty. Ltd.) in MOPS buffer (50mM, pH 7.0) was added and vortexed vigorously prior to incubating at 100°C for 6 minutes, vortexing vigorously again every 2 minutes. The tubes were then transferred into a 50°C water bath and 4ml of sodium acetate buffer (200mM, pH 4.5) added followed by 0.1ml of amyloglucosidase (EC 3.2.1.3; 20 units; Megazyme Australia Pty. Ltd.). The contents of the tube were vortexed and incubated at 50°C for 30 minutes. After incubation the volume of the tube contents was adjusted to the 10.0ml volumetric mark using distilled water. The tube was then vortexed and centrifuged at 3000rpm for 10 minutes. Duplicate aliquots of the tube contents (0.1ml) were transferred to the bottom of glass test tubes (16x100mm). 3.0ml of GOPOD reagent (glucose oxidase, >12 000units/L; Peroxidase, >650units/L; 4-Aminoantipyrine, 0.4mM; Megazyme Australia Pty. Ltd.) was added to each tube, including the glucose standard solution (0.1ml, 1mg/mL; Megazyme Pty. Ltd.) and the reagent blank (0.1mL, distilled water). The tubes were incubated at 50°C for 20 minutes and the absorbance of each sample, including the glucose standard were read against the

reagent blank at 510nm with a spectrophotometer (Shimadzu). Calculation of percentage starch and mg/g dry weight starch in the sample was achieved using the following equations:

%Starch **= ΔE x F x 100 x 1/1000 x 100/W x 162/180**

where :

ΔE	= absorbance of sample read against the reagent blank
F	= 100(μg of glucose)/absorbance for 100μg of glucose, conversion from absorbance to μg
100	= volume correction (0.1mL taken from 10mL)
1/1000	= conversion from μg to mg
W	= the weight in mg of tissue analysed
100/W	= factor to express starch as a percentage of dry weight
162/180	= adjustment from free glucose to anhydro glucose, as occurs in starch

%Starch = ΔE x F/W x 9
Starch (mg/g) = %Starch x 10

A starch standard (regular maize starch, known starch content = 98% dry weight; Megazyme Australia Pty. Ltd.) was run initially to validate the technique. The calculated starch content of the standard was 97.5%.

5.3 Results

5.3.a Effect of Cutting Collection at Different Times in the Year

The rooting percentage of cuttings collected from two year old stock plants on 3/8/94, 14/9/94, 26/10/94 and 14/12/94 after 16 weeks of growth in the phytotron was 91, 76, 0 and 11% respectively (Section IV.2.3.a). In cuttings which were collected on the 3/8/94 and 14/9/94, visible callus tissue (SRG=1) was observed approximately 8 or 9 weeks after setting. All cutting mortality occurred in the weeks prior to callus formation (SRG=1). In cuttings which were collected on 26/10/96, the leaves of all cuttings became chlorotic 3 weeks after excision. In the 4th week after excision, tissue necrosis and secondary infection was observed in all cuttings and no further samples were collected for carbohydrate analysis. In the cuttings which were

collected on the 14/12/94, leaf chlorosis was observed in approximately 30% of cuttings 4 weeks after excision. The cuttings which were chlorotic in week 4 were entirely necrotic by week 5, however, at week 5 more cuttings had become chlorotic. The cycle continued during weeks 5 to 7 until all cuttings allocated for carbohydrate sampling were necrotic, at which point no further samples were collected for carbohydrate analysis.

The components of the soluble carbohydrate fraction were sucrose, glucose, fructose, pinitol, sequoitol, myo-inositol and pinpollitol (Figure IV.5.1). The concentration of total carbohydrate in the basal stem region of the cutting is presented graphically by adding together the concentration of total soluble carbohydrate and starch in the basal stem region of the cutting (Figure IV.5.1). At the time of excision (week 0), the total carbohydrate content in the basal stem region of the cutting was the highest in cuttings collected on 26/10/94 (Figure IV.5.1.c: 137.6mg/g DW), and the lowest in cuttings collected on the 3/8/94 (Figure IV.5.a: 60.1mg/g DW). In those cuttings which exhibited a high rooting percentage, the concentration of total carbohydrate in basal stem region generally decreased from the time of excision (week 0), until visible callus tissue was observed on the base of the cutting (week 8 to 9) (Figures IV.5.1.a and IV.5.1.b). In those cuttings which exhibited a low rooting percentage, the initial concentration of total carbohydrate in the basal stem region of the cutting was high (Figures IV.5.1.c and IV.5.1.d). However, the concentration of total carbohydrate in these cuttings decreased rapidly soon after excision. The concentration of total carbohydrate observed in the basal stem region of these cuttings prior to necrosis was similar to that observed in the population of cuttings which survived and rooted.

The total soluble carbohydrate fraction was calculated by adding together the individual concentrations of sucrose, glucose, fructose, pinitol, sequoitol, myo-inositol and pinpollitol in each sample. In general, the concentration of total soluble carbohydrate closely mirrored the changes which occurred in the total carbohydrate levels (Figure IV.5.2). The concentration of starch in the basal stem region of the cutting at the time of excision varied markedly between two year old cuttings collected on different dates (Figure IV.5.2). The concentration of starch was the highest in cuttings collected on 14/9/94 (Figure IV.5.2.b: 42.3mg/g DW) and the lowest in cuttings collected on 3/8/94 (Figure IV.5.2.a: 10.3mg/g DW). However, the concentration of starch in the basal stem region of the cutting decreased significantly, within a week of setting, to a similar basal concentration in all cuttings

Figure IV.5.1 - Change in the mean concentration of starch, sucrose, glucose, fructose, pinitol, sequoitol, myo-inositol and pinpollitol during the callus formation period in *P. radiata* cuttings collected from 2 year old stock plants at four different times in the year; **(a)** cuttings collected on the 3rd of August 1994 (winter), **(b)** cuttings collected on the 14th of September 1994 (early-spring) **(c)** cuttings collected on the 26th of October (mid-spring) and **(d)** cuttings collected on the 7th of December (summer). Cuttings were propagated under controlled environment conditions in the phytotron (mean data, standard errors and LSD's included in Appendices 20, 22, 23 and 24).

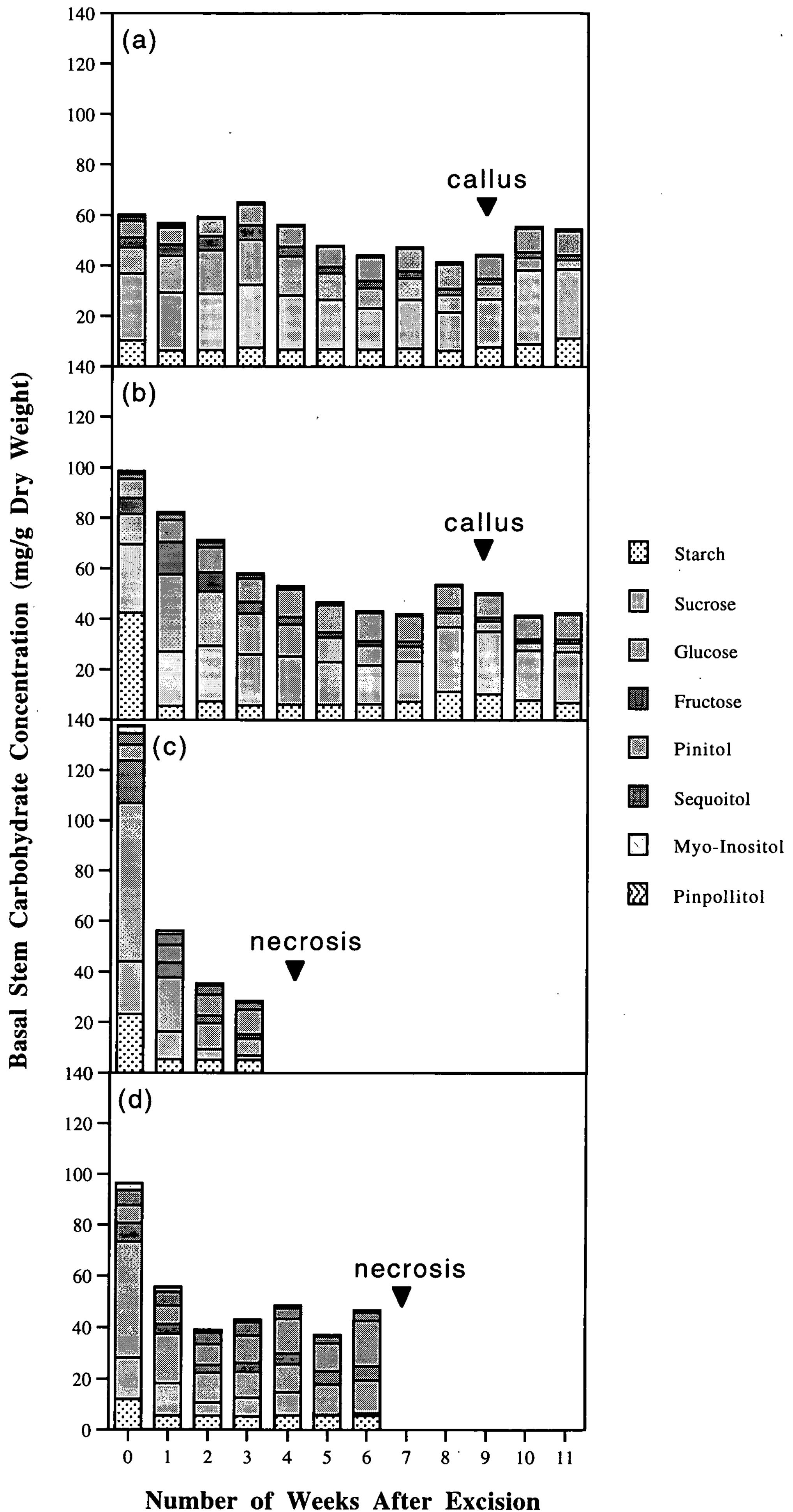
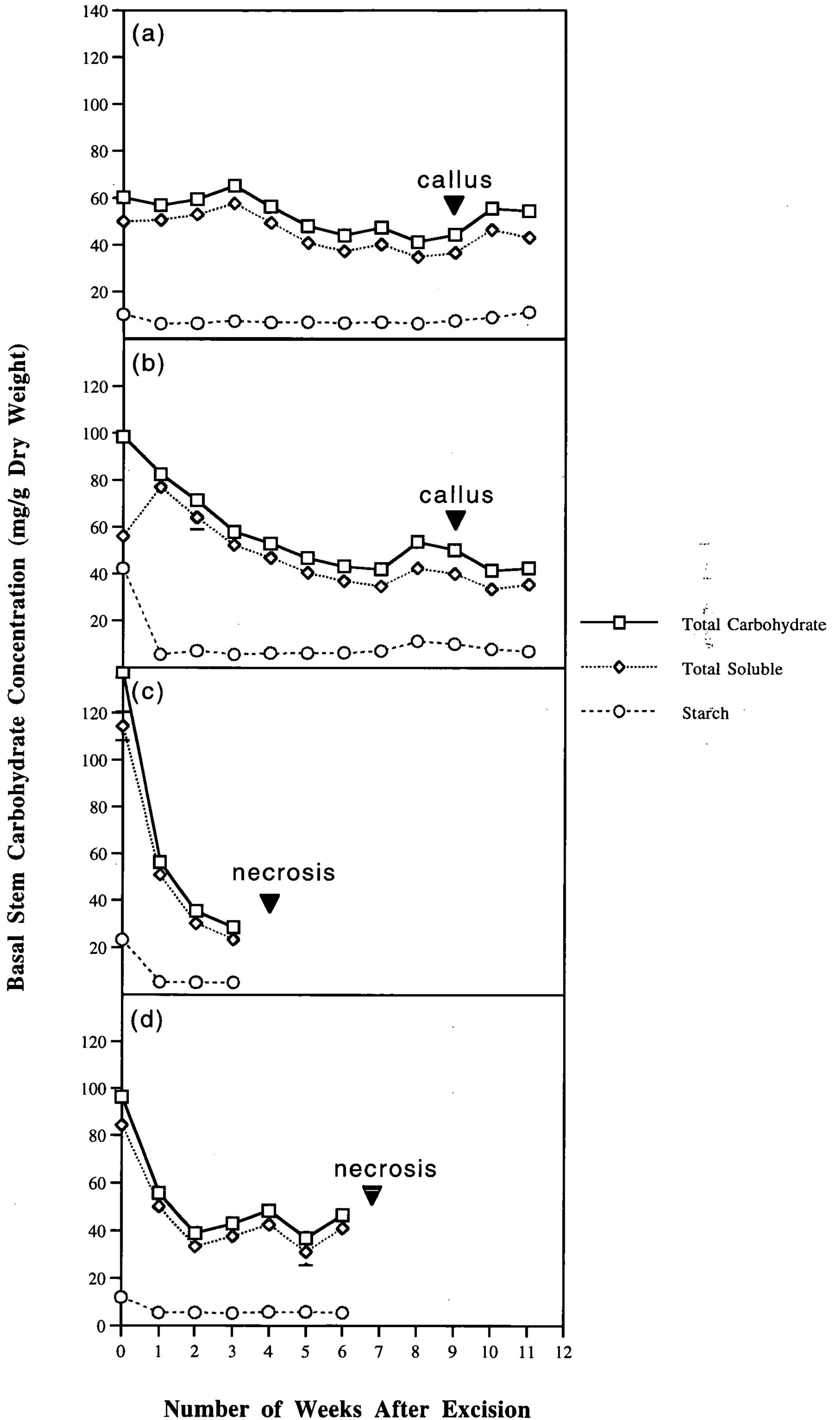

(a)
callus
(b)
callus
(c)
necrosis
(d)
necrosis
Basal Stem Carbohydrate Concentration (mg/g Dry Weight)
Number of Weeks After Excision
0
1
2
3
4
5
6
7
8
9
10
11
0
20
40
60
80
100
120
140
Starch
Sucrose
Glucose
Fructose
Pinitol
Sequoitol
Myo-Inositol
Pinpollitol

Figure IV.5.2 - Change in the mean concentration of total carbohydrate, total soluble carbohydrate and starch (including standard error) during the callus formation period in *P. radiata* cuttings collected from 2 year old stock plants at different times in the year; **(a)** cuttings collected on the 3rd of August 1994 (winter; $LSD_{0.05}$=6.172 for total soluble and 1.138 for starch), **(b)** cuttings collected on the 14th of September 1994 (early-spring; $LSD_{0.05}$=7.217 for total soluble and 2.264 for starch) **(c)** cuttings collected on the 26th of October (mid-spring; $LSD_{0.05}$=10.807 for total soluble and 2.344 for starch) and **(d)** cuttings collected on the 7th of December (summer; $LSD_{0.05}$=8.264 for total soluble and 1.446 for starch). Cuttings were propagated under controlled environment conditions in the phytotron (mean data, standard errors and LSD's included in Appendices 20, 22, 23 and 24).

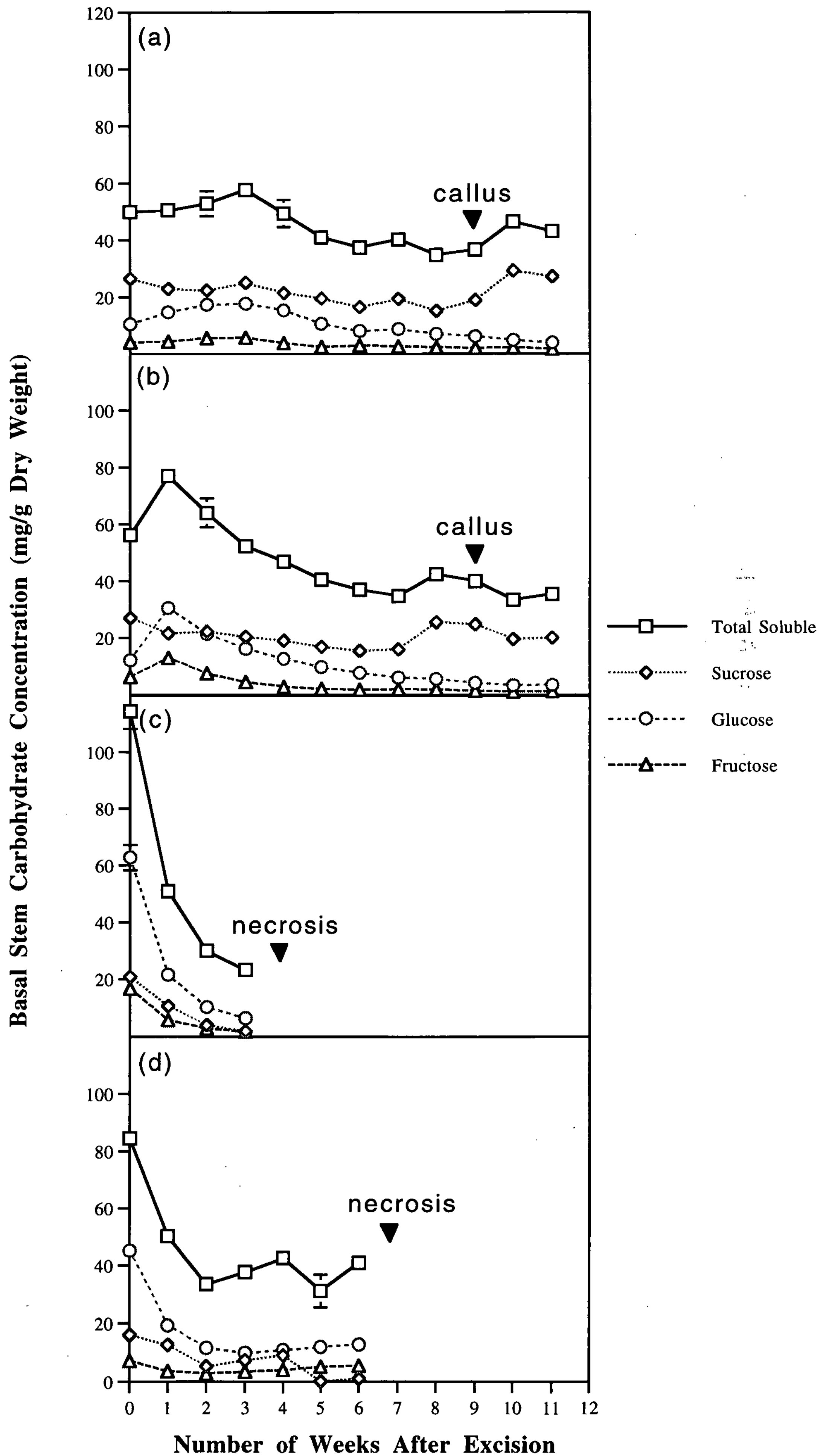

(a)
callus
(b)
callus
(c)
necrosis
(d)
necrosis
Basal Stem Carbohydrate Concentration (mg/g Dry Weight)
Number of Weeks After Excision
Total Carbohydrate
Total Soluble
Starch

collected at different dates. There was no further decrease in starch concentration below the concentration of approximately 5.5mg/g DW. A similar decrease in starch concentration was observed during the first week in the basal leaves of cuttings collected at different dates (Appendices 20, 22, 23 and 24). This indicated that the initial concentration of total carbohydrate at the time of excision was not limiting to the growth and development of the adventitious root system. In fact, the concentration of total carbohydrate was higher at the time of excision in cuttings which exhibited a low rooting percentage (Figures IV.5.2.c and IV.5.2.d), than in those cuttings which exhibited a high rooting percentage (Figures IV.5.2.a and IV.5.2.b). Therefore, the change in carbohydrate concentration during the callus formation period may be more important than the absolute concentration at the time of excision. The results illustrated in Figure IV.5.2 demonstrate that the change in the soluble carbohydrate concentration was mainly responsible for the change observed in the total carbohydrate concentration.

The examination of the major soluble carbohydrate components (sucrose, glucose and fructose) indicated that the concentration of sucrose during ARF may be important for the successful development of an adventitious root system. In those cuttings which were collected from two year old stock plants and exhibited a high rooting percentage, the lowest concentration of sucrose observed in cuttings during the callus formation period was approximately 15mg/g DW (Figures IV.5.3.a and IV.5.3.b). The change in the concentration of total soluble carbohydrate, which was observed between weeks 7 and 11, was mainly attributed to changes which occurred in the concentration of sucrose (Figures IV.5.3.a and IV.5.3.b). In those cuttings which exhibited a low rooting percentage, the concentration of sucrose in the basal stem region of the cutting decreased from approximately 18.5mg/g DW at the time of excision to approximately 1.4mg/g in the week prior to tissue necrosis (Figures IV.5.3.c and IV.5.3.d). The low concentration of sucrose observed in the week prior to tissue necrosis indicated that the concentration of sucrose may be limiting to ARF in cuttings collected on the 26/10/94 and 7/12/94. Therefore, the maintenance of sucrose concentrations in the basal stem region above approximately 15mg/g DW may be important to the successful development of an adventitious root system.

At the time of excision, the concentration of glucose and fructose in the basal stem region was generally higher in cuttings which exhibited a low rooting percentage, than in cuttings which exhibited a high rooting percentage (Figure IV.5.3). This may indicate that high concentrations of glucose and fructose at the

Figure IV.5.3 - Change in the mean concentration of total soluble carbohydrate, sucrose, glucose and fructose (including standard error) during the callus formation period in *P. radiata* cuttings collected from 2 year old stock plants at different times in the year; **(a)** cuttings collected on the 3rd of August 1994 (winter; $LSD_{0.05}$=6.172 for total soluble, 4.035 for sucrose, 3.060 for glucose and 0.998 for fructose), **(b)** cuttings collected on the 14th of September 1994 (early-spring; $LSD_{0.05}$=7.217 for total soluble, 4.743 for sucrose, 3.894 for glucose and 1.439 for fructose) **(c)** cuttings collected on the 26th of October (mid-spring; $LSD_{0.05}$=10.807 for total soluble, 2.286 for sucrose, 7.137 for glucose and 3.892 for fructose) and **(d)** cuttings collected on the 7th of December (summer; $LSD_{0.05}$=8.264 for total soluble, 2.784 for sucrose, 3.492 for glucose and 1.785 for fructose). Cuttings were propagated under controlled environment conditions in the phytotron (mean data, standard errors and LSD's included in Appendices 20, 22, 23 and 24).


(a)
callus
(b)
callus
(c)
necrosis
(d)
necrosis
Basal Stem Carbohydrate Concentration (mg/g Dry Weight)
Number of Weeks After Excision
Total Soluble
Sucrose
Glucose
Fructose
0
20
40
60
80
100
120
0 1 2 3 4 5 6 7 8 9 10 11 12

time of excision are inhibitory to ARF. This will be investigated in future experiments. In general, the concentration of glucose in the basal stem region of cuttings was higher than the concentration of fructose. However, the concentration of glucose and fructose changed in similar ways throughout the callus formation period. This indicated that the metabolism/catabolism of fructose and glucose are closely linked.

The other major component of the soluble carbohydrate fraction was pinitol. In those cuttings which exhibited a high rooting percentage, the concentration of pinitol in the basal stem region increased significantly between week 0 and week 11 (Figures IV.5.4.a and IV.5.4.b). In cuttings which exhibited a low rooting percentage, the concentration of pinitol increased significantly between week 0 and the week prior to tissue necrosis (Figures IV.5.4.c and IV.5.4.d). Bieleski (1994) demonstrated the importance of pinitol in other plant species as a compatible solute under conditions of water stress. The accumulation of pinitol in *P. radiata* cuttings during ARF may indicate that cuttings are in a state of water stress.

In general, sucrose, glucose, fructose and pinitol comprised over 90% of all the soluble carbohydrate fraction. The remainder of the soluble carbohydrate fraction consisted of the minor components, sequoitol, myo-inositol and pinpollitol. In general, the concentration of sequoitol and myo-inositol decreased significantly, from the time of cutting excision, during the callus formation period and at all dates which cuttings were collected (Figure IV.5.5). The concentration of sequoitol and myo-inositol was much higher in those cuttings which exhibited a low rooting percentage, compared to those cuttings which exhibited a high rooting percentage. The concentration of pinpollitol in the basal stem region increased significantly during the callus formation period for cuttings collected on all dates (Figure IV.5.5). The accumulation of pinpollitol during the period after setting indicated that metabolism/catabolism of pinpollitol may be similar to that of pinitol.

Figure IV.5.4 - Change in the mean concentration of total soluble carbohydrate and pinitol (including standard errors) during the callus formation period in *P. radiata* cuttings collected from 2 year old stock plants at different times in the year; **(a)** cuttings collected on the 3rd of August 1994 (winter; $LSD_{0.05}$=1.559 for pinitol), **(b)** cuttings collected on the 14th of September 1994 (early-spring; $LSD_{0.05}$=1.427 for pinitol) **(c)** cuttings collected on the 26th of October (mid-spring; $LSD_{0.05}$=0.959 for pinitol) and **(d)** cuttings collected on the 7th of December (summer; $LSD_{0.05}$=3.245 for pinitol). Cuttings were propagated under controlled environment conditions in the phytotron (mean data, standard errors and LSD's included in Appendices 20, 22, 23 and 24).

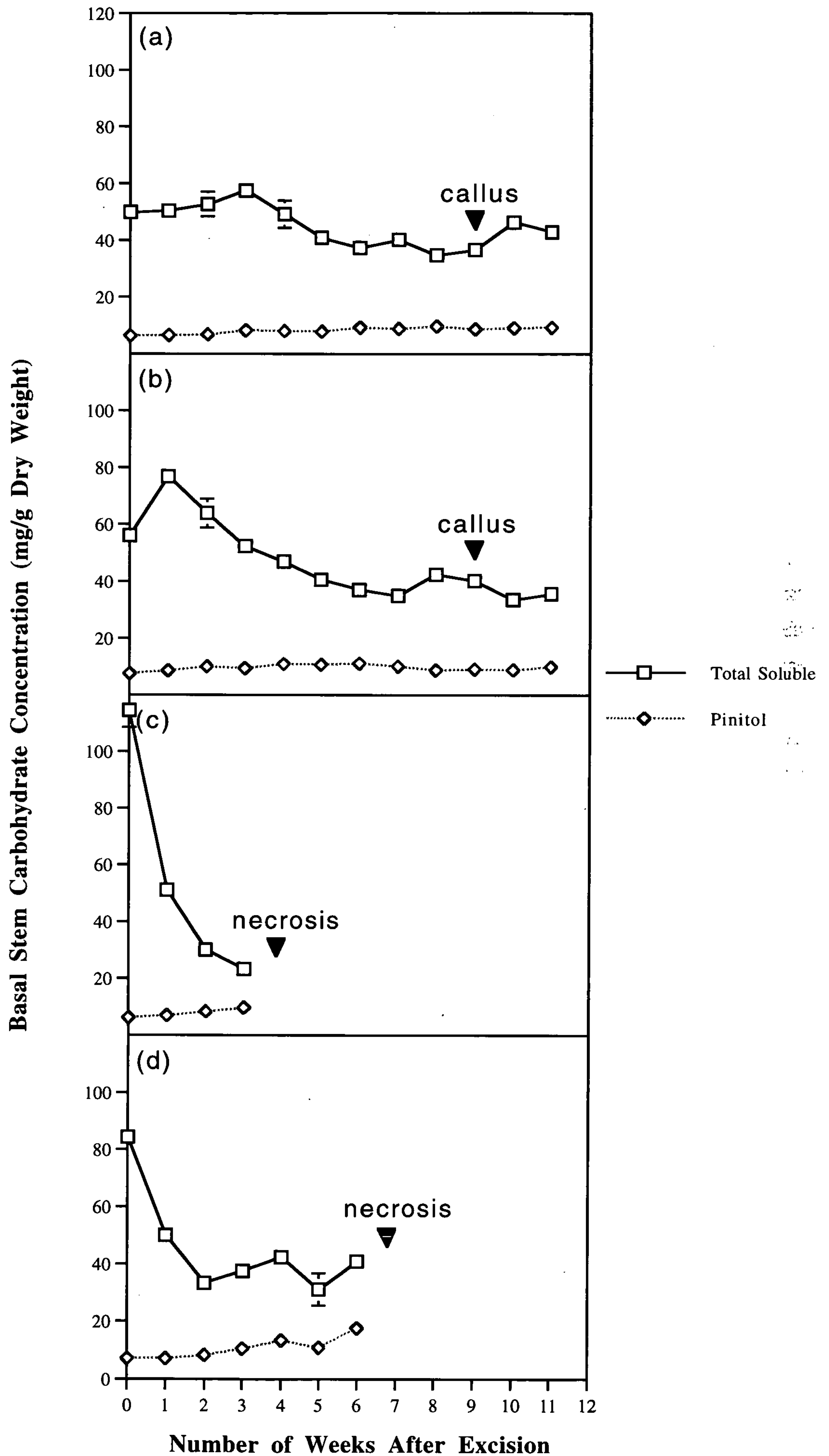
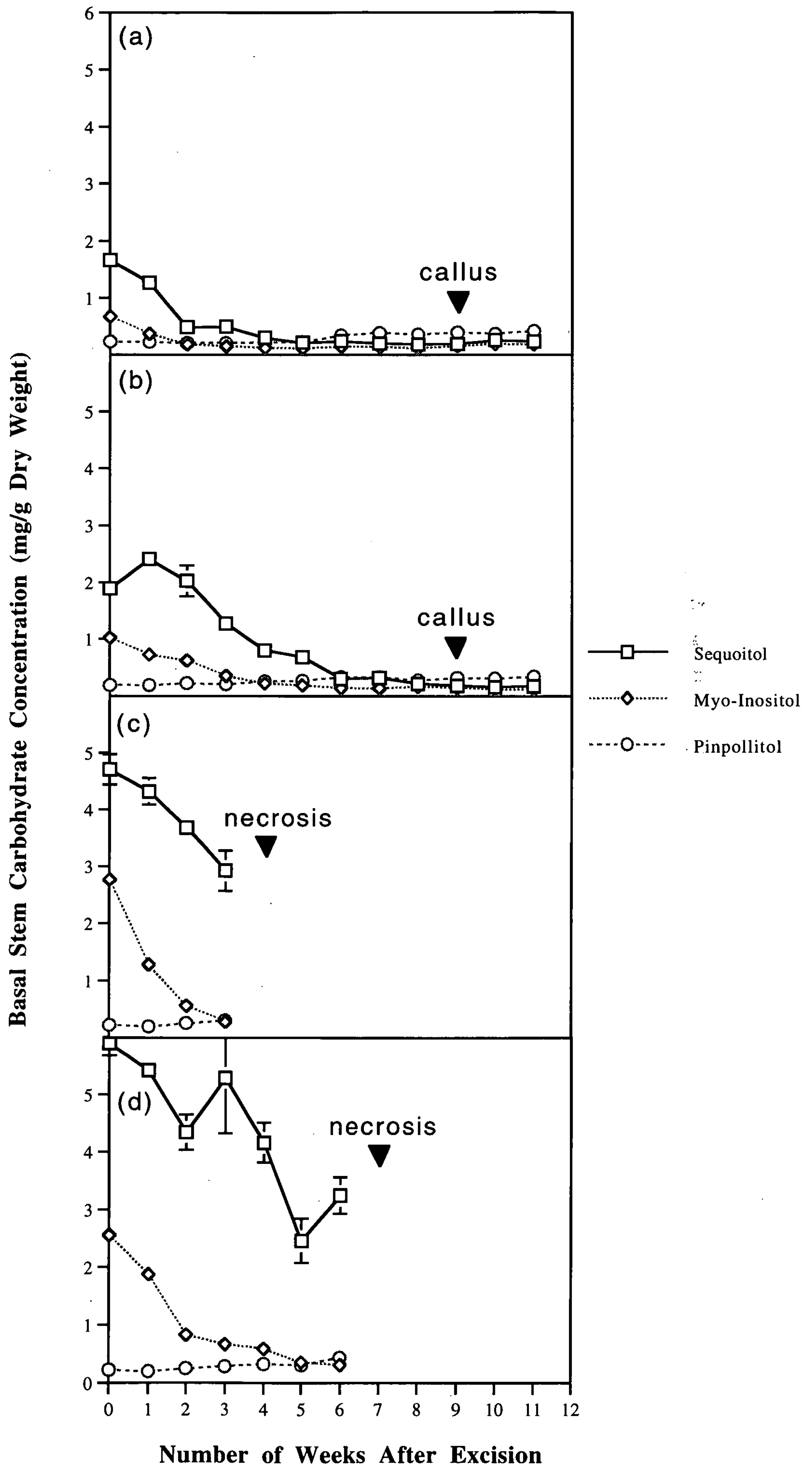

(a)
callus
(b)
callus
(c)
necrosis
(d)
necrosis
Basal Stem Carbohydrate Concentration (mg/g Dry Weight)
Number of Weeks After Excision
Total Soluble
Pinitol
0
20
40
60
80
100
120
0 1 2 3 4 5 6 7 8 9 10 11 12

Figure IV.5.5 - Change in the mean concentration of sequoitol, myo-inositol and pinpollitol (including standard errors) during the callus formation period in *P. radiata* cuttings collected from 2 year old stock plants at different times in the year; **(a)** cuttings collected on the 3rd of August 1994 (winter; $LSD_{0.05}$=0.198 for sequoitol, 0.040 for myo-inositol and 0.070 for pinpollitol), **(b)** cuttings collected on the 14th of September 1994 (early-spring; $LSD_{0.05}$=0.283 for sequoitol, 0.081 for myo-inositol and 0.057 for pinpollitol) **(c)** cuttings collected on the 26th of October (mid-spring; $LSD_{0.05}$=0.864 for sequoitol, 0.269 for myo-inositol and 0.044 for pinpollitol) and **(d)** cuttings collected on the 7th of December (summer; $LSD_{0.05}$=1.022 for sequoitol, 0.314 for myo-inositol and 0.058 for pinpollitol). Cuttings were propagated under controlled environment conditions in the phytotron (mean data, standard errors and LSD's included in Appendices 20, 22, 23 and 24).


(a)
callus
(b)
callus
(c)
necrosis
(d)
necrosis
Sequoitol
Myo-Inositol
Pinpollitol
Basal Stem Carbohydrate Concentration (mg/g Dry Weight)
Number of Weeks After Excision

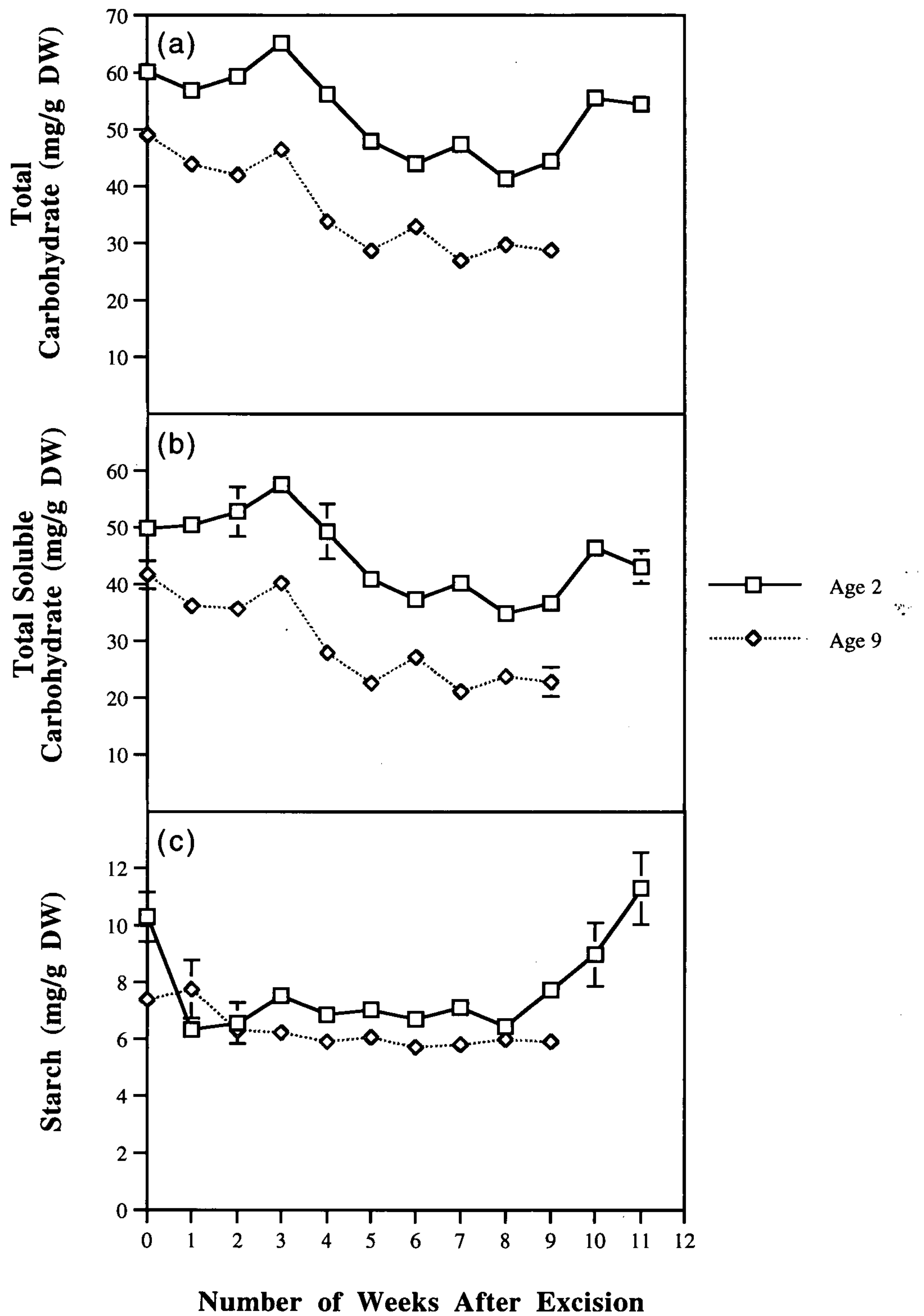
5.3.b Effect of Parent Stock Plant Age

The rooting percentage of cuttings which were collected from two and nine year old stock plants on the 3/8/94, after 16 weeks of growth in the phytotron, was 91% and 30% respectively. In cuttings which were collected from two year old stock plants, visible callus tissue (SRG=1) was observed on the base of cuttings at approximately week 8 or 9 after setting. All cutting mortality was observed in the weeks prior to callus formation. In cuttings which were collected from nine year old stock plants on the 3/8/94, leaf chlorosis was observed in approximately 20% of cuttings 4 weeks after excision. The cuttings which displayed signs of leaf chlorosis in week 4 were entirely necrotic 5 weeks after excision. However, during week 5 more cuttings appeared chlorotic and the cycle continued during weeks 5 to 9 until all cuttings allocated for carbohydrate sampling had become necrotic, at which point no further samples were collected for carbohydrate analysis. The samples collected during week 9 after excision displayed no visible signs of adventitious root development.

The concentration of total carbohydrate in the basal stem region of the cutting was generally higher in cuttings collected from two year old stock plants in comparison to those collected from nine year old stock plants at all times during the period of measurement (Figure IV.5.6.a). The concentration of total carbohydrate observed in cuttings collected from nine year old stock plants decreased from 49.0mg/g DW at the time of excision to 28.8mg/g DW in week 9. In cuttings collected from two year old stock plants on the same date, the concentration of total carbohydrate decreased between weeks 0 and 8. However, the concentration of total carbohydrate did increase from 41.3mg/g DW in week 8 to 55.4mg/g DW in week 10. In general, the concentration of total soluble carbohydrate observed during the callus formation period closely mirrored the changes which were observed in the total carbohydrate concentrations (Figure IV.5.6.b). This was observed in cuttings collected from both two and nine year old stock plants.

The concentration of starch in the basal stem region of cuttings collected from nine year old stock plants decreased at a slow rate, relative to cuttings collected from two year old stock plants, to a basal concentration of approximately 5mg/g DW which was significantly less than that observed in cuttings collected from two year old stock plants (Figure IV.5.6.c). In cuttings collected from two year old stock plants, the concentration of starch increased significantly from 6.4mg/g DW in week

Figure IV.5.6 - Change in the mean concentration of **(a)** total carbohydrate, **(b)** total soluble carbohydrate (age comparison $LSD_{0.05}$=5.587) and **(c)** starch (age comparison $LSD_{0.05}$=1.138) (including standard errors) during the callus formation period in *P. radiata* cuttings collected from stock plants which were two and nine years old on the 3/8/94 (mean data and standard errors included in Appendices 20 and 21).



(a)
Total Carbohydrate (mg/g DW)
70
60
50
40
30
20
10
(b)
Total Soluble Carbohydrate (mg/g DW)
60
50
40
30
20
10
(c)
Starch (mg/g DW)
12
10
8
6
4
2
0
0 1 2 3 4 5 6 7 8 9 10 11 12
Number of Weeks After Excision
Age 2
Age 9

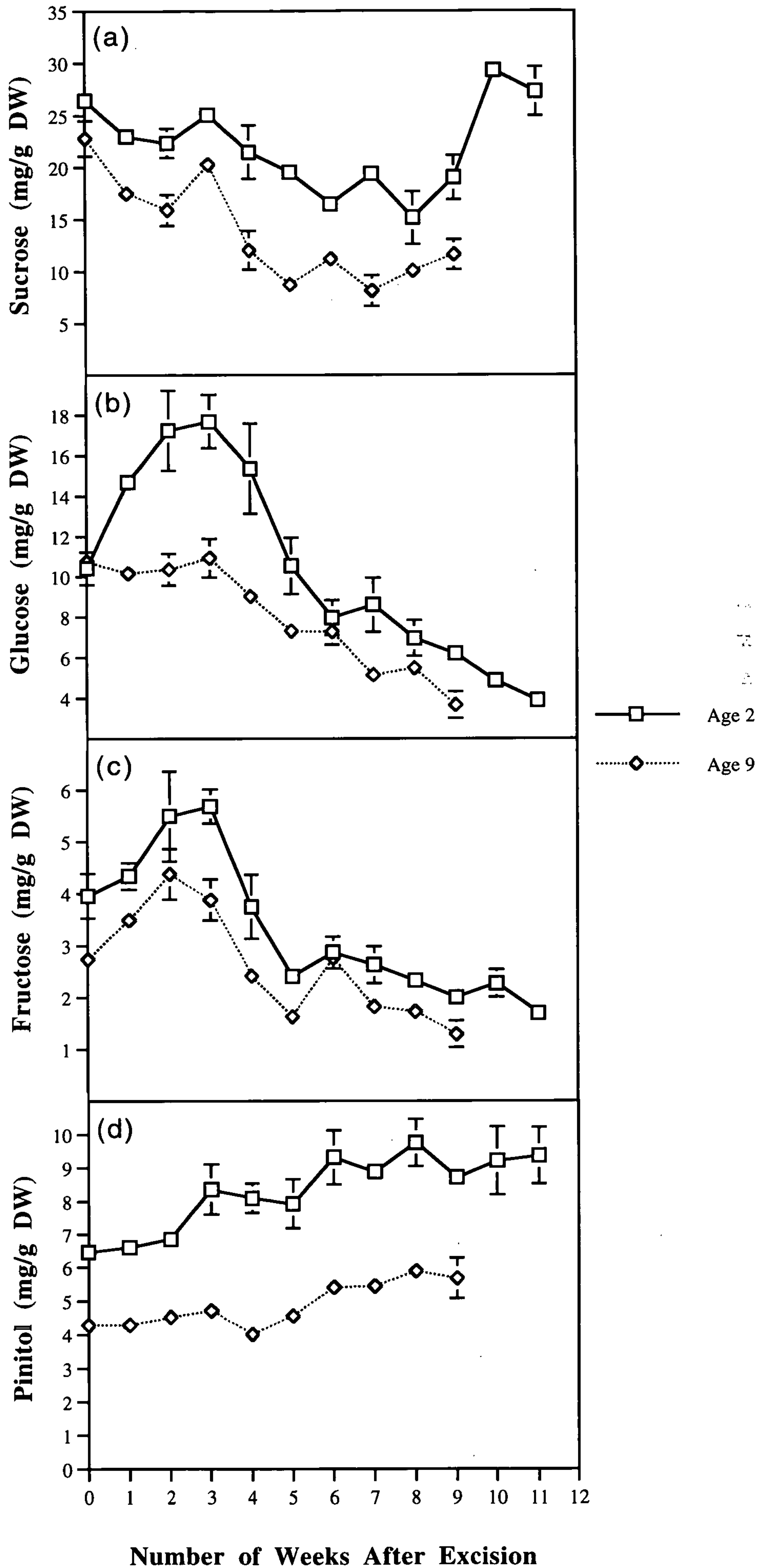
8 to 11.3mg/g DW in week 11 after setting. However, the concentration of starch in cuttings collected from nine year old stock plants did not change significantly between weeks 2 and 9 after setting.

The concentration of major soluble carbohydrate components, sucrose, glucose, fructose and pinitol, was always observed to be lower in cuttings collected from nine year old stock plants, compared to cuttings collected from two year old stock plants (Figure IV.5.7). The concentration of sucrose in the basal stem region of cuttings taken from nine year old stock plants decreased significantly from 22.8mg/g DW at the time of excision to 11.7mg/g DW at week 9 (Figure IV.5.7.a). In general, the concentration of sucrose was significantly higher in cuttings collected from two year old stock plants than in cuttings collected from nine year old stock plants (Figure IV.5.7.a). In cuttings collected from two year old stock plants, the concentration of sucrose was maintained above approximately 15mg/g DW throughout the entire callus formation period. This was similar to the result mentioned previously in Section IV.5.3.a. The successful growth and development of an adventitious root system in cuttings collected from nine year old stock plants may be inhibited because of a low sucrose concentration in the basal stem region of the cutting.

In cuttings collected from nine year old stock plants, the concentration of glucose in the basal stem region decreased significantly, at a relatively linear rate, from 10.7mg/g DW at the time of excision to 3.7mg/g DW by week 9 (Figure IV.5.7.b). In cuttings collected from two year old stock plants, the concentration of glucose increased significantly from 10.4mg/g DW at the time of excision to 17.7mg/g DW in week 3 after excision, before declining significantly at a relatively linear rate between week 3 and week 11 to a concentration of 3.9mg/g DW (Figure IV.5.7.b). The concentration of fructose was significantly higher in cuttings collected from two year old stock plants than in cuttings collected from nine year old stock plants (Figure IV.5.7.c). Although differing in absolute value, a similar trend in concentration of fructose was observed throughout the callus formation period in cuttings collected from both two and nine year old stock plants.

In cuttings collected from nine year old stock plants, the concentration of pinitol in the basal stem region increased significantly from 4.3mg/g DW at week 0 to 5.6mg/g DW at 9 weeks after excision (Figure IV.5.7.d). In cuttings collected from two year old stock plants, the concentration of pinitol in the basal stem region

Figure IV.5.7 - Change in the mean concentration of **(a)** sucrose (age comparison $LSD_{0.05}$=3.686), **(b)** glucose (age comparison $LSD_{0.05}$=2.595), **(c)** fructose (age comparison $LSD_{0.05}$=0.625) and **(d)** pinitol (age comparison $LSD_{0.05}$=0.795) (including standard errors) during the callus formation period in *P. radiata* cuttings collected from stock plants which were two and nine years old on the 3/8/94 (mean data and standard errors included in Appendices 20 and 21).

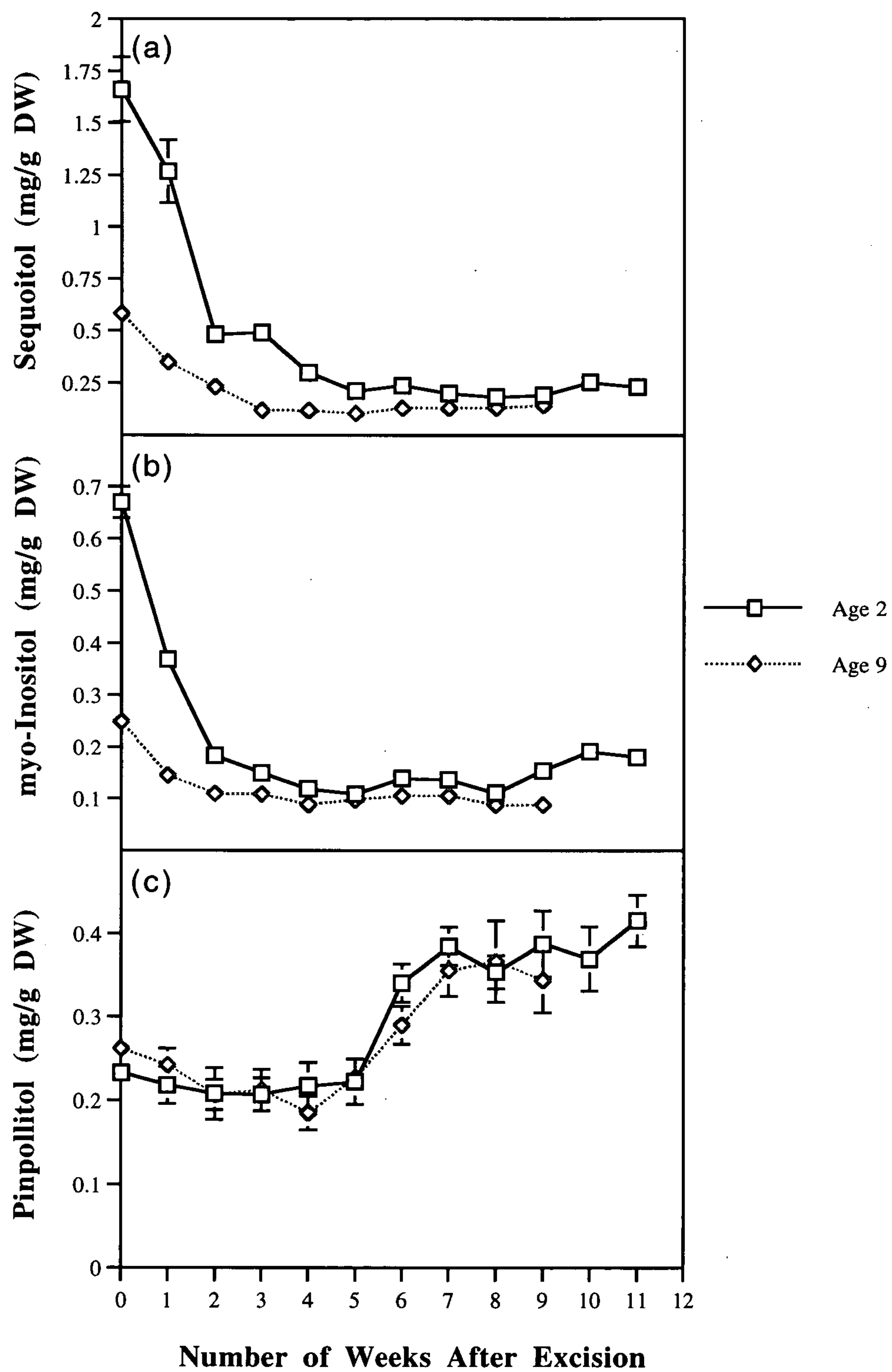

(a)
Sucrose (mg/g DW)
35
30
25
20
15
10
5
(b)
Glucose (mg/g DW)
18
16
14
12
10
8
6
4
(c)
Fructose (mg/g DW)
6
5
4
3
2
1
(d)
Pinitol (mg/g DW)
10
9
8
7
6
5
4
3
2
1
0
0 1 2 3 4 5 6 7 8 9 10 11 12
Number of Weeks After Excision
Age 2
Age 9

of the cutting increased significantly from 6.5mg/g DW at the time of excision to 9.4mg/g DW by week 11. The concentration of pinitol increased in cuttings collected from both two and nine year old stock plants, while the concentration of other major soluble carbohydrates generally declined. Again, this indicated that the carbohydrate pinitol may have a physiological function other than as a energy source during ARF.

The concentration of minor carbohydrates sequoitol and myo-inositol were significantly higher in cuttings collected from two year old stock plants, compared with those collected from nine year old stock plants (Figures IV.5.8.a and IV.5.8.b respectively). Although the initial concentration of sequoitol and myo-inositol was significantly higher in cuttings taken from two year old stock plants, the concentration decreased rapidly between the time of excision and week 5 to approximately the same concentration as that observed in cuttings collected from nine year old stock plants (Figures IV.5.8.a and IV.5.8.b). No further change was observed in the concentration of sequoitol or myo-inositol during the remaining weeks of measurement.

The concentration of pinpollitol in the basal stem region increased significantly between week 0 and week 9 in cuttings collected from both two and nine year old stock plants (Figure IV.5.8.c). However, no significant difference was observed between the concentration of pinpollitol in cuttings collected from different aged stock plants. Although the concentration of pinpollitol and pinitol differed in absolute value, a similar trend in concentration change was observed throughout the callus formation period.

Figure IV.5.8 - Change in the mean concentration of **(a)** sequoitol (age comparison $LSD_{0.05}$=0.155), **(b)** myo-inositol (age comparison $LSD_{0.05}$=0.040) and **(c)** pinpollitol (no significant differences p=0.468) (including standard errors) during the callus formation period in *P. radiata* cuttings collected from stock plants which were two and nine years old on the 3/8/94 (mean data and standard errors included in Appendices 20 and 21).



Sequoitol (mg/g DW)
myo-Inositol (mg/g DW)
Pinpollitol (mg/g DW)
(a)
(b)
(c)
2
1.75
1.5
1.25
1
0.75
0.5
0.25
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.4
0.3
0.2
0.1
0
0 1 2 3 4 5 6 7 8 9 10 11 12
Number of Weeks After Excision
Age 2
Age 9

5.4 Discussion

A poor relationship was observed between the initial concentration of carbohydrate (soluble carbohydrates and starch) and the rooting percentage of cuttings collected from both two and nine year old stock plants. In fact, the highest total carbohydrate concentration was observed in cuttings collected on the 26/10/94, when the rooting percentage of cuttings was the lowest. The concentration of carbohydrates measured at the time of excision did not appear to be a good indicator of rooting ability in *P. radiata* cuttings. However, the ability to change or maintain the concentration of different carbohydrates after setting may be more important than the absolute concentration of carbohydrates at the time of excision. A similar result was observed in the PGR studies (Section IV.4).

In cuttings collected from two year old stock plants which exhibited a high rooting percentage, the concentration of sucrose was maintained above approximately 15mg/g DW throughout the callus formation period. However, in cuttings collected from two year old stock plants which exhibited a low rooting percentage, the concentration of sucrose decreased rapidly soon after setting to a very low level in the week preceding cutting necrosis. Assuming that sucrose is being utilised as an energy source by the developing callus and that the main form of carbohydrate transported in the phloem of *P. radiata* is sucrose, then the maintenance of adequate sucrose concentrations in the basal region of cuttings would require the transport of sucrose from other regions within the cutting. In cuttings which exhibited a low rooting percentage, a problem associated with either the transport or mobilisation of carbohydrates from other regions within the cutting may limit the rooting success. This will be investigated in future experiments.

In cuttings collected from two and nine year old stock plants at different dates, the concentration of pinitol and pinpollitol was observed to increase significantly during the callus formation period. While pinitol and pinpollitol were being accumulated in the basal stem region of the cutting during ARF, other soluble carbohydrates were generally decreasing in concentration. This indicated that pinitol and pinpollitol may have a function other than as a source of metabolisable energy. In other plant species, Bieleski (1994) demonstrated the importance of pinitol as a compatible solute under conditions of water stress, through the maintenance of cellular osmotic potential. Therefore, the accumulation of pinitol and pinpollitol in *P. radiata* cuttings during ARF may be a response to water stress.

In general, the concentration of carbohydrates measured in the basal stem region of cuttings during ARF was lower in cuttings collected from nine year old stock plants than cuttings collected from two year old stock plants. This indicated that the low concentration of carbohydrate observed in the basal stem region of cuttings collected from nine year old stock plants may have been responsible for the low rooting percentage. While a lower concentration of carbohydrates (dry weight basis) were generally observed in cuttings collected from nine year old stock plants, the physiological concentrations may not have differed significantly from that observed in cuttings collected from two year old stock plants, because of the differences in secondary thickening of cuttings.

IV.6 Water Relations

6.1 Introduction

In Section IV.4 and IV.5, a common theme has emerged in the search for the physiological and/or anatomical (endogenous) factor responsible for the variation in rooting percentage of *P. radiata* cuttings. In searching for the endogenous factor, the concentration of PGR's or carbohydrates measured at the time of excision did not relate well to the rooting ability of cuttings. However, the ability to respond, as seen with the 'peak' in auxin concentration and now the ability to maintain adequate sucrose concentrations at the site of ARF, may be more important than the absolute concentration measured at the time of excision. The endogenous factor may be the ability to react to excision rather than being actually present at the time of excision.

The excision of a cutting from the stock plant removes the existing root system, and therefore the normal pathway of water and nutrient uptake. Although the root system may no longer exist, transpiration through the leaves will continue (Hartmann and Kester, 1983). In cuttings, the loss of water through transpiration may reduce the water content to such a low level that cuttings may fail to survive. In cuttings, the rate of water loss is mainly dependent on the difference in water vapour pressure between the leaf and the surrounding air (Andersen, 1986). This is also modified by the stomatal resistance of the cutting leaves. The level of irradiance has also been demonstrated to effect the rate of water loss through increasing temperature (Andersen, 1986). Cameron and Rook (1974) observed that *P. radiata* cuttings are often in a state of intense water stress at the time of setting (-2.5 to -3.0MPa). Cameron and Rook (1974) demonstrated that most of the water entry into *P. radiata* cutting occurred through the base of the cut stem and through the foliage in contact with the moist rooting medium. Maintenance of adequate inter- and intra-cellular water relation status within the cutting during ARF may play a significant role in influencing the final rooting percentage. Therefore the seasonal variation and stock plant age related effects observed in the rooting of *P. radiata* cuttings may be related to water relations during propagation. To extend the "developing model", the ability of cuttings to maintain adequate water relations during ARF may be more important than the water relations of cuttings at the time of excision.

The aim of this experiment was firstly to examine the water relations of cuttings collected from stock plants of the same age at different times in the year when the rooting percentage was known to differ, and secondly to examine the water relations of cuttings collected from different aged stock plants at a time in the year when the rooting percentage of cuttings was known to differ. A comparison of cutting water status, between cuttings which exhibit a high rooting percentage and cuttings which exhibit a low rooting percentage, may indicate why cuttings have problems with ARF at different times in the year and also between cuttings collected from stock plants of different ages. The examination of cutting water relations was conducted during the period of callus formation, since in previous studies, most of the cutting mortality was observed during this period.

6.2 Materials and Methods

6.2.a Experimental Design

Two experiments were designed to investigate the change in water relations of *P. radiata* cuttings during ARF . In the first experiment, cuttings were collected from two year old stock plants, at Westerway, Tasmania, at four different times in the year when the rooting percentage was known to differ within the same age (3rd of August, 14th of September, 26th of October and 7th of December: Section IV.1). The cuttings were collected at dawn, transported to the phytotron and set in standard root trainer cells and rooting media and propagated under standard conditions (Section III.2.2). In the second experiment, cuttings were collected from two year old stock plants, at Westerway, and nine year old stock plants, at National Park, at a time in the year when the rooting percentage of cuttings was known to differ between ages (August 3rd: Section IV.1). The cuttings were collected at dawn, transported to the phytotron and set in standard root trainer cells and rooting media and propagated under standard conditions (Section III.2.2).

6.2.b Measurement of Cutting Water Potential

At the time of cutting collection and at weekly intervals thereafter, 10 basal leaves were randomly sampled within each population and the leaf water potential (LWP) was measured using the pressure chamber technique (Section III.8). The readings were recorded in units of mega pascals (MPa) and expressed as a negative numerical value to reflect that water in the cutting was under tension. Water potential measurements were recorded pre-dawn in the field and after transport.

6.3 Results

6.3.a Stock Plant Water Potential

The water potential of the stock plants at the time of cutting excision may influence the rooting success of *P. radiata* cuttings. However, no relationship between the LWP and the rooting percentage was observed at different times in the year (Table IV.6.1). The LWP of two year old stock plants increased generally from approximately zero MPa on the 3/8/94 to -1.325MPa on the 7/12/94. However, the LWP at the time of excision was higher in those cuttings collected from two year old stock plants on the 26/10/94 (-0.635MPa), than those collected from two year old stock plants on the 14/9/94 (-0.965MPa), even though the rooting percentage was significantly lower in cuttings collected on the 26/10/94 than those collected on the 14/9/94. This suggested that the initial water content of cuttings at the time of excision was not a major determinant of the rooting success in *P. radiata* cuttings. The LWP of stock plants were measured at dawn, when the stock plant water potential would be in equilibrium with the soil water potential. Therefore, stock plant LWP measurements should also reflect the soil water potential at the time of cutting collection.

Collection Date	Stock Plant Age	Leaf Water Potential (MPa)	Rooting Percentage
3/8/94	2	0.000	91
3/8/94	9	-0.945	30
14/9/94	2	-0.965	76
26/10/94	2	-0.635	0
7/12/94	2	-1.325	11

Table IV.6.1 - The average LWP (MPa) of stock plants at the time of cutting collection, collected from two year old stock plants on the 3/8/94, 14/9/94, 26/10/94 and 7/12/94, and nine year old stock plants on the 3/8/94 (data included in Appendix 29).

The average LWP of nine year old stock plants was significantly lower than that observed in two year old stock plants measured on the same date (Table IV.6.1).

This corresponded to a lower rooting percentage in cuttings collected from nine year old stock plants. Although a significantly lower water potential was observed in cuttings collected from nine year old stock plants, this may only reflect a lower soil water potential. The poor relationship observed between stock plant water potential and the rooting percentage of cuttings collected from two year old stock plants at different times in the year, indicated that stock plant water potential may not influence the final rooting percentage.

6.3.b Changes in Water Potential during Transport

Despite care taken, the average LWP of cuttings, after being transported between the field and the phytotron, was always lower than that observed at the time of excision (Table IV.6.2). During transport, all cuttings remained in styrofoam transport containers for approximately the same period of time (2hours). The change in cutting LWP after transport was generally greater with higher temperatures (Table IV.6.2). The only exception was observed in cuttings collected on the 7/12/94, where the reduction in LWP after transport was not significant even though the cuttings were collected on the warmest day (Table IV.6.2). The change in LWP during transport did not relate well with the final rooting percentage, indicating that under the conditions employed in this study, water loss during transport did not exert a pronounced effect on the rooting percentage of the cuttings.

Collection Date	Initial LWP (MPa)	After Transport LWP (MPa)	Temperature min - max (°C)	Rooting Percentage
3/8/94	0.000 a	-0.025 a	2-11	91
14/9/94	-0.965 a	-2.003 b	8-18	76
26/10/94	-0.635 a	-1.278 b	11-21	0
7/12/94	-1.325 a	-1.408 a	14-33	11

Table IV.6.2 - The change in average LWP of cuttings collected from two year old stock, at different times in the year, after being transported between the field and the phytotron. During transport all cuttings remained in styrofoam transport boxes for approximately the same period of time (2 hours). The temperature on the day of cutting collection increased significantly between the 3/8/94 and the 7/12/94. Significance is indicated using (a, b).

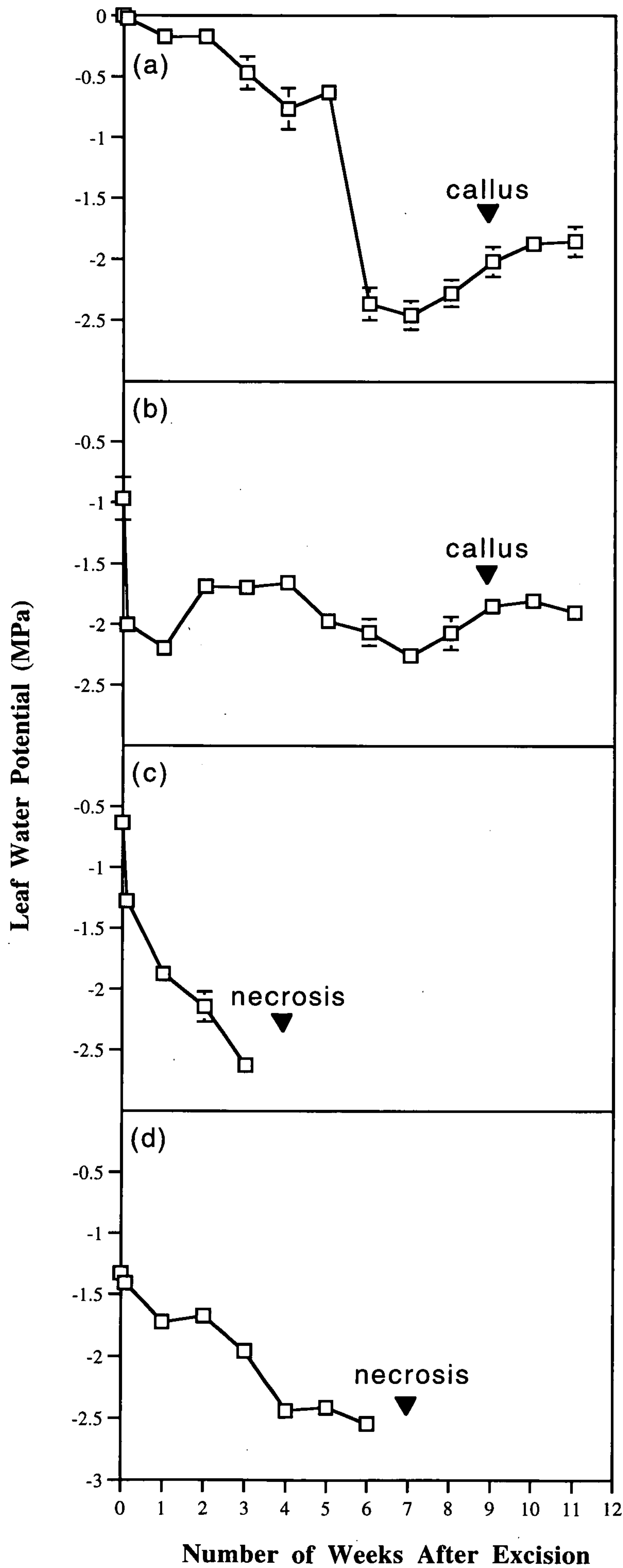
6.3.c Changes in Water Potential of Cuttings Collected at Different Times in the Year

The rooting percentage of cuttings collected from two year old stock plants at Westerway on the 3/8/94, 14/8/94, 26/10/94 and 7/12/94, after 16 weeks of growth in the phytotron, was 91, 76, 0 and 11% respectively (Section IV.2.3.a). In cuttings which were collected on the 3/8/94 and the 14/9/94, visible callus tissue was observed at approximately 8 or 9 weeks following excision. In these cuttings, most of the mortality occurred between weeks 4 and 9, after which no further tissue necrosis was observed. In those cuttings which did not root, the leaves appeared dull in lustre prior to displaying chlorotic symptoms, ultimately leading to tissue necrosis being observed. The necrotic cuttings appeared dry and brown in colour. In those cuttings collected on the 26/10/94, the leaves of all cuttings became chlorotic 3 weeks after excision. In the 4th week following excision, tissue necrosis and secondary infection was observed in all cuttings and no further leaf water potential measurements were recorded. In those cuttings collected on the 7/12/94, leaf chlorosis was observed in approximately 30% of the cuttings 4 weeks following excision from the stock plant. The cuttings which were chlorotic in week 4 were entirely necrotic by week 5, however, at week 5 more cuttings had become chlorotic. The chlorotic-necrotic cycle continued during weeks 5 to 6 until all cuttings allocated for sampling were necrotic, at which point no further leaf water potential measurements were recorded. Again, the necrotic cuttings appeared dry and brown in colour.

In those cuttings collected from two year old stock plants which exhibited a high rooting percentage, a significant decrease in the LWP was observed between week 0 and week 7 (Figures IV.6.1.a and IV.6.1.b). Although the LWP changed differently during this period, the LWP increased significantly in a similar way between week 7 and 11. This increase in LWP corresponded to the appearance of callus tissue on the base of cutting. The increase in LWP during weeks 7 to 11 may suggest that cuttings have an increased ability to regulate their water potential, either through increased water uptake or decreased water loss.

In those cuttings collected from two year old stock plants on the 26/10/94, the average LWP decreased from -0.635MPa at the time of excision to -2.623MPa by week 3, at a relatively linear rate (Figure IV.6.1.c). No further LWP measurements were recorded after week 3 because all cuttings which were collected on the 26/10/94 and allocated for LWP sampling were necrotic.

Figure IV.6.1 - Changes in the mean leaf water potential (MPa) (including standard errors) of cuttings collected from 2 year old stock plants at different times in the year; **(a)** cuttings collected on 3/8/94 (winter); $LSD_{0.05}$=0.303, **(b)** cuttings collected on the 14/9/94 (early-spring); $LSD_{0.05}$=0.269, **(c)** cuttings collected on the 26/10/94 (mid-spring); $LSD_{0.05}$=0.219 and **(d)** cuttings collected on the 7/12/94 (summer); $LSD_{0.05}$=0.192. The cuttings were set under controlled environment conditions in the phytotron. The mean of 10 leaf water potential measurements and the associated standard error are displayed at each point (data included in Appendix 25).


0
-0.5
-1
-1.5
-2
-2.5
(a)
callus
(b)
callus
(c)
necrosis
(d)
necrosis
-3
0 1 2 3 4 5 6 7 8 9 10 11 12
Leaf Water Potential (MPa)
Number of Weeks After Excision

In those cuttings collected from two year old stock plants on the 7/12/94, the average LWP decreased from -1.325MPa at the time of excision to -2.548MPa at week 6, at a relatively linear rate (Figure IV.6.1.d). The rooting percentage of cuttings collected from two year old stock plants on the 7/12/94 was 11%. No further LWP measurements were recorded after week 6 because all cuttings which were collected on the 7/12/94 and allocated for LWP sampling were necrotic. In treatments where cuttings exhibited a low rooting percentage, the average LWP decreased in a relatively linear rate from the time of excision until the week prior to tissue necrosis (Figures IV.6.1.c and IV.6.1.d). In treatments where cuttings exhibited a high rooting percentage, the average LWP did not decrease below -2.500MPa. However, in treatments where cuttings a low rooting percentage was observed, the average LWP observed in the week prior to tissue necrosis was below -2.500MPa, indicating that there may be a threshold value in LWP, beyond which cuttings fail to survive. The atmospheric water potential at 95% relative humidity and 20°C equalled -6.530 MPa (T.log[100/RH]). Therefore, without the ability of cuttings to regulate their water potential, the LWP would be expected to equilibrate with the atmospheric water potential of -6.530MPa.

6.3.d Effect of Parent Stock Plant Age

The rooting percentage of cuttings collected from two and nine year old stock plants on the 3/8/94 was 91 and 30% respectively. In those cuttings collected from two year old stock plants, the LWP decreased significantly from approximately 0.000MPa at the time of excision to -2.458MPa at week 7. However, the LWP increased significantly from -2.458MPa in week 7 to -1.853MPa in week 11, which was approximately the time when callus tissue was observed on the base of cutting (Figure IV.6.2). In those cuttings collected from nine year old stock plants, the LWP decreased significantly from -0.945MPa at the time of excision to -2.650MPa in week 3. The average LWP of cuttings collected from nine year old stock plants increased from -2.650MPa in week 3 to -1.910MPa in week 10 (Figure IV.6.2). However, in week 4 approximately 15-20% of the cuttings collected from nine year old stock plants developed signs of leaf chlorosis. In week 5 the cuttings which were chlorotic had become necrotic and further cuttings had also developed chlorotic symptoms. This cycle continued during weeks 7 to 10, until all cuttings allocated for LWP measurement were necrotic, at which point no further LWP measurements were recorded. The cuttings which displayed signs of chlorosis were not selected for the assessment of cutting LWP because of visible leaf degradation and non-

repeatability of LWP measurements. The average LWP of chlorotic leaves was demonstrated to be less than -2.795MPa. In transverse section the leaves contained a hollow region between the vascular bundle and the external epidermis, indicating that tissue dehydration had occurred.

The LWP of *P. radiata* cuttings, collected on the 3/8/94 from two and nine year old stock plants, differed significantly during the first 5 weeks of propagation in the phytotron. However, between weeks 6 and 10 no significant difference was observed in the LWP of cuttings collected from two and nine year old stock plants (Figure IV.6.2).

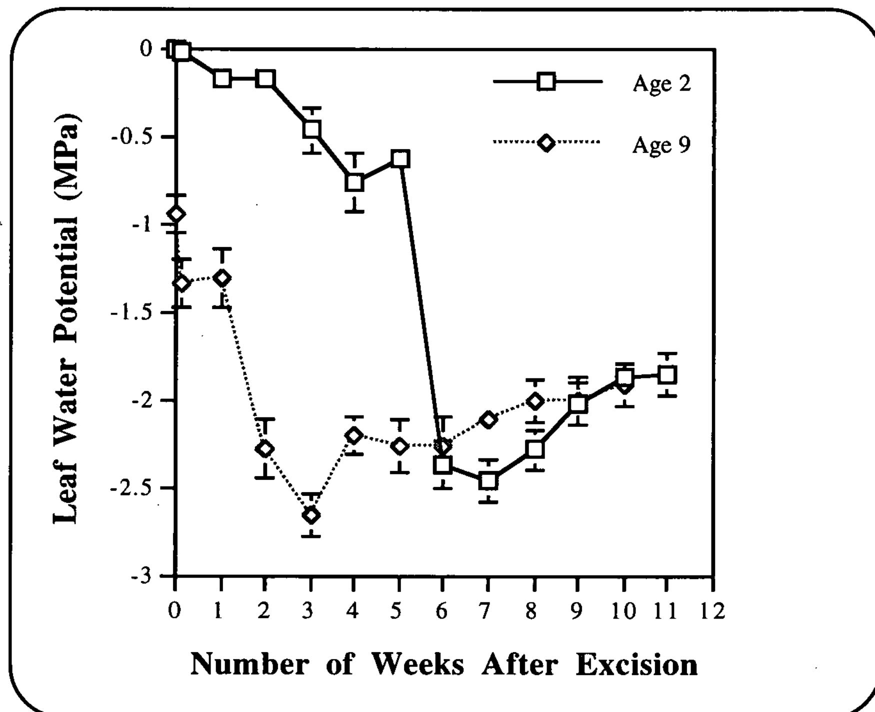


Figure IV.6.2 - Change in the mean LWP (MPa) of cuttings collected from two and nine year old stock plants on the 3/8/94 during the period of callus formation. The cuttings were propagated under controlled environment conditions in the phytotron. The mean and standard error of 10 leaf water potential measurements were represented at each point in Figure IV.6.2 (data included in Appendix 25). $LSD_{0.05}$= -0.414MPa.

6.4 Discussion

The LWP of *P. radiata* cuttings, at the time of excision from the stock plant, did not appear to be related to the rooting percentage of cuttings. In general, at the time of excision, a higher LWP was observed in cuttings collected from stock plants at later times in the year. At the time of excision, the LWP of cuttings collected from juvenile stock plants was significantly lower than that observed in the cuttings collected from mature stock plants. Although a significantly higher water potential was observed in those cuttings collected from nine year old stock plants at the time of excision, the poor relationship observed between stock plant water potential and the rooting percentage of cuttings collected at different times in the year indicated that stock plant water potential may not influence the final rooting percentage.

In general, the loss of water from cuttings during transport between the field and the phytotron was larger as the temperature on the day of collection increased. However, there was no relationship between the rate of LWP loss during transport and the final rooting percentage after 16 week of propagation under constant environment conditions in the phytotron.

In those cuttings which were collected from two year old stock plants on the 3/8/94 and the 14/9/94, the average LWP decreased significantly between the time of excision and week 7. However, between week 7 and 11 the average LWP increased significantly, which corresponded to the time when callus tissue was observed at the base of the cutting. This indicated that the callus tissue or associated changes within the cutting at the time of callusing may enhance the rate of water uptake from the rooting media. The average LWP of cuttings collected on the 3/8/94 and the 14/9/94 did not go below -2.500MPa.

In those cuttings which were collected from two year old stock plants on the 26/10/94 and the 7/12/94, the average LWP decreased at a relatively linear rate between the time of excision until the week prior to cutting necrosis. The low LWP in the week prior to tissue necrosis indicated that the water content of the cuttings may have reached a level where permanent and irreversible damage had occurred. In the week prior to cutting necrosis, the average LWP of cuttings collected on the 26/10/94 and 7/12/94 was less than -2.500MPa. This evidence suggests that a threshold value of LWP may exist beyond which cutting necrosis may occur.

The LWP of cuttings collected from nine year old stock plants was significantly less than that observed in cuttings collected from two year old stock plants during the first five weeks of propagation. However, after week 5 the LWP of cuttings collected from two and nine year old stock plants did not differ significantly. The similar LWP observed during weeks 6-11 may have resulted from the biased selection process which only sampled those cuttings which were not either chlorotic or necrotic. That is, only those cuttings from nine year old stock plants which were more likely to root were sampled.

In summary, the ability of cuttings to regulate water potential during ARF may be a better indicator of rooting ability than the water potential of cuttings at the time of excision. This finding was similar to that observed in PGR and carbohydrate studies, where the ability to respond to excision may be more important to ARF than the absolute levels at the time of excision.

IV.7 Photosynthesis and Water Relations in Cuttings During Callus Development

7.1 Introduction

In a previous experiment (Section IV.5), the maintenance of sucrose and other soluble carbohydrate concentrations in the basal stem region of the cutting, where anatomical changes were observed to occur, appeared to be important in the successful development of an adventitious root system. Although the concentration of soluble carbohydrates were observed to be maintained at a relatively constant concentration throughout the callus formation period, the concentration of endogenous starch in the basal stem section and their associated fascicle needles were observed to decrease in concentration, within a week of excision, to a point where no further change occurred until callus was visible on the base of the cutting. Assuming that soluble sugars are constantly being utilised as an energy and carbon source, the maintenance of constant soluble sugar concentrations in the basal stem section suggests that sucrose is being transported either from starch reserves in other parts of the cutting or supplied from current photosynthate being produced in the leaves. The rate of photosynthesis has been demonstrated to influence the rooting of certain leafy cuttings (Davis and Potter, 1981; Davis, 1988). However, others have suggested that current photosynthesis is of little or no importance to ARF (Hansen *et. al.*, 1978).

The effects of water stress on ARF in *P. radiata* cuttings was demonstrated in Section IV.6 of this thesis. Other research has demonstrated that even mild water stress can also have dramatic effects on photosynthetic rates (Cameron and Rook, 1974; Hartmann and Kester, 1983; Andersen, 1986). The aims of this research were to investigate rates of photosynthesis in *P. radiata* cuttings in an attempt to elucidate the route of assimilate supply to the basal stem region of the cutting. Also, the measurement of needle water potential was used to indicate whether changes in photosynthetic rate arise as a result of water stress. The measurement of photosynthetic rate in fascicle needles of cuttings should indicate whether current photosynthate or starch stores (located in other regions of the cutting) are the major pathway of carbohydrate supply to the basal stem region of the cutting during callus development.

7.2 Materials and Methods

7.2.a Infra Red Gas Analyser

At the time of excision, one day after excision and at weekly intervals after excision for 12 weeks, five randomly selected stock plants or cuttings were used for photosynthetic measurements. Photosynthetic measurements were recorded using an LCA-3 portable Infra Red Gas Analyser (IRGA) (Analytical Development Co. Ltd., England). The LCA-3 is a battery powered, 'open' system IRGA for the measurement of transpiration and photosynthesis.

Reference air containing 370ppm CO_2, 21%O_2 and nitrogen balance was released directly from a portable pressurised gas cylinder via a gas regulator and adjustable flowmeter at a constant rate (150ml/min) into the reference port of the LCA-3. The reference air was humidified by the humidity controller (iron (II) sulphate 7 hydrate column) on the LCA-3. The reference air was then pumped at a controlled rate (120ml/min) determined by the mass flowmeter in the LCA-3 to the leaf chamber (Parkinsons Leaf Chamber, PLC(B), Analytical Development Co. Ltd., England). The flow rate was adjusted to give carbon dioxide differential readings of 10-15 percent or less of the carbon dioxide concentration in the atmosphere. On its return, either the leaf chamber air or reference air is selected by the solenoid valve for analysis as dictated by the LCA-3 analyser operating mode. The air to be analysed was then drawn by a second pump at the rate determined by the mass flow sensor and delivered to the analysis section. The absorption by the sample of infrared radiation in the 4.26μm band, emitted by the modulated source, was measured by a pyroelectric detector. Measurements and results were shown on the analyser display panel and also recorded on the data storage card located in the base of the LCA-3. Temperature in the leaf chamber was calculated by an energy/balance equation in the LCA-3.

7.2.b Measurement of Photon Flux Density and Light Saturation Curves

Each time a CO_2 differential was recorded on the LCA-3 a light intensity or photon flux density (PFD) measurement was recorded using a Lamba LI-185 meter fitted with a quantum flux sensor (the sensor on the leaf chamber was found to be inaccurate at high PFD's). The quantum flux sensor measured photosynthetically active radiation (PAR) in the 400-700nm spectrum range and results were expressed

in $\mu mol.m^{-2}.s^{-1}$. Measurements of PFD were made at leaf height and were corrected for the clear perspex housing on the leaf chamber (PLC).

The light saturation curve was conducted using *P. radiata* seedlings (40-50cm in height) which were grown in a glasshouse (25/15°C day/night temperature) under natural light conditions for approximately 3 months. The seedlings were transferred to a controlled environment room, which was maintained at 20°C, prior to measurement. A copper water jacket was fitted to the base of the leaf chamber to control the temperature in the chamber. The reference air line from the gas cylinder to the LCA-3 was passed through a copper coil which was immersed in a water bath set at 20°C. The water in the water bath was pumped into the copper water jacket to aid in maintenance of constant temperature in the leaf chamber. Light for the experiment was supplied by a 400 W mercury vapour lamp suspended above the leaf chamber. A glass water table separated the light source from the leaf chamber to prevent excessive heating of the leaf chamber by the lamp. The PFD was controlled by inserting varying thicknesses of 'Sarlon' shade cloth between the light source and the leaf chamber, and measured using a Lamba LI-185 meter mentioned previously in this section.

7.2.c Measurement of Leaf Area

The photosynthetic rate of a plant is generally expressed as amount of carbon dioxide exchanged per unit leaf area, per unit time ($\mu mol\ CO_2.m^{-2}.s^{-1}$). In *P. radiata*, trees have three needles per fascicle, with the cross-section of each needle approximating one-third segment of a circle and stomata are present on each of the three surfaces. Wood and Brittain (1972) expressed rates of photosynthesis in *P. radiata* per unit leaf volume ($mgCO_2.cm^{-3}.hr^{-1}$), Stupendick and Shepherd (1980) expressed rates of photosynthesis in per unit dry weight of leaf material ($mgCO_2.g^{-1}.hr^{-1}$). However, Bennett and Rook (1978) and Conroy *et. al.* (1986) expressed rates on a leaf area basis and stated that to compare rates of photosynthesis in *P.radiata* with rates based on projected leaf area, data should be multiplied by a factor of 2.4.

In current work, leaf area was calculated by photocopying needles carefully separated between two sheets of A4 transparency paper. The photocopies were then scanned using an Apple Macintosh linked to a OneScanner with Ofoto software, picture files were then converted to GIF files before being opened in NIH Image v1.58 for measurement of leaf area using a reference length (50mm) drawn on the scanned image. The error, as calculated from a known area, was found to be +/- 2%.

The leaf area may be converted to a dry weight if required because of the linear relationship (R^2=0.999) which exists between leaf area and leaf dry weight (Figure IV.7.1).

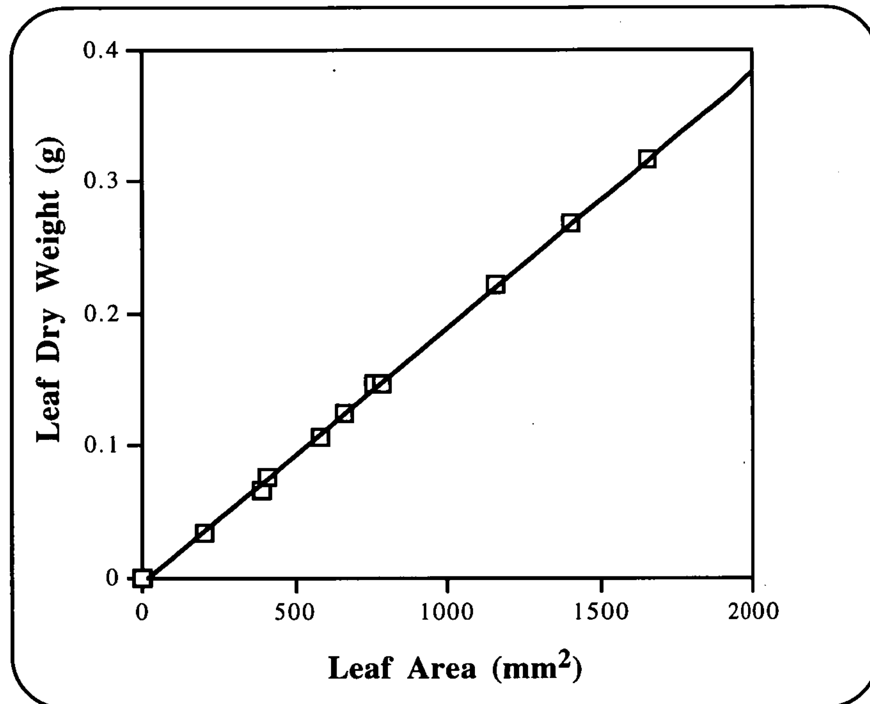


Figure IV.7.1 - The linear relationship between leaf area (mm^2) and leaf dry weight (g) (r^2=0.999). The photosynthetic rate may be converted from per leaf area basis to per leaf dry weight basis using the formula;

$$\text{Leaf Dry Weight (g)} = (1.9501 \times 10^{-4} \times \text{Leaf Area mm}^2) - 4.979 \times 10^{-3}$$

7.2.d Calculation of Net Assimilation Rates

The rate of CO_2 flux into plant tissue ('apparent' photosynthesis) is a measure of the net assimilation rate. The net assimilation rate, or rate of CO_2 exchange, is calculated by the formula :

A = (f/s) x ΔC

where A = assimilation rate (μmol $CO_2.g^{-1}.s^{-1}$)

f = mole flow of air ($mol.s^{-1}$)

= f_v x (1/22.4) x [273.15 / (273.15+T)] x (p / 101.3)

f_v = volumetric flow of air ($l.s^{-1}$)

T = temperature recorded during measurement (°C)
p = atmospheric pressure during measurement (kPa)
s = photosynthetic weight of leaf measured (g)
ΔC = CO_2 differential (ppm)

Net CO_2 exchange was calculated for fascicle leaves of *P. radiata* seedlings over a range of PFD's between 0 and 1250 $\mu mol.m^{-2}.s^{-1}$ at a constant temperature of 20°C. The calculation of CO_2 exchange rates was conducted on stock plants grown in the field and on cuttings set in the phytotron.

7.2.e Porometer Measurements

Stomatal resistance ($s.cm^{-1}$) was measured on single three-needle fascicle from the mid-stem section of cuttings or stock plants using an automatic porometer (Delta-T Devices, Mk3, Cambridge, England). Measurements were recorded during the mid-afternoon on the same plant or cutting which was randomly selected for the measurement of photosynthesis. Calibration was achieved using a moulded polypropylene calibration plate with diffusion resistances of known value at 20°C. For each plant or cutting an average of ten time readings from the 200Hz counter in the automatic porometer between 40 and 50% relative humidity, were recorded. The three-needle fascicle was considered to approximately occupy the area of the automatic porometer sensor, but because of the structure of *P. radiata* leaves the measurement of stomatal resistance was difficult to express accurately in terms of leaf area. Stomatal resistances were recorded using a standard curve.

7.2.f Water Potential Measurements

The leaf water potential (LWP) of individual needles selected from the mid-section of the cutting were measured using the pressure chamber (Section III.8). Measurements were recorded during the mid-afternoon (maximum stress) on the same cuttings randomly selected for photosynthesis and porometer measurements.

7.3 Results

Stem cuttings were collected from three year-old stock plants in the field during mid-May when the rooting percentage of cuttings was known to be high (Section IV.1). The cuttings were collected in the early morning (0700 - 0830hrs)

and the weather on the day of cutting collection was moderately overcast, with an average temperature ranging between 15 - 17°C. At the time of excision, the average rate of net CO_2 exchange was 4.49μmol.m^{-2}.s^{-1}. In the 30 minute period following excision, the rate of net CO_2 exchange did not decrease significantly in comparison to the net rate of CO_2 exchange at the time of excision (Figure IV.7.2). In fact, the rate of net CO_2 exchange, during the 30 minute period following excision, appeared to be more related to the photon flux density (PFD) (Figure IV.7.2). The average LWP at the time of excision was observed to be -0.92MPa and the average stomatal resistance was observed to be approximately 6.42s.cm^{-1} (Appendix 26).

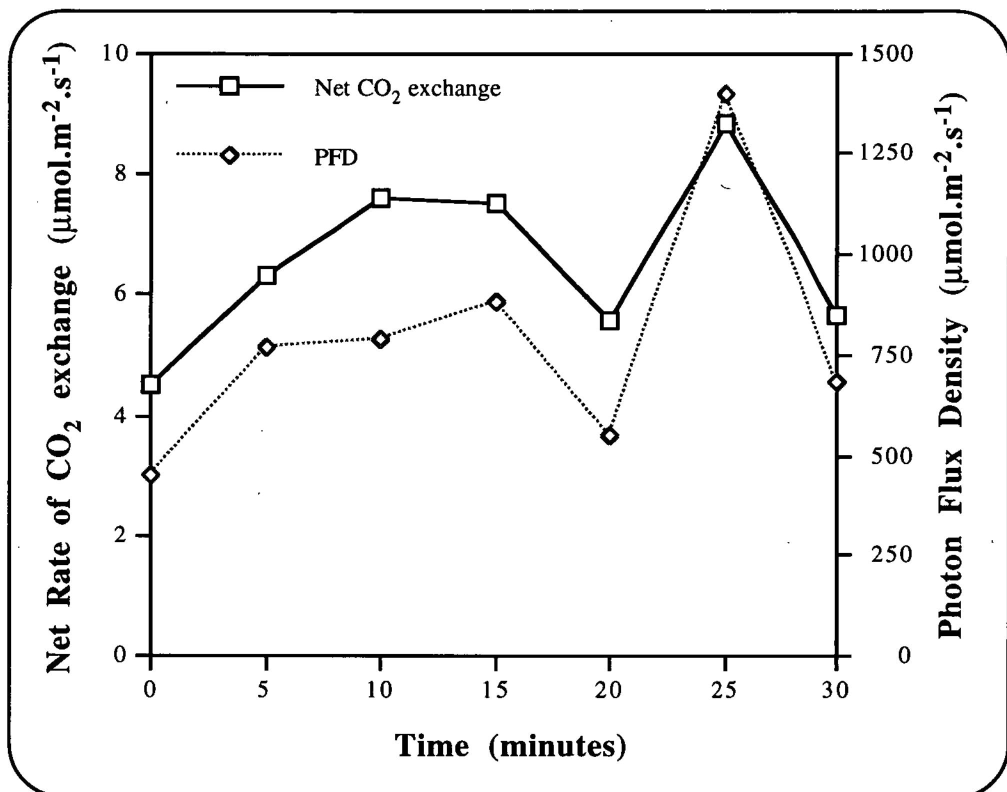


Figure IV.7.2 - Change in the rate of net CO_2 exchange and PFD during a 30 minute period following excision. The rate of net CO_2 exchange was not reduced significantly during the 30 minute period following excision, however, the rate of net CO_2 exchange did vary in relation to the PFD (data included in Appendix 27).

Cuttings were collected from field grown stock plants and set in standard containers and rooting media in the phytotron (Section III.2.2). After one day in the

phytotron, the rate of net CO_2 exchange in cuttings had decreased from 4.49 to -0.38μmol.m^{-2}.s^{-1} (Appendix 26). Therefore, at this time cuttings were in a state of net CO_2 efflux. The PFD, at leaf height, in the phytotron was known to be approximately 45μmol.m^{-2}.s^{-1}. The low PFD within the phytotron was thought to limit the rate of net CO_2 exchange. A light saturation curve was conducted to determine if PFD, in the phytotron, was limiting the rate of net CO_2 exchange (Figure IV.7.3). At 20°C, light saturation occurred at PFD's of between 550 and 700μmol.m^{-2}.s^{-1}.

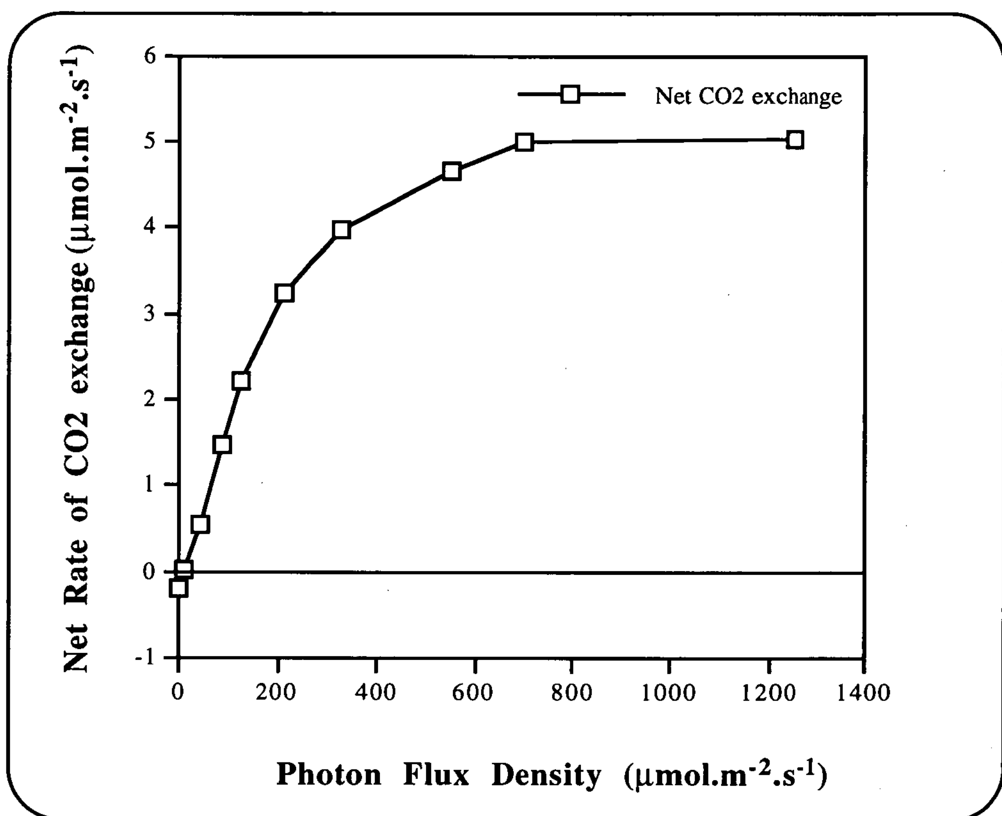


Figure IV.7.3. Rate of net CO_2 exchange in *P. radiata* seedlings at 20°C, PFD was varied between 0 and 1250μmol.m^{-2}.s^{-1}. Light saturation occurred between 550 and 700μmol.m^{-2}.s^{-1} (mean data included in Appendix 28).

However, at a PFD of 42μmol.m^{-2}.s^{-1}, which is a similar PFD to that experienced within the phytotron, the average rate of net CO_2 exchange was 0.563 μmol.m^{-2}.s^{-1}. The light compensation point occurred at a PFD of approximately 8-10μmol.m^{-2}.s^{-1} (Figure IV.7.3).

The average LWP decreased from -0.92 to -1.46MPa within a day after cuttings were collected from the field and set in the phytotron (Figure IV.7.5). The corresponding stomatal resistance increased from 6.42 to 113.18s.cm^{-1}, indicating that the stomates had closed in response to a water stress situation (Appendix 26). In the dark and at 20°C, the rate of net CO_2 exchange in cuttings and seedlings was -0.38μmol.m^{-2}.s^{-1} and -0.201μmol.m^{-2}.s^{-1} respectively. This indicated that the rate of respiration may have increased in response to cutting excision.

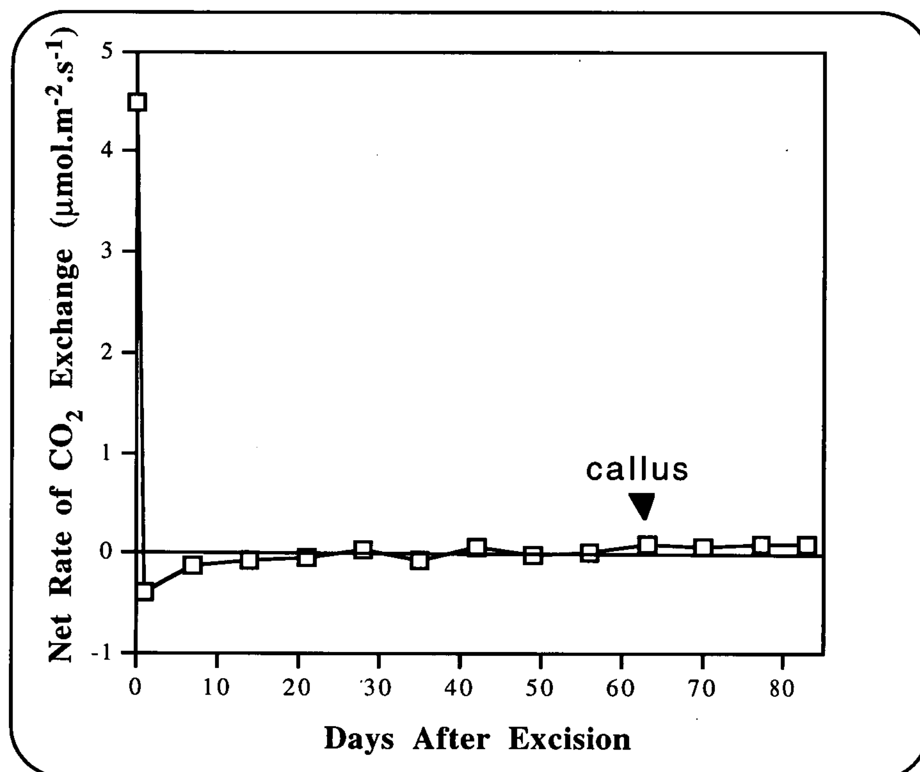


Figure IV.7.4 - Change in the rate of net CO_2 exchange (μmol.m^{-2}.s^{-1}) during the callus formation period. The rate of net CO_2 exchange decreased dramatically from the time of excision (4.49μmol.m^{-2}.s^{-1}) to one day after excision (-0.38μmol.m^{-2}.s^{-1}), $LSD_{0.05}$=0.46 (mean data and standard errors included in Appendix 26).

In the phytotron, the PFD was well below the level of light saturation, but still high enough to expect some net CO_2 exchange to occur. The rate of net CO_2 exchange and LWP were monitored on a weekly basis during the callus formation period (Figures IV.7.4 and IV.7.5 respectively). The rate of net CO_2 exchange increased gradually from -0.38μmol.m^{-2}.s^{-1} during the first day after excision, to the

compensation point by approximately day 28 after excision. By day 63, the rate of net CO_2 exchange had increased to $0.1\mu mol.m^{-2}.s^{-1}$ and callus tissue was visible at the base of the cuttings (Figure IV.7.4). The rooting percentage of cuttings after 16 weeks in the phytotron was 89.0%. The low rate of net CO_2 exchange during the callus formation period indicated that the production of current photosynthate may not be essential for ARF in *P. radiata* cuttings.

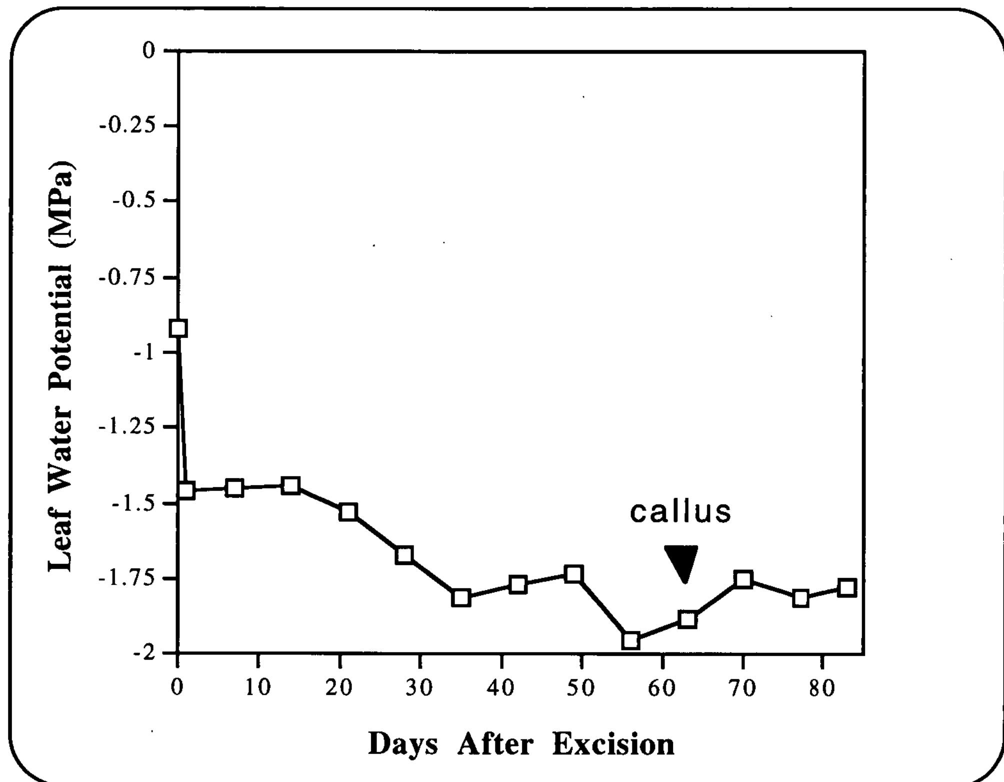


Figure IV.7.5 - The change in average LWP (MPa) of cuttings during the callus formation period. The LWP decreased rapidly soon after excision, then declined at a slower rate until day 56, where the lowest average leaf water potential was recorded (-1.95MPa). The average leaf water potential increased once callus tissue becomes visible at the base of the cuttings (data included in Appendix 26).

The average LWP of cuttings decreased significantly from -0.92 to -1.46MPa within a day after cutting excision (Figure IV.7.5). The average LWP then decreased gradually from -1.46 to -1.95MPa by day 56 after excision. After day 56, the average LWP increased gradually from -1.95 to -1.78Mpa. The increase in LWP

corresponded to the appearance of callus tissue, which became visible at approximately 60 days after excision.

At day 70 after excision, the average stomatal resistance of cuttings was 10.54s.cm^{-1} (Appendix 26). This was significantly ($p < 0.01$) less than the stomatal resistance recorded one day after excision (113.18s.cm^{-1}; Appendix 26).

7.4 Discussion

In the field, under conditions of high PFD, stock plants were demonstrated to have a low stomatal resistance (6.42s.cm^{-1}), a water potential of -0.92MPa, and a net CO_2 exchange rate of 4.49μmol.m^{-2}.s^{-1}. However, after a day in the phytotron, cuttings were observed to be in a state of net CO_2 efflux (-0.38μmol.m^{-2}.s^{-1}). Initially, it was thought that the low PFD in the phytotron (45μmol.m^{-2}.s^{-1}) may have been totally responsible for this net CO_2 efflux. However, a light saturation curve conducted on a *P. radiata* seedling (which was grown under similar natural light conditions as was experienced by the stock plants) demonstrated that at phytotron temperature (20°C) and PFD, a net CO_2 exchange rate in the order of 11 - 12% of the maximum may be expected.

In the first week after excision, the LWP decreased significantly and the stomatal resistance increased. This indicated that the cuttings were in a state of water stress. The basal rate of respiration also appeared to increase in response to excision.

The rate of net CO_2 exchange increased significantly between weeks 1 and 12 following excision. The rate of CO_2 exchange did not reach the compensation point until approximately day 28 after excision. A net CO_2 influx was observed at approximately day 63, which corresponded to the time that callus tissue was visible on the base of the cutting. The LWP of cuttings decreased gradually from the time of excision until approximately day 63, where upon the LWP tended to increase. The stomatal resistance decreased significantly between day 1 and day 70. This indicated that basal callus tissue may allow cuttings to increase the rate of water uptake from the propagation environment. The increased LWP and the reduced stomatal resistance, may have enabled cuttings to resume CO_2 exchange at higher rates.

The negative and near zero rates of net CO_2 exchange observed throughout the callus formation period indicated that the production of current photosynthate was not the major pathway of carbohydrate supply to the basal region during callus formation. Therefore, in those cuttings which rooted well, the high level of carbohydrate observed in the basal region of the cutting during callus formation may be supplied from stored forms, such as starch, which may be located in other parts of the cutting.

IV.8 Individual Cutting Water Relations

8.1 Introduction

While in this study, a cutting was considered to not have survived when 100% of the leaf surface area became necrotic, irreversible damage would obviously have occurred some time prior to tissue necrosis being observed. The metabolic processes leading to cutting necrosis are not well understood.

In a previous experiment (Section IV.6), the random selection of apparently healthy cuttings was used to assess cutting water potential during the callus formation, but it was acknowledged that this only gave an indication of the water status in those cuttings which rooted. This was particularly apparent in cuttings which were physiologically older (nine years old), where the mortality of cuttings was high and only a relatively few healthy cuttings could be sampled for water potential assessment. In Section IV.6, results indicated that the water relations of cuttings during ARF may strongly influence the final rooting percentage. A low average leaf water potential (LWP) was observed prior to periods of high cutting mortality. However, the results were somewhat inconclusive because they did not demonstrate specifically that a low LWP was associated with a high rate of mortality. Where cutting mortality was low, the random sampling method employed in Section IV.6 for water potential assessment was considered a satisfactory indicator of cutting water status. However, when cutting mortality was high, the random selection process used previously was considered unsatisfactory to monitor water potential change in cuttings which may not survive. Therefore, monitoring the water status of individual cuttings during ARF may provide an insight into the processes occurring prior to cutting necrosis and provide information on the critical period during ARF.

This experiment aimed to examine the changes in water potential of individual cuttings in an attempt to better understand the changes in water relations which occur prior to cutting necrosis. This may be achieved by monitoring changes in LWP during callus formation, in a population of cuttings where at least some mortality may be expected.

8.2 Materials and Methods

8.2.a Experimental Design

The cutting material was collected from five year old stock plants located at Mount Lloyd on the 9/5/96. Although the rooting percentage of the stock plant population was not known, work by Jacobs (1939), Fielding (1954) and Fielding (1969) indicated that approximately 20-40% mortality could be expected with cuttings collected from five year-old stock plants. The experiment monitored changes in LWP of 20 cuttings on a weekly basis during the period of callus formation (12 weeks). In a previous experiment (Section IV.1), most of the mortality was observed to occur during the callus formation period. Each cutting was collected from a different stock plant in the field. A further 20 cuttings were collected and used to investigate whether the weekly sampling of needles from the same cutting for LWP assessment, had any significant effect on the final rooting percentage (control cuttings).

Cuttings were set in standard pots and rooting media and propagated under standard conditions in the phytotron (Section III.2.2). The LWP was measured pre-dawn using the pressure chamber technique (Section III.8) and the rooting percentage determined after 16 weeks (Section III.3.1). The first LWP measurements were recorded after one day in the phytotron (Week 0). A cutting which displayed visible signs of necrosis over the entire leaf area of the cutting was deemed to have failed to survive. Once a cutting was entirely necrotic, no further LWP measurements were recorded on that particular cutting.

8.3 Results

The LWP of *P. radiata* cuttings propagated in the phytotron were monitored on a weekly basis during the callus formation period (Appendix 29). The rooting percentage of cuttings used to monitor the LWP was 75% (Table IV.8.1). In the cuttings where the LWP was not monitored (control cuttings), the rooting percentage was 70% (Table IV.8.1). This indicated that the weekly sampling of needles from the same cutting had no adverse effects on the final rooting percentage. Therefore, the final rooting percentage of cuttings collected from five year old stock plants was demonstrated to be between 70-75%. This was in agreement with results of other researchers (Jacobs, 1939; Fielding, 1954; Fielding, 1969).

Treatment	%Rooted	%Mortality	Significance
Control	70	30	a
LWP Monitored	75	25	a

Table IV.8.1 - The percentage rooting and mortality results of control and LWP monitored cuttings after 16 weeks in the phytotron. There was no significant difference between the control and LWP monitored cuttings.

The LWP of 20 individual cuttings were monitored on a weekly basis during the period of callus formation (Appendix 29). During this period five of the original 20 cuttings displayed the typical necrotic symptom of cuttings which failed to survive (Plate 30: cutting numbers 1, 6, 8, 13 and 15). The average LWP of all 20 cuttings (total average) displayed a linear decrease from -1.41MPa at the time of setting (week 0) to -1.89MPa in week 4 (Figure IV.8.1). The rate of LWP loss during this period was approximately -0.12MPa/week (Figure IV.8.2). During the remaining weeks (5-12) the average LWP of the total population remained at a relatively constant potential between -1.80 and -1.95MPa (Figure IV.8.1).

In those cuttings which formed an adventitious root system (Plate 30: cutting numbers 2-5, 7, 9-12, 14, 16-20), the average LWP (rooted average) decreased from -1.40MPa at the time of setting to -1.85MPa in week 4 (Figure IV.8.1). The rate of LWP loss during this period was approximately -0.11MPa/week (Figure IV.8.2). The average LWP increased gradually from -1.85MPa in week five to approximately -1.70MPa in week eight. During the remaining weeks (8-12) the average LWP of the rooted cuttings remained at a relatively constant potential of between -1.70 and -1.75MPa. The lowest LWP observed in those cuttings which rooted was -2.38MPa (Appendix 29, cutting number 18, week number 6).

In those cuttings which did not root, the average LWP (necrotic average) decreased from -1.44MPa at the time of setting to -2.04MPa in week 4 at a linear rate of -0.15MPa/week (Figures IV.8.1 and IV.8.2). The average LWP, in these cuttings, increased slightly from -2.06MPa in week 5 to -1.93MPa in week 6. However, from week 6 onwards, the average LWP decreased from -1.93MPa to -2.73MPa in week 12 at a linear rate of approximately -0.13MPa/week until the entire leaf area became necrotic in week 13 (Figure IV.8.1 : Plate 30, cutting

numbers 1, 6, 8, 13 and 15). The leaves on these cuttings appeared dull in lustre and were slightly chlorotic in the weeks preceding leaf necrosis. This indicated that the cutting LWP may have fallen below a critical potential for survival sometime prior to leaf necrosis being observed.

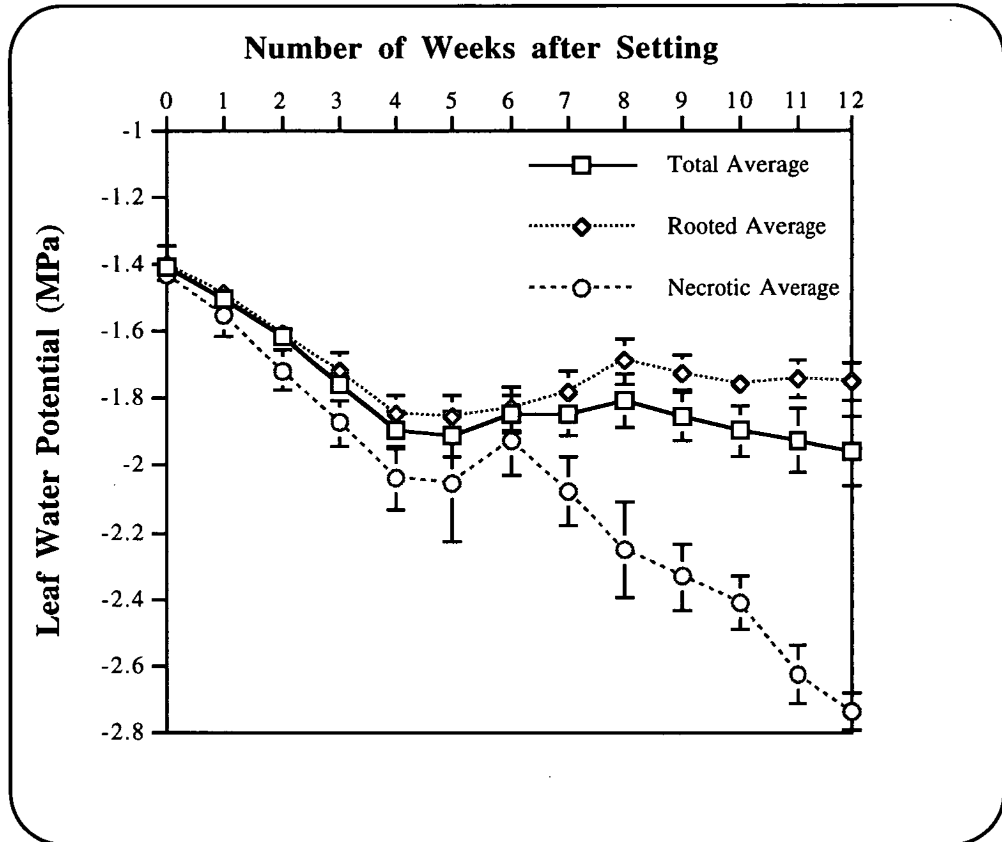


Figure IV.8.1 - Change in the average LWP of all cuttings (Total Average), those cuttings which rooted (Rooted Average) and those cuttings which failed to root and became necrotic (Necrotic Average). Means and standard errors displayed (data included in Appendix 29).

In those cuttings which did not survive, the LWP recorded prior to leaf necrosis was less than -2.60MPa (Figure IV.8.3). The lowest LWP observed in cuttings which rooted was -2.38MPa (cutting number 18, week 6). This indicated that a critical threshold value of LWP may exist, and if exceeded cuttings may fail to survive. The threshold value appeared to be between -2.40MPa and -2.60MPa, although the exact value may differ between individual cuttings.

Plate 30 - The 20 cuttings used in the individual water relations monitoring experiment after 16 weeks of growth in the phytotron. Note that cutting numbers 1, 6, 8, 13 and 15 have become necrotic. The surviving cuttings had formed an adventitious root system and produced a small amount of top growth.

1
2
3
4
5
CUTTING NUMBER
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CUTTING NUMBER
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CUTTING NUMBER
30

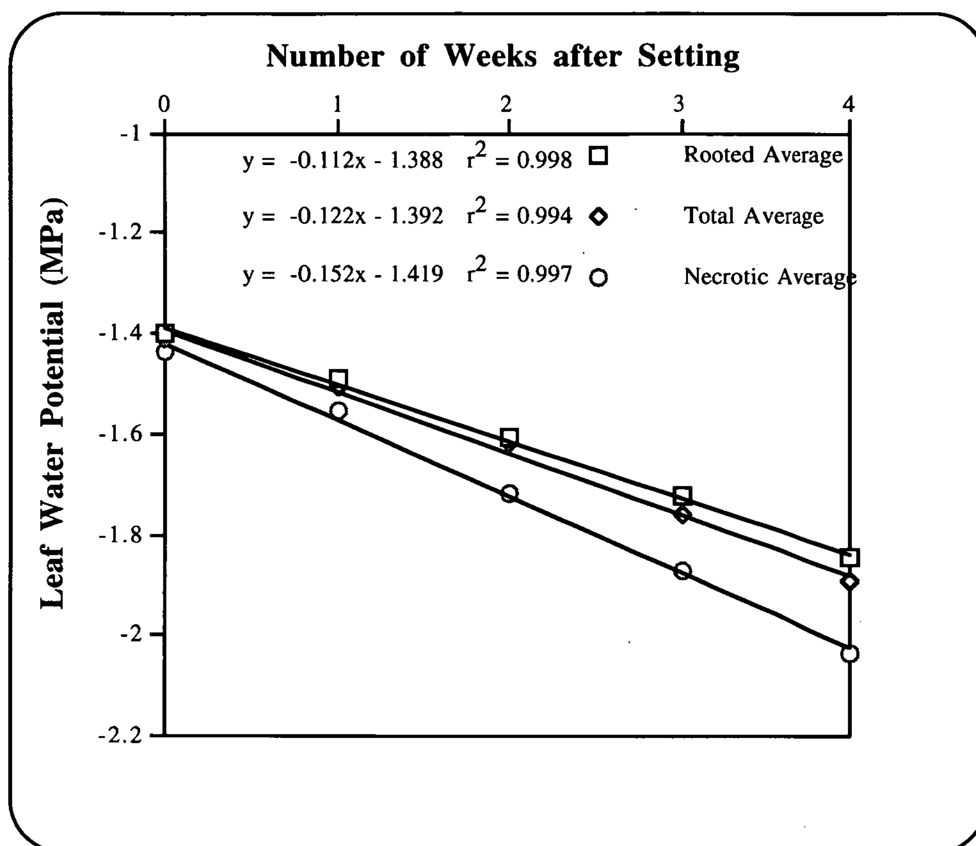

Figure IV.8.2 - The average rate of decline in LWP of all 20 cuttings (Total Average: -0.122MPa/week), those cuttings which rooted (Rooted Average: -0.112MPa/week) and those cuttings which failed to survive (Necrotic Average: -0.152MPa/week) (data included in Appendix 29).

In those cuttings which failed to survive, cutting number 6 was observed to be entirely necrotic after 5 weeks (Plate 30). The LWP in cutting number 6 decreased at a rate of approximately -0.21MPa/week during the first four weeks following excision (Figure IV.8.3). The decrease in LWP of cutting numbers 1, 8, 13 and 15 (Plate 30), which also failed to survive, occurred at a much slower rate (average of -0.14MPa/week) during the first four weeks following excision (Figure IV.8.3). During week five and six cutting numbers 1, 8, 13 and 15 displayed either an increase or plateau in LWP, as was observed in those cuttings which rooted successfully. However, from week 6 onwards cutting numbers 1, 8, 13 and 15 displayed an almost linear decline in LWP until leaf necrosis was observed in week 13 (Figure IV.8.3 and Plate 30).

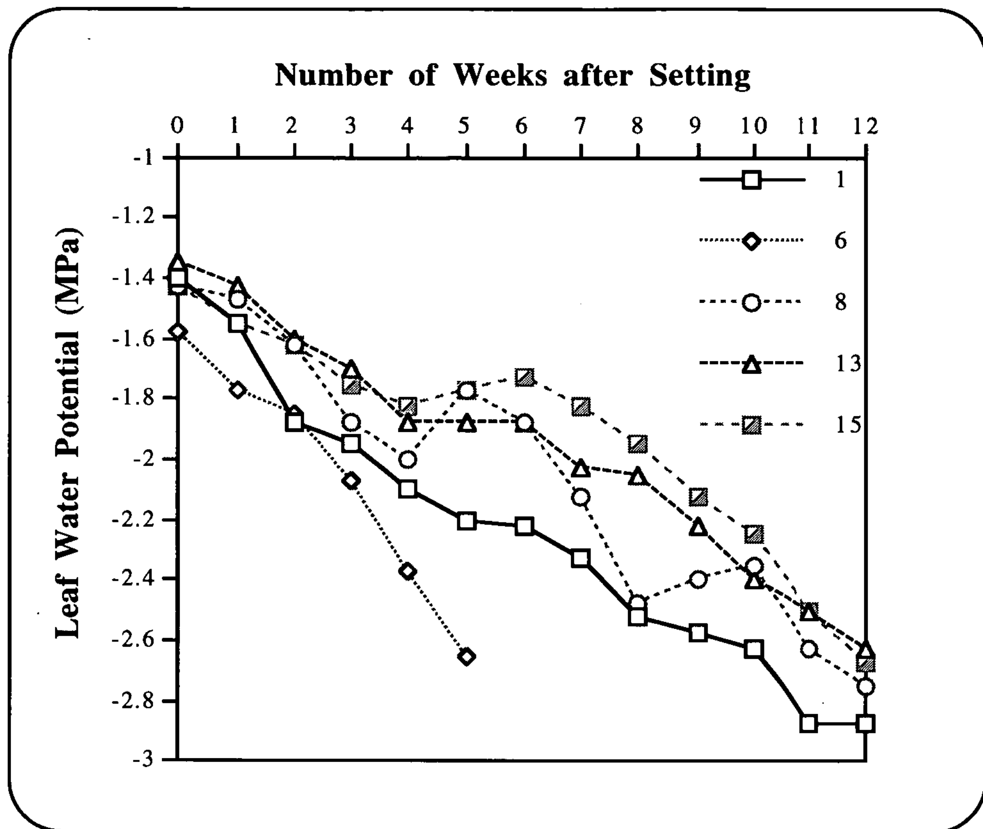

Figure IV.8.3 - The change in LWP of the cuttings which failed to survive (cutting numbers 1, 6, 8, 13 and 15: Plate 30). In cutting number 6 the LWP decreased rapidly until leaf necrosis was observed in week 5. The LWP of cutting numbers 1, 8, 13 and 15 decreased at a slower rate until week 13 when total leaf necrosis was observed (data included in Appendix 29).

8.4 Discussion

A final rooting percentage of 70-75% occurred in cuttings collected from five year old stock plants during May. This was in agreement with results of other researchers (Jacobs, 1939; Fielding, 1954; Fielding, 1969). The weekly sampling of individual needles from the same cutting, for water potential assessment, was demonstrated to have no significant effect on the final rooting percentage.

There was a pronounced difference in the way LWP changed after setting in those cuttings which rooted and those cuttings which failed to survive. In cuttings which rooted, the average LWP declined at a rate of -0.11MPa/week during the first four weeks after excision. However, between weeks 5 and 8 after setting the

average LWP increased gradually from -1.86Mpa to approximately -1.69MPa, indicating that cuttings may be controlling their water loss and absorbing water from the rooting media. During the remainder of the callus formation period (weeks 8 to 12), the average LWP remained relatively constant, between -1.69MPa and -1.76MPa During this period, the water status of cuttings appeared to be in a state of equilibrium.

In those cuttings which failed to survive, the average LWP declined at a rate of approximately -0.15MPa/week during the first four weeks after excision. This was higher than that observed in cuttings which rooted, despite all cuttings having approximately the same LWP at the time of setting. This indicated that a higher rate of water loss and/or a lower rate of water uptake may have occurred in cuttings which failed to survive. The major differences in LWP, between those cuttings which rooted and those cuttings which failed to root, occurred between weeks 6-12 after setting. In those cuttings which rooted, the LWP was maintained at a relatively constant level between approximately -1.70 and -1.80MPa. However, in those cuttings which failed to survive, the LWP declined at a relatively linear rate of -0.13MPa/week until leaf necrosis was observed in week 13. This indicated that cuttings which have the ability to regulate their water potential are more likely to develop an adventitious root system than cuttings which may not be able to regulate their water potential.

Results suggested that there may be a threshold value of LWP beyond which cuttings fail to survive. This threshold value appeared to be between approximately -2.40MPa and -2.60MPa. However, it is acknowledged that the absolute value may vary between individual cuttings and may be influenced by other factors, including stock plant age and season of cutting collection. The monitoring of LWP in individual cuttings demonstrated the actual changes in water potential which occurred in cuttings which rooted and those which failed to survive. It also demonstrated how the average result may be skewed if the rooted and non rooting cuttings are not partitioned.

IV.9 Manipulation of Rooting

9.1 Introduction

In Section IV.1 of this thesis, the rooting of juvenile *Pinus radiata* cuttings was demonstrated to decline significantly between winter and spring. The differences observed in the concentration of auxin and carbohydrate during this period was thought to be related to the rooting success (Section IV.4 and IV.5 respectively). In cuttings which were collected from two year old stock plants on the 3/8/94 and the 14/9/94 (winter-early spring), where the rooting percentage was 91 and 76% respectively, a 'peak' in basal stem auxin (IAA) concentration was observed during weeks 2 and 3 after excision. Also, the basal stem sucrose concentration was maintained above 15mg/g DW throughout the callus formation period. This is in contrast to cuttings which were collected from two year old stock plants on the 26/10/94 and the 7/12/94 (mid spring-summer) where the rooting percentage was 0 and 14% respectively. In the latter group of cuttings, a 'peak' in the basal stem IAA concentration was not observed and the basal stem concentration of sucrose declined rapidly from approximately 20mg/g DW to almost zero within a few weeks following excision. This indicated that a failure to regulate auxin and sucrose levels may be a limitation to the rooting success of cuttings collected in October and December.

In cuttings which were collected from two year old stock plants on the 26/10/94 and the 7/12/94, a high concentration of glucose and fructose was observed in the basal stem region of cuttings at the time of excision when compared with cuttings collected on the 3/8/94 and the 14/9/94 (Section IV.5). It was thought that the high concentration of glucose and fructose observed in cuttings may have be an indication that cuttings are unlikely to undergo ARF.

The application of exogenous auxin to *P. radiata* cuttings has been examined previously (Jacobs, 1939; Fielding, 1954; Libby and Conkle, 1966; Fielding, 1969; Smith and Thorpe, 1975b). In general, the application of exogenous auxin-like compounds appeared to decrease the time taken to form roots and increase the number of roots produced per cutting. However, the effect of applied auxins on the percentage rooting remains debatable. The application of auxin to juvenile cuttings

which have been collected at a time in the year when the rooting percentage is known to be low has not been examined. The influence of applied carbohydrates on the rooting of *P. radiata* cuttings has not been examined. In other plant species, the application of exogenous sucrose generally enhanced the rooting of cuttings (Veierskov, 1988). However, the influence of applied carbohydrates appeared to be highly dependant upon the concentration used, too high and toxic effects are usually observed (Veierskov, 1988). In olive cuttings, the application of sucrose and IBA together significantly enhanced the rooting response over IBA alone (Wiesman and Lavee, 1995).

The usual sites of auxin biosynthesis in vascular plants are the meristems and enlarging tissues (Leopold and Kriedemann, 1985). Given that in *P. radiata* cuttings, the main site of auxin formation is likely to be the terminal bud and nearby expanding needles, the auxin 'peak' observed in the basal stem region of cuttings collected from 2 year old stock plants on the 3/8/94 and the 14/9/94 may have originated from auxin synthesised in the stem apex and then translocated basipetally to the basal region of the cutting. The auxin transport inhibitor 2,3,5-triiodobenzoic acid (TIBA) has been demonstrated to inhibit ARF in a number of plant species by inhibiting polar auxin transport to the lower stem region (Wample and Ried, 1979; Yamamoto and Kozlowski, 1987; McNamara and Mitchell, 1991). The influence of TIBA on ARF in *P. radiata* cuttings has not been investigated.

A series of experiments were designed in an attempt to manipulate the rooting success of cuttings collected from juvenile stock plants at different times in the year, when the rooting was known to differ greatly, in an attempt to better understand the importance and involvement of auxin and carbohydrates in the rooting of *P. radiata* cuttings.

9.2 Materials and Methods

9.2.1 Triiodobenzoic acid (TIBA) Experiment

An experiment was undertaken to investigate the influence of the auxin transport inhibitor 2, 3, 5-triiodobenzoic acid (TIBA) on the rooting success and IAA concentration in *P. radiata* cuttings. The cuttings were collected from 2 year old stock plants at Westerway on the 21/7/95, when the rooting percentage was known to be high, and set in the standard rooting medium and under standard environmental

conditions in the phytotron (Section III.2.2). A complete randomised block design was utilised, where there were 4 treatments (untreated control, lanolin control, TIBA and apex removed) and 5 replicates of 40 cuttings per replicate. The TIBA was incorporated into lanolin paste (0.5% w/v) and approximately 0.2-0.3g applied as a 1cm wide ring around the mid-stem region of a 10cm long cutting. In the lanolin control treatment, a 1cm wide ring of lanolin paste was applied around the mid-stem region of the cutting. In the apex removed treatment, the apical meristem and approximately 1cm of terminal stem and associated leaves were excised using hand secateurs. The cuttings were propagated for 16 weeks, after which, the rooting percentage was determined (Section III.3.1).

At the time of excision and at weekly intervals thereafter for 4 weeks, apical and basal stem samples were collected for IAA analysis. Each sample unit consisted of five 1cm apical stem or basal stem sections which were bulked together, snap frozen in liquid nitrogen, freeze dried and stored at -20°C prior to RIA determination of IAA concentrations (Section III.7). At each sample time 5 replicates of basal and apical samples were collected.

9.2.2 Application of Exogenous Auxin (Concentration x Timing Experiment)

An experiment was designed to investigate the influence of different concentrations of exogenously applied auxins and at different times after excision on the percentage rooting of *P. radiata* cuttings. The cuttings were collected from 2 year old stock plants at Westerway on the 14/12/95, when the rooting percentage was known to be low, and set in the standard rooting medium and under standard environmental conditions in the phytotron (Section III.2.2). The experiment was duplicated in a glasshouse which was located at Westerway, Tasmania. The cuttings were set in the standard rooting medium and root trainer cells (Section III.2.2) and placed on a 20°C heated propagation bench under mist. The misting was controlled using a gold leaf-mercury switch and sarlon 50% shade cloth was positioned 1m above the cuttings to protect them from direct sunlight. The daytime temperature ranged from 15-27°C and the night time temperature ranged from 4-15°C.

A (3 x 3) factorial experiment was designed which examined three different concentrations of exogenously applied auxin in the form of indole-3-butyric acid (IBA) (0, 3000 and 8000ppm) and three different times of application (at the time of excision, 2 weeks after excision and 4 weeks after excision). The 3000 and

8000ppm IBA (Rootex®) was applied as a basal powder dip to the cuttings. A talc (Faulding) powder dip was used as the 0ppm treatment. Four replicates of 20 cuttings per replicate were set in the phytotron. Three replicates of 20 cuttings per replicate were set in the glasshouse. The cuttings were propagated for 16 weeks under phytotron and glasshouse conditions, after which, the rooting percentage was determined (Section III.3.1).

9.2.3 Interaction of Auxin, Sucrose and Timing of Application

An experiment was designed to investigate the influence of exogenously applied auxins and sucrose at different times after excision on the percentage rooting of *P. radiata* cuttings. The cuttings were collected from 2 year old stock plants at Westerway on the 14/12/95, when the rooting percentage was known to be low, and set in the standard rooting medium and under standard environmental conditions in the phytotron (Section III.2.2). The experiment was duplicated in a glasshouse which was located at Westerway, Tasmania. The cuttings were set in the standard rooting medium and root trainer cells (Section III.2.2) and placed on a 20°C heated propagation bench under mist (mentioned previously in Section IV.5.2.2).

A (4 x 2) factorial experiment was designed to examine the influence of applying exogenous auxin (3000ppm IBA), at different times after excision (a control with no auxin applied, auxin applied at the time of excision, 2 weeks after excision and 4 weeks after excision) and with or without a sucrose basal powder dip applied every week during the period of callus formation (40% w/w). Four replicates of 20 cuttings per replicate were set in the phytotron. Three replicates of 20 cuttings per replicate were set in the glasshouse. The cuttings were propagated for 16 weeks under phytotron and glasshouse conditions, after which, the rooting percentage was determined (Section III.3.1).

The experiments outlined in Section IV.5.2.2 and IV.5.2.3 require the 'lifting' of cuttings from the rooting media at different times after setting. Therefore a separate experiment was designed to examine the effect of 'lifting' on the rooting success of *P. radiata* cuttings. The experiment consisted of 3 treatments (control, lifted and re-set 2 weeks after originally setting and lifted and re-set 4 weeks after originally setting). The experiment consisted of 3 replicates of 20 cuttings per replicate. The cuttings were collected from 2 year old stock plants at Westerway on the 14/12/95, when the rooting percentage was known to be low, and set in the

standard rooting medium and under standard environmental conditions in the phytotron (Section III.2.2). The experiment was also duplicated in a glasshouse which was located at Westerway, Tasmania. The cuttings were set in the standard rooting medium and root trainer cells (Section III.2.2) and placed on a 20°C heated propagation bench under mist (mentioned previously in Section IV.5.2.2). The cuttings were propagated for 16 weeks under phytotron and glasshouse conditions, after which, the rooting percentage was determined (Section III.3.1).

Another experiment was designed to examine the change in leaf water potential (LWP) of *P. radiata* cuttings which were set under both phytotron and glasshouse conditions. At the time of excision and at weekly intervals thereafter for 6 weeks, 5 cuttings were randomly selected from each environment and the LWP measured using the pressure chamber technique (Section III.8). Although it is acknowledged that there was no true replication of environments, the results obtained may highlight differences in LWP between the two different environments.

9.2.4 Glucose and Fructose Application Experiments

An experiment was designed to investigate the application of exogenous glucose and fructose on the rooting success of *P. radiata* cuttings in the phytotron. The cuttings were collected from 3 year old stock plants located at Mt Lloyd on the 23/4/96, when the rooting percentage was expected to be high, and set in the standard rooting medium and under standard environmental conditions in the phytotron (Section III.2.2). The experiment consisted of 4 treatments (control, glucose, fructose and a combined glucose and fructose dip) with 4 replicates of 20 cuttings per replicate. The glucose and fructose were applied as powder dips (40% w/w) to the basal region of the cutting at the time of excision. The cuttings were propagated for 16 weeks under phytotron conditions, after which, the rooting percentage was determined (Section III.3.1).

9.3 Results

9.3.1 Effect of Triiodobenzoic Acid (TIBA)

9.3.1.a Effect on Percentage Rooting

The application of 0.5% TIBA in lanolin to the mid-stem region of cuttings

collected from 2 year old stock plants significantly reduced the rooting percentage in comparison with the control group of cuttings (Figure IV.9.1; Plate 31). There was no significant difference between the control and lanolin treated cuttings. The removal of the cutting apex significantly reduced the rooting percentage of the cuttings in comparison to the control treatments. However, the TIBA treated cuttings had a significantly lower rooting percentage in comparison to the apex removed treatment.

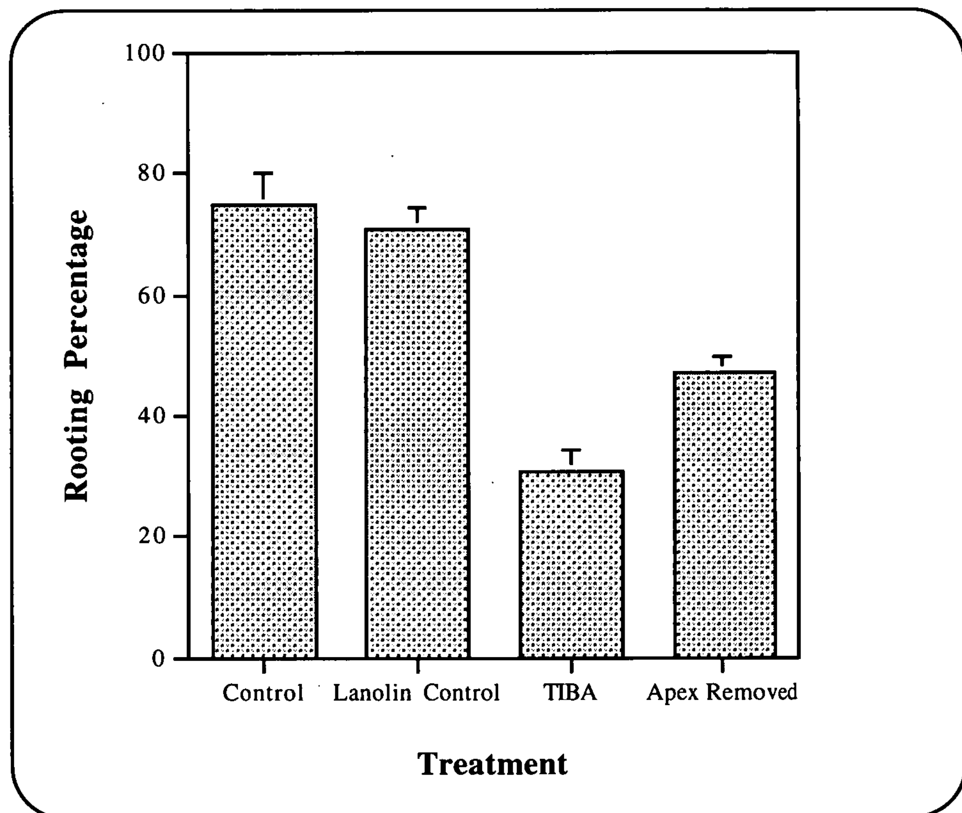

Figure IV.9.1 - The mean rooting percentage of cuttings collected from 2 year old stock plants on the 21/7/95 (including standard errors), set in the phytotron under standard conditions (Section III.2.2). The treatments were (a) control, (b) lanolin control, (c) TIBA in a 0.5% w/w lanolin paste and (d) apex of cutting removed. The $LSD_{0.05}$=11.35 (data included in Appendix 30).

In week 3 after setting, approximately 30-40% of the TIBA treated cuttings displayed signs of chlorosis in the leaves above the zone of TIBA application. In the following week (week 4), approximately 50-60% of the TIBA treated cuttings displayed signs of leaf chlorosis in the zone where the TIBA was applied. However,

Plate 31 - The rooting of cuttings in the TIBA experiment after 16 weeks in the phytotron (bar = 20cm).

the terminal bud and surrounding leaves remained green and appeared unaffected. In week 6 following excision, the cuttings which had become chlorotic during week 3 and 4 were completely chlorotic and tissue necrosis was evident.

9.3.1.b The Effect of TIBA on IAA Concentration

A significantly lower concentration of IAA was recorded in the basal stem region of TIBA treated cuttings in comparison to the control group cuttings during weeks 1, 2 and 3 after excision (Figure IV.9.2). There was no significant difference observed in the concentration of IAA in apical tissues of both control and TIBA treated cuttings.

In the control cuttings, the concentration of IAA in the apical and basal stem tissue increased significantly from 1435.56 ng/g DW and 2200.36 ng/g DW respectively in week 1 to 1739.08 ng/g DW and 2629.54 ng/g DW respectively in week 2 after excision (Figure IV.9.2.a). The concentration of IAA in the apical and basal stem region decreased significantly from 1733.56 ng/g DW and 2345.62 ng/g DW respectively in week 3 to 1352.92 ng/g DW and 1475.04 ng/g DW in week 4 (Figure IV.9.2.a). This 'peak' in IAA concentration was similar to that observed previously in cuttings which exhibited a high rooting percentage (Section IV.4).

In the TIBA treated cuttings, the concentration of IAA in the apical region of the cutting increased significantly from 1501.48 ng/g DW at the time of excision to 1799.56 ng/g DW in week 3 (Figure IV.9.2.b). The concentration of IAA in the apical region decreased significantly from 1799.56 ng/g DW in week 3 to 1450.42 ng/g DW in week 4. However, the concentration of IAA in the basal stem region of the cutting decreased from 2239.7 ng/g DW at the time of excision to 1441.82 ng/g DW in week 4 following excision (Figure IV.9.2.b).

The low rooting percentage in TIBA treated cuttings corresponded with low concentrations of IAA in the basal stem region of the cuttings in comparison to the control group of cuttings. This indicated that TIBA appeared successful at inhibiting the translocation of IAA to the basal stem region. The reduced concentration of IAA observed in the basal region of TIBA treated cuttings presents more evidence which suggests that IAA may play an important role in the rooting of *P radiata* cuttings. The concentration of TIBA used in the experiment was not considered toxic. Concentrations of 1-2% TIBA in lanolin (w/w) have been used in other plant species

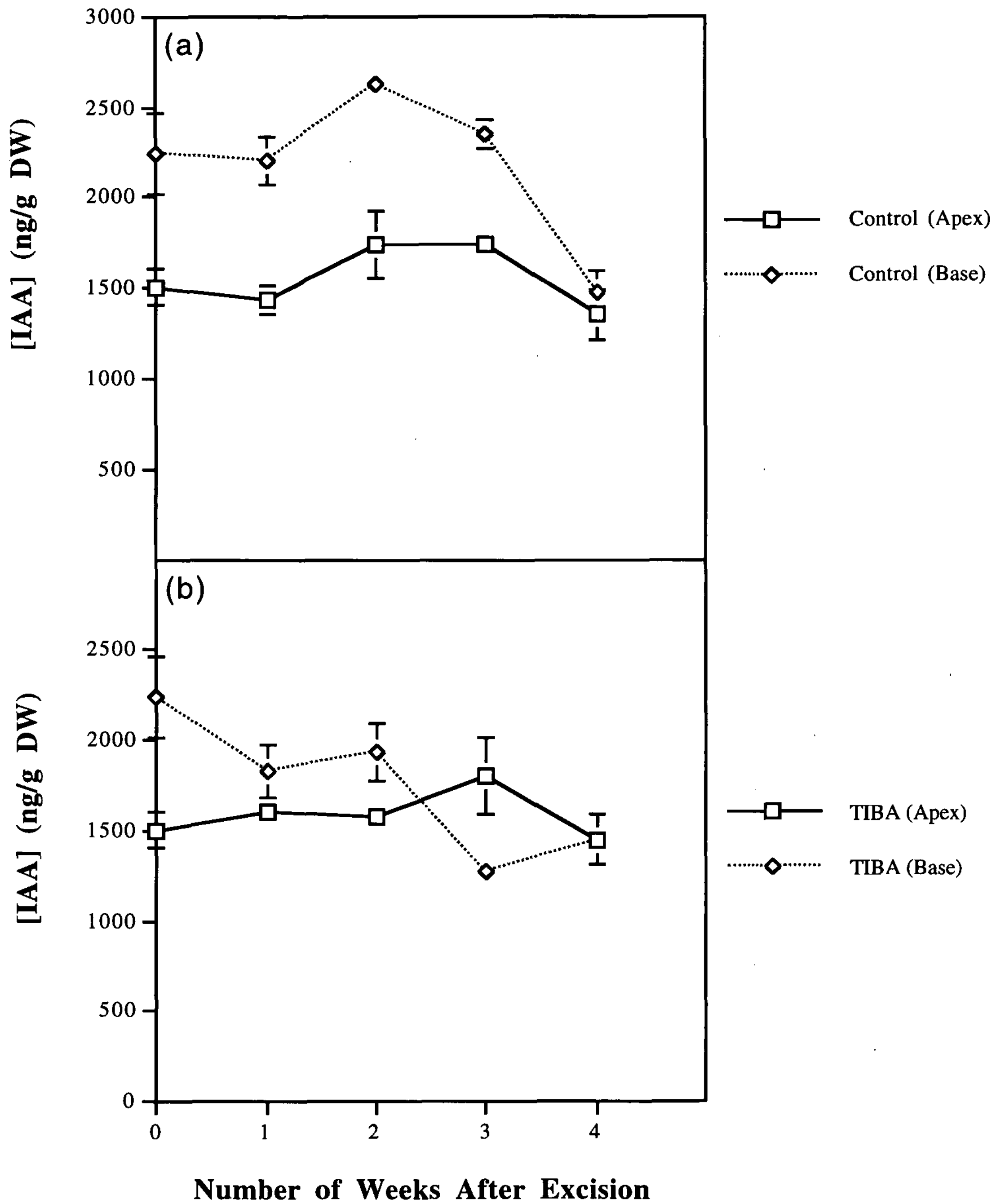

Figure IV.9.2 - The change in mean apical and basal stem IAA concentrations (including standard errors) in *P. radiata* cuttings collected from two year old stock plants on the 21/7/95 and propagated under standard conditions in the phytotron; **(a)** control cuttings and **(b)** TIBA treated cuttings (interaction $LSD_{0.05}$=223.7) (data included in Appendix 31).

without any harmful effects (Prasad *et al.*, 1989; McNamara and Mitchell, 1991). The cross-reactivity of the IAA antibody used in the RIA technique with TIBA was not known. The concentrations of IAA observed in this experiment were similar to that observed in Section IV.4. However, the interpretation of the IAA concentration data must still be viewed with some caution.

9.3.2 Auxin Applications

9.3.2.a Concentration and Timing Experiment

There was a significant interaction between the concentration of IBA used and the timing of auxin application under phytotron conditions (Figure IV.9.3.a). The rooting percentage of cutting was significantly higher when the auxin was applied 2 weeks after setting (mean equals 33.75%), compared to the application of auxin at the time of excision and 4 weeks after setting (means equal 15.42% and 8.75% respectively). There was also a significant reduction in the rooting percentage of cuttings treated with talc 4 weeks after setting in comparison to being treated with talc 2 weeks after setting. In the phytotron, the 'lifting' and re-setting of cuttings at different times after setting did not significantly influence the rooting percentage of cuttings (Appendix 36.a).

In the glasshouse, both the concentration of auxin and the timing of application did not significantly influence the rooting of *P. radiata* cuttings collected from 3 year old stock plants (Figure IV.9.3.b). The rooting percentage of cuttings propagated under glasshouse conditions was much higher than that observed in the phytotron. In the glasshouse, 'lifting' of cuttings 2 weeks after setting reduced the rooting percentage significantly in comparison to the control and 'lifted' 4 weeks after setting treatment (Appendix 36.b).

9.3.2.b Auxin-Sucrose and Timing Experiment

In the phytotron, there was a significant interaction between the timing of IBA application and the application of sucrose (Figure IV.9.4.a). In comparison to the control group of cuttings, the rooting percentage was significantly reduced if the IBA (3000ppm) was applied at the time of setting (T0), whereas no significant difference in the rooting percentage was observed if IBA was applied at week 2 after setting (T2). The application of sucrose alone or in combination with IBA at different times after setting generally resulted in a reduction of the rooting percentage. The only

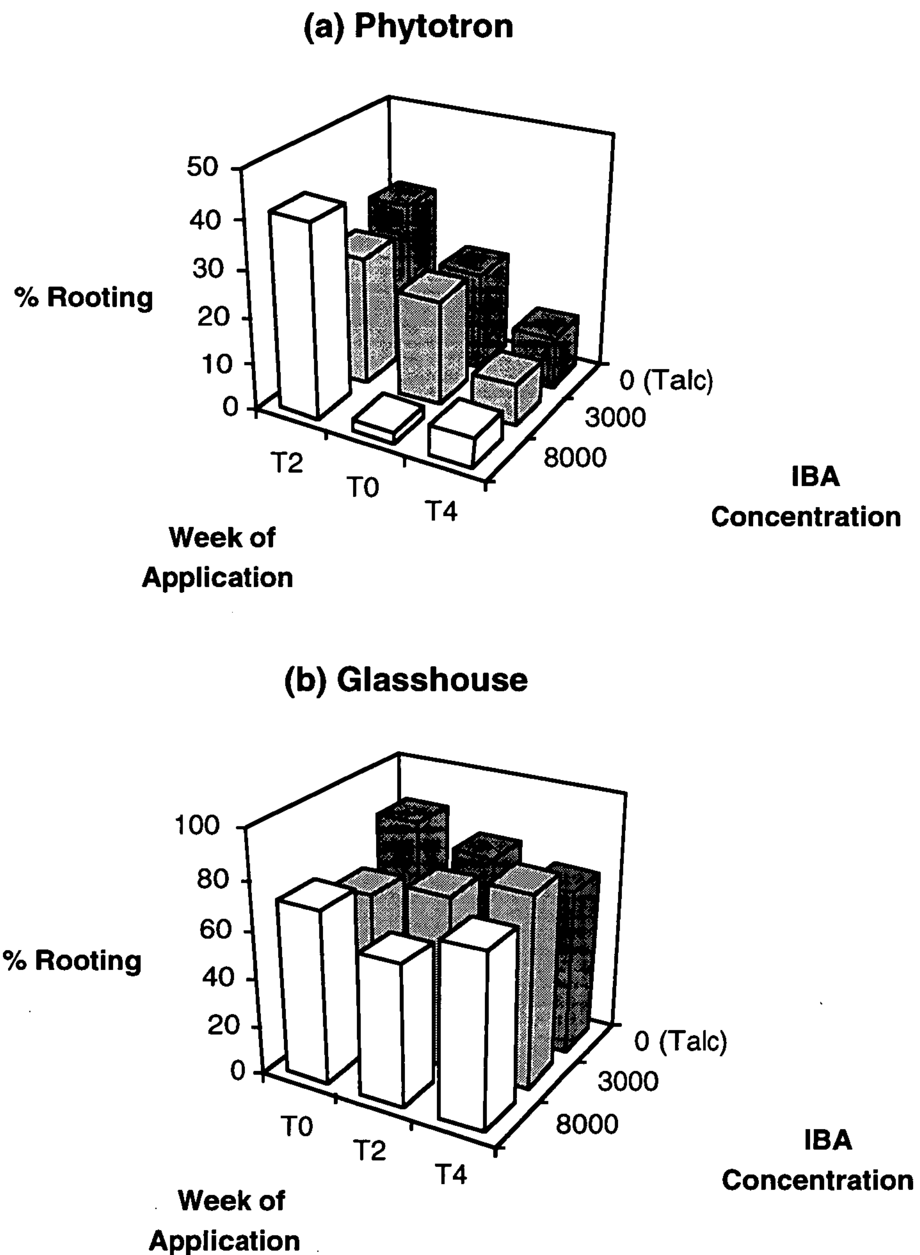

Figure IV.9.3 - The effect of IBA concentration and timing of application on the rooting percentage of cuttings collected from three year old stock plants on the 14/12/95. Cuttings were propagated under **(a)** phytotron (interaction $LSD_{0.05}$=12.43) and **(b)** glasshouse conditions. There was no significant difference observed between treatments under glasshouse conditions (means and standard errors included in Appendices 32 and 33).

exception to this was observed in week 4, when the application of IBA and sucrose together resulted in a significantly higher rooting percentage than just IBA alone. However, based on these results, it was difficult to draw many conclusions about the effect of auxin and sucrose on the rooting percentage of *P. radiata* cuttings.

In the glasshouse, both the timing of auxin application and the application of sucrose did not significantly influence the rooting of *P. radiata* cuttings collected from 3 year old stock plants (Figure IV.9.4.b). The rooting percentage of cuttings propagated under glasshouse conditions was much higher than that observed in the phytotron.

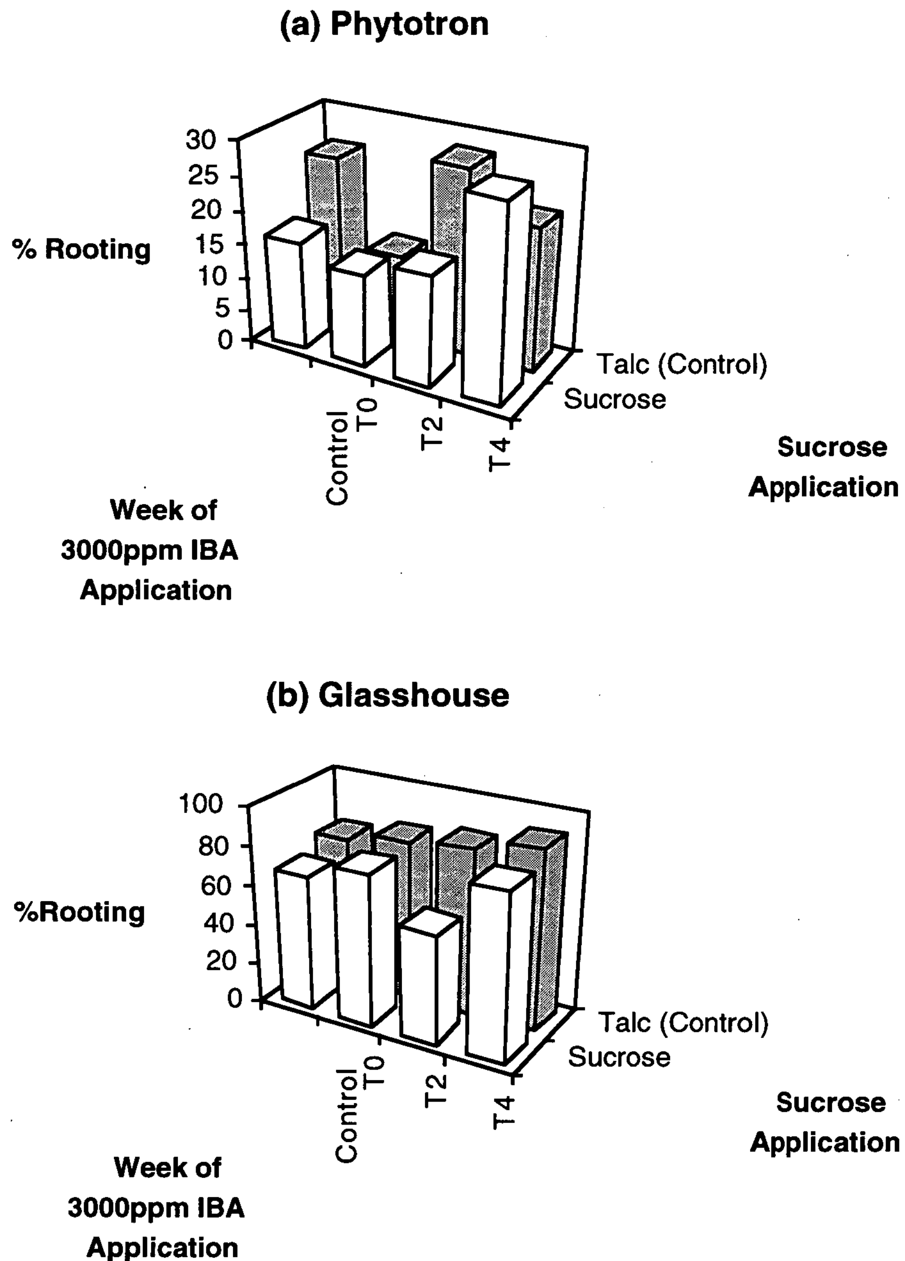

Figure IV.9.4 - The effect of IBA (3000ppm) application time and sucrose application on the rooting percentage of cuttings collected from three year old stock plants on the 14/12/95. Cuttings were propagated under **(a)** phytotron (interaction $LSD_{0.05}$=9.17) and **(b)** glasshouse conditions. There was no significant difference observed between treatments under glasshouse conditions (means and standard errors included in Appendices 34 and 35).

9.3.2.c Comparison of Cutting Water Potential

In general, a higher rooting percentage of cuttings was observed in the glasshouse (Figures IV.9.3 and IV.9.4). This observation was somewhat contradictory to the findings discussed in Section IV.1 of this thesis, where a physiological and/or anatomical factor was considered more responsible for influencing the rooting percentage than the propagation environment of the cutting. Although no valid statistical comparison may be made between the two different environments, the LWP of cuttings propagated under glasshouse conditions was much higher at all times after excision than that observed in the phytotron (Figure IV.9.5).

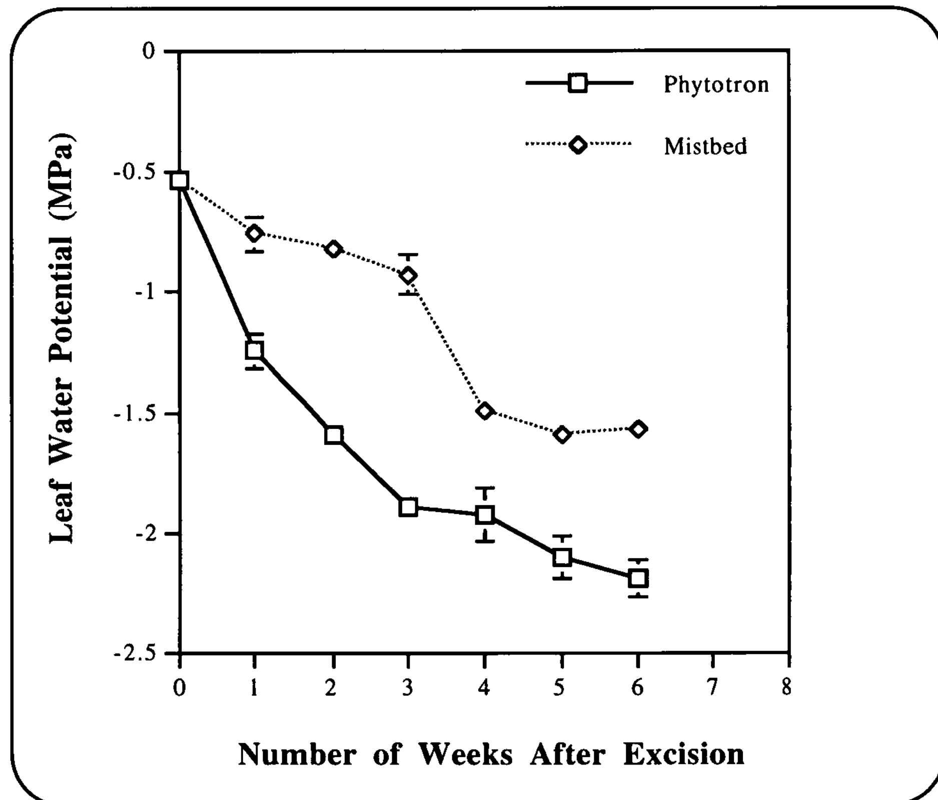

Figure IV.9.5 - The change in mean leaf water potential (MPa) of *P. radiata* cuttings during a 6 week period following excision. The cuttings were collected from 3 year old stock plants at Mt Lloyd and set under both phytotron and glasshouse conditions. The $LSD_{0.05}$=0.210 and 0.165 for weekly changes in the LWP of phytotron and glasshouse set cuttings respectively. The means and standard errors in Figure IV.5.5 were obtained from Appendix 37. No statistical comparison could be made between different cutting environments because there was no true replication of environments.

9.3.3 Glucose and Fructose Applications

The application of 40% (w/w) glucose, fructose and glucose and fructose combined basal powder dips at the time of setting significantly reduced the rooting percentage of *P. radiata* cuttings in comparison to the control group of cuttings (Figure IV.9.6; $LSD_{0.05}$=11.75). The cuttings were collected from 3 year old stock plants on the 23/4/96 and set under standard conditions in the phytotron. However, there was no significant difference between the glucose, fructose and glucose and fructose combined basal powder dip.

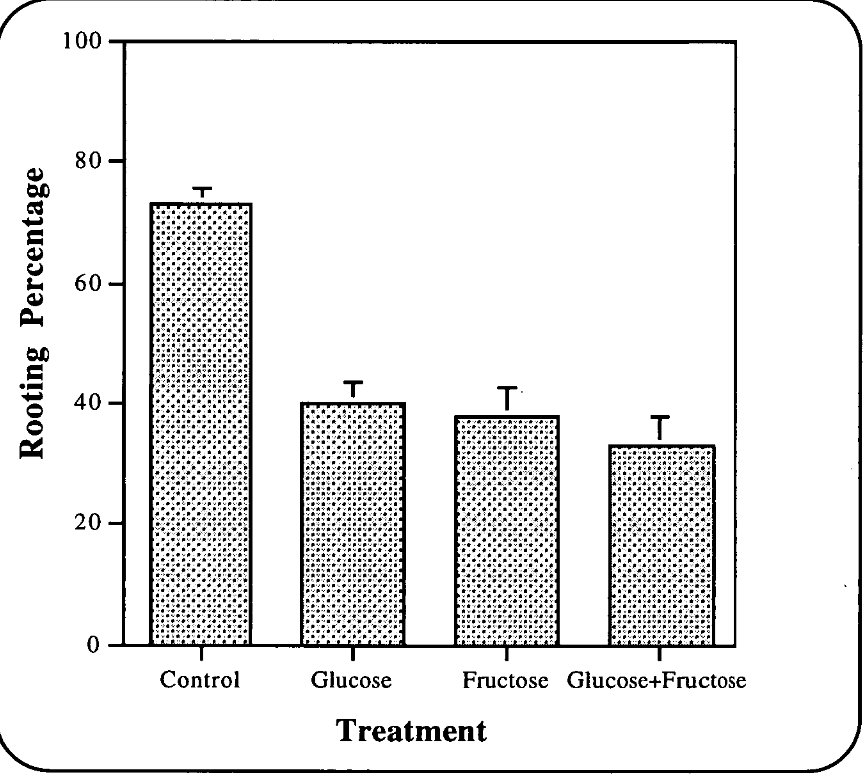

Figure IV.9.5 - The mean effect of applied glucose and fructose (including standard errors) on the rooting percentage of *P. radiata* cuttings. The glucose and fructose were applied as a basal talc dip (400g carbohydrate/kg talc) to cuttings collected from 3 year old stock plants on the 23/4/96. The cuttings were propagated in the phytotron ($LSD_{0.05}$=11.75) (means and standard errors included in Appendix 38).

9.4 Discussion

The application of TIBA to the mid-stem region of *P. radiata* cuttings collected from two year old stock plants on the 21/7/95 significantly lowered the rooting percentage in comparison to the control group of cuttings. A reduced rooting percentage in TIBA treated cuttings was related to a reduced IAA concentration in the basal region of the cutting during weeks 1, 2 and 3 after setting. However, no significant difference in IAA concentration was observed in the apical region of both TIBA and control treated cuttings throughout the 4 week period of measurement. This indicated that TIBA may have been successful at inhibiting the transport of IAA from the apical to the basal stem region of the cutting. Similar findings have been reported by other researchers (Hernandez and Driss-Ecole, 1989; Botia *et. al.*, 1992; Grochowska *et. al.*, 1994).

In the phytotron, the removal of the stem apex containing the apical meristem significantly reduced the rooting percentage of cuttings collected from two year old stock plants on the 21/7/95, when the rooting percentage was known to be high, in comparison to the control group of cuttings. Although the concentration of IAA in the apex removed treatment was not assessed, the excision of the terminal bud may limit the amount of auxin available for translocation to the basal stem region. In commercial *P. radiata* cuttings nurseries, the setting of cuttings without a apical meristem is a common practice. Those cuttings which do not possess a apical meristem are termed second order shoots or secondary cuttings. However, the rooting percentage of secondary cuttings set under field conditions is not reported to differ significantly to first order shoots (cuttings which possess an apical meristem) (Fielding, 1954; Menzies *et. al.*, 1985).

In Section IV.4. and IV.5 of this thesis, results indicated that the concentration of endogenous auxin and sucrose may limit the rooting percentage of cuttings collected from juvenile stock plants in spring and early summer. Therefore, in an attempt to promote the rooting of *P. radiata* cuttings collected at times in the year when the rooting percentage was known to be low, various combinations and timings of basal auxin and sucrose applications were investigated. In the phytotron, the application of auxin 2 weeks after setting significantly increased the rooting percentage in comparison to other treatments. This indicated that auxin may be required for ARF during this period. This result is in agreement with the findings presented in Section IV.4, where a 'peak' of endogenous auxin was observed, during

week 2 and 3 after excision, in cuttings that exhibited a high rooting percentage. However, under glasshouse conditions, no significant difference in the rooting percentage of cuttings was observed between different auxin treatments. In general, the application of sucrose alone or in combination with auxin did not appear to influence the rooting percentage of cuttings set under both phytotron and glasshouse conditions.

In comparison to cuttings set in the phytotron, a higher rooting percentage was observed in cuttings set under glasshouse conditions. In Section IV.1 of this thesis, the cutting environment was not thought to influence the rooting percentage of *P. radiata* cuttings. However, the difference observed in cuttings set under glasshouse and phytotron conditions indicated that environmental conditions may influence the rooting percentage of *P. radiata* cuttings. A higher LWP was observed in glasshouse set cuttings compared to phytotron set cuttings. This indicated that cuttings which were propagated under glasshouse conditions had a more favourable water potential during ARF, which as mentioned in Section IV.8 was thought to be important for the development of callus tissue. Therefore, the maintenance of a high water potential during ARF may be the most important factor necessary for the successful rooting of *P. radiata* cuttings.

In general, the lifting and re-setting of cuttings at different times after excision did not appear to influence the rooting percentage of *P. radiata* cuttings under phytotron conditions. In the glasshouse however, the lifting and re-setting of cuttings 2 weeks after excision significantly reduced the rooting percentage in comparison to the other lifting treatments. The significance of this result may require further investigation.

The application of glucose, fructose and glucose and fructose combined significantly reduced the rooting percentage of cuttings in comparison to the control cuttings. This indicated that high concentrations of glucose and fructose at the time of excision may be responsible for the inhibition of ARF, a result which was initially considered in Section IV.5 of this thesis. However, the concentration of carbohydrate used in the basal powder dips may have been toxic to the cutting.

V. General Discussion

V. General Discussion

While the results presented in each chapter have been previously discussed, the focus of this section is to bring together the relevant findings and discuss the physiological factors affecting the rooting success of *Pinus radiata* cuttings. Although many cultural factors affecting the commercial production of *P. radiata* cuttings have been researched previously, this project focused on gaining an increased understanding of the physiological mechanisms associated with ARF. Throughout this discussion commercial implications and recommendations are provided.

The rooting ability of cuttings appeared to be influenced mainly by two factors; (1) the age of the parent stock plant from which the cutting was collected and (2) the time in the year when the cuttings was collected. Firstly, there is a loss in the rooting ability of cuttings associated with an increase in the age of the parent stock plant. Secondly, there is a seasonal variation in the percentage of cuttings forming roots. These finding were similar to previous studies which have examined the influence of stock plant age and season of cutting collection (Jacobs, 1939; Fielding, 1954; Fielding, 1969; Menzies *et al.*, 1988). These factors restrict the type of shoots which can be selected as cutting material and limit the commercial production of *P. radiata* cuttings to only certain times in the year. Although the effects of these factors have been demonstrated, the underlying physiological and morphological mechanisms controlling adventitious rooting in *P. radiata* have not been elucidated. The current nursery practice aims to manage the factors which may influence the rooting potential of cuttings to achieve a commercial production of rooted cuttings.

The rooting percentage of *P. radiata* cuttings was influenced by both stock plant age and time of cutting collection. The rooting percentage of cuttings collected from different aged stock plants displayed a similar trend throughout the year, with maximum rooting in June, decreasing through Tasmanian spring to a minimum survival in October, then increasing in November and December. The amplitude of the seasonal change was much less in cuttings collected from mature stock plants. This trend is in broad agreement with earlier reports (Jacobs, 1939; Fielding, 1954; Fielding, 1969; Menzies *et al.*, 1988). The interaction between stock plant age and time of collection was considered an important finding because previous researchers have tended to treat these factors separately. From a commercial perspective, the trial

demonstrated that it was possible to collect and set cuttings and achieve a high rooting percentage as late as August. The late setting of cuttings was considered an advantage in avoiding the wet conditions which occur during winter under Tasmanian field conditions.

The growth characteristics of the cuttings after setting were demonstrated to be influenced by the age of the stock plant from which the cutting was collected. The nursery growth of cuttings was generally reduced with increased age of the parent stock plant. This finding was in agreement with Fielding (1954) and Fielding (1969). The reduced rate of growth in cuttings collected from older stock plants, in comparison to those collected from juvenile stock plants, was attributed to both an increased time taken to initiate an adventitious root system and also a slower root growth after initiation. The cuttings begin to elongate (top growth) soon after callus growth has commenced. However, the amount of elongation (top growth) was influenced by the age of the stock plant from which the cutting was collected, where the more juvenile cutting material produced the most top growth. This differed from other reports which have demonstrated that a cutting will approximately double its height before root growth has occurred (Menzies *et al.*, 1988). Therefore from a commercial perspective, not only do cuttings collected from mature stock plants (8 year old) during winter have a lower rooting percentage than cuttings collected from juvenile stock plants, even if they form roots they generally grow much slower in the nursery. This means that cuttings collected from older stock plants may have problems achieving a transplantable size during the normal commercial production timetable.

The length of the cutting was demonstrated to influence the rooting percentage of *P. radiata* cuttings. Cuttings which were 5cm in length did not root as well as those cuttings which were 10 and 15cm in length. However, Fielding (1969) concluded that cutting size *per se* did not influence the rooting percentage. While, from an industry perspective, the collection of smaller cutting material is more desirable, the collection of cuttings below 10cm may adversely affect the rooting percentage. The length of the cutting also had a significant influence on many of the growth characteristics of the cuttings. The longer cuttings produced more callus tissue, higher root numbers, more top growth, and greater root dry weight. The thickness of the cuttings did not influence the rooting percentage of cuttings, however, it did influence the number of roots produced by each cutting and the amount of top growth produced. The increased growth of larger cuttings in the nursery is in agreement with the findings of Fielding (1969). From a commercial perspective, not only does the initial size of the cutting

influence the rooting percentage, but it also determines other important growth characteristics of the cutting, including the number of roots and the amount of top growth that a cutting will produce. The behaviour of cuttings with respect to stock plant age and seasonality under Tasmanian conditions is similar to that reported in the literature for other temperate climatic conditions (Jacobs, 1939; Fielding, 1954; Fielding, 1969; Menzies *et al.*, 1988). This provides the basis for further studies into the physiological basis of ARF in *P. radiata.*

The evidence presented suggests that the ability of *P. radiata* cuttings to form an adventitious root system is controlled by some endogenous factor(s). A similar rooting percentage of cuttings was observed in cuttings set under both field and controlled environment (phytotron) conditions This indicated that the factor(s) responsible for the seasonal variation in rooting percentage were present at the time of excision and appeared to be of physiological and/or anatomical origin (endogenous factor). Adventitious root formation (ARF) in other species is known to be influenced by many physiological and anatomical factors. The most important physiological factors appeared to be PGR's, carbohydrates and water relations. In this discussion the anatomical and physiological changes occurring during ARF will be evaluated independently, and then interactions between each of these factors discussed.

The successful development of an adventitious root system was dependant on the formation of callus tissue in the rooting zone. There was no callus tissue observed in the rooting zone of cuttings which did not root. This observation prompted the focus of research effort to the investigation of the processes leading to callus formation in cuttings. Cameron and Thomson (1969) also demonstrated that the first stage of adventitious root formation in *P. radiata* cuttings was the formation of callus tissue near the basal region of the cutting.

The growth of callus tissue was observed as the first step in the formation of an adventitious root system. The initiation of callus in those cuttings which rooted successfully at 20°C occurred at approximately 4 weeks after excision. The callus tissue appeared to initiate near the cambial region. Once initiated, callus parenchyma rapidly multiplied and grew in both the transverse and longitudinal planes. In the callus tissue, the first signs of vascular development (root initiation) were observed at approximately 11 weeks after excision. The evidence of vascular development included the appearance of callus xylem (tracheids) within the callus tissue. Cameron and Thomson (1969) reported similar anatomical changes occurring during the

development of an adventitious root system in *P. radiata*. In most other plant species, there is usually a short amount of time between callus initiation and root initiation (Hartmann and Kester, 1983). However, in *P. radiata*, callus initiation and root initiation were separated by approximately 6-7 weeks. In those cuttings which exhibited a low rooting percentage, no signs of callus development were observed in the weeks prior to cutting necrosis. This indicated that callus initiation had not occurred in these cuttings.

In cuttings collected from nine year old stock plants, much larger resin ducts were observed in the stem region between the phloem and the epidermis, than in cuttings collected from two year old stock plants. Lovell and White (1986) suggested that resin ducts may act as a barrier to ARF by occupying potential sites of callus initiation. In this study, in cuttings collected from both two and nine year old stock plants, the resin ducts did not appear close enough to the cambial region, the region where callus development is initiated, to reduce the number of potential callus initiation sites.

The concentration of the plant growth regulators (PGR's) auxin, abscisic acid, gibberellic acid, zeatin riboside and dihydrozeatin riboside at the time of excision was demonstrated to change significantly at different times in the season and between different ages. Although significant differences were observed at the time of excision, no relationship between PGR concentration and the subsequent rooting percentage was observed. In other plant species, many researchers have demonstrated that the concentration of PGR's at the time of excision may be related to the rooting percentage of cuttings (Boerjan *et al.*, 1995; Nanda and Anand, 1970; Wu and Barnes, 1981). However, a poor relationship between PGR concentration and rooting percentage has been reported in many other plant species (Blakesley, 1991a; Van Staden and Harty, 1988). If PGR's were involved in the ARF of *P. radiata* cuttings, then it was not reflected by PGR concentrations at the time of excision.

The ratio of different PGR's did not appear to influence the rooting ability of *P. radiata* cuttings. This finding appeared to disagree with the findings of other researchers who have demonstrated that the ratio of PGR's is an important factor in controlling the rooting of other plant species, eg. the ratio of auxin to cytokinin (Hartmann and Kester, 1983).

The concentration of PGR's in the basal stem region of *P. radiata* cuttings were demonstrated to change significantly in concentration after excision. This is in agreement with other researchers who have examined the changes in PGR's during ARF in other plant species (Blakesley *et al.*, 1991a; Blakesley *et al.*, 1991b; Hausman, 1993; Moncousin *et al.*, 1989;). Of all the PGR's assayed, the change in the concentration of auxin during the callus formation period was considered the most important. Because of the relatively specific root promoting properties of applied auxin, it is thought to play an important role in the rooting of cuttings in many plant species (Gaspar and Hofinger, 1988; Hartmann and Kester, 1983) The concentration of auxin in the basal stem region of *P. radiata* cuttings during ARF was though to influence the rooting success of the cuttings. In those cuttings which exhibited a high rooting percentage, a transient rise in auxin (indole-3-acetic acid) concentration was observed 2 and 3 weeks after excision. A transient increase in endogenous auxin concentration was previously reported by Blakesley *et al.* (1991b) as being the first biochemical event leading to root initiation in *Phaseolus aureus* hypocotyl cuttings. However, root initiation in these hypocotyl cuttings occurred within 24 hours after excision. Therefore, it may be too difficult to determine if the transient rise in auxin concentration was related to the callus initiation or root initiation stage of ARF in these hypocotyl cuttings. In *P. radiata* cuttings, because of the time difference between callus initiation and root initiation it appeared that the transient rise in auxin concentration may have been responsible for the callus initiation stage of ARF. This evidence indicated that the increase in endogenous auxin concentration may be the first sign of callus initiation in *P. radiata* cuttings. There is evidence to suggest that auxin may be required for cell division (Leopold and Kriedemann, 1985). Therefore, the presence of callus tissue in week 4 after excision indicated that the transient rise in auxin may have been responsible for the cell division in the cambial region.

In those cuttings which exhibited a low rooting percentage, a transient rise in endogenous auxin concentration was not observed during the period prior to cutting necrosis. These cuttings may lack the ability to react to excision.

Although the concentration of auxin at the time of excision did not relate well with the rooting percentage of cuttings, the change in the concentration of auxin after excision did. This suggested that the ability to change after excision may be more important than the absolute concentrations of auxin at the time of excision.

The application of TIBA, a auxin transport inhibitor, was demonstrated to significantly reduce the rooting percentage of cuttings. A lower concentration of endogenous auxin was observed in the basal stem region of TIBA treated cuttings. This indicated that TIBA may reduce the basipetal transport of auxin to the basal stem region of the cutting. In other plant species, the application of TIBA has also been demonstrated to reduce rooting percentage of cuttings (Wample and Ried, 1979; Yamamato and Kozlowski, 1987, McNamara and Mitchell, 1991). This was also thought to be a result of reduced basipetal auxin transport. The reduced basipetal transport of auxin appeared to inhibit the pulse of auxin which was observed in the control cuttings. In the control cuttings, a transient rise in endogenous auxin concentration was observed in the basal stem region of cuttings 2 and 3 weeks after excision. This finding supported that observed in previous experiments. The measurement of endogenous IAA levels in both the apical meristem and the basal stem region of the cutting suggested that the synthesis of IAA occurred in the apical meristem.

In general, the exogenous application of auxin to *P. radiata* cuttings, at a time in the year when the rooting percentage was known to be low, did not influence the rooting percentage. This finding was in agreement with other research which demonstrated that applied auxin does not influence the rooting percentage of *P. radiata* cuttings (Fielding, 1954; Fielding, 1969; Jacobs, 1939; Libby and Conkle, 1966). However, in a wide range of plant species, the application of exogenous auxin has been demonstrated to promote the rooting percentage of cuttings (Hartmann and Kester, 1983). Other researchers have demonstrated that the application of exogenous auxin increases the number of roots produced by a *P. radiata* cutting and speeds up the ARF process (Fielding, 1969; Libby and Conkle, 1966). This result was not observed under our experimental conditions. It is not known whether the auxin applied to the basal stem region of *P. radiata* cuttings reached the target tissues. Assuming that the applied auxin did reach the target tissue, this may suggest that auxin is not involved in the ARF process. Therefore, the transient rise in auxin concentration observed may only be an artefact of other biochemical events occurring in the cutting at the same time.

The other PGR's analysed during the propagation of the cuttings did not appear to have a regulatory role in the rooting of *P. radiata* cuttings. In general, the concentration of abscisic acid (ABA) increased within a week of excision. This had decreased by week 2 to a similar level to that observed at the time of excision. This

transient rise in ABA has been reported in many other plant species (Leopold and Kriedemann, 1985). The transient rise in ABA may be water-stress related or as a response to severance itself. However, the same general trend was observed in all cuttings therefore it would appear that ABA had little or no influence on the final rooting percentage. The cytokinins zeatin (riboside) and dihydrozeatin riboside appeared to have little or no influence on the final rooting percentage. Although cytokinins are generally considered to be inhibitors of ARF (Van Staden and Harty, 1988), they did not appear to be involved in the ARF of *P. radiata* cuttings.

The response of plant tissues to changes in PGR concentrations is also dependant upon the sensitivity of the cells to that particular PGR. The sensitivity of the target cells to a particular PGR may be influenced by many factors, including the number of PGR receptor sites, the receptor binding ability and the ability initiate the cascade of biochemical events which ultimately leads to anatomical or physiological changes. Trewavas (1981) indicated that the sensitivity of plant tissues to the PGR may be more important to plant growth and development than just the concentration of the PGR present in the plant tissue. Therefore, the relationship between PGR's and a growth response must not be over-interpreted, as tissue sensitivity issues have not been investigated in this study, only acknowledged.

The initial carbohydrate content of cuttings did not appear to influence the rooting percentage of cuttings. The results indicated that a weak inverse relationship may exist between the total carbohydrate content of cuttings at the time of excision and the rooting percentage. Further analysis of the individual components of total cutting carbohydrate revealed that fluctuations in the concentration of sucrose appeared to be related with ARF events. In those cuttings which exhibited a high rooting percentage, the concentration of sucrose was maintained above approximately 15mg/g DW throughout the callus formation period. However, in those cuttings which exhibited a low rooting percentage, the concentration of sucrose in the basal region of the cutting appeared to decline rapidly to almost zero in the week prior to cutting necrosis. This indicated that sucrose availability may be limiting the rooting success of *P. radiata* cuttings. Sucrose was main transport form of carbohydrate found in the phloem. The sucrose in the phloem may arrive at the basal stem region of the cutting via the release from stored forms such as starch, or from the formation of current photosynthate. The concentration of starch in the basal stem region and the attached basal leaves decreased rapidly to a basal concentration within the first week. This indicated that starch may be catabolised rapidly to supply the developing adventitious root system. A low rate of

carbon dioxide exchange observed in cuttings propagated under phytotron conditions indicated that current photosynthate is unlikely to be responsible for the maintenance of sucrose concentrations in the basal region of cuttings which exhibited a high rooting percentage. Therefore, the cutting must be able to mobilise starch and transport it from other regions within the cutting to the basal region.

The rapid metabolisation of starch in the basal stem and basal leaf region of cuttings collected at different times in the season and from different aged stock plants indicated that the conversion of starch to soluble sugars occurred readily. Also, the concentration of starch in cuttings collected at different times in the season did not appear to limiting to ARF. This indicated that the translocation of carbohydrate to the basal region of the cutting may limit the rooting percentage of cuttings at particular times in the year and in cuttings collected from mature stock plants.

In cuttings where a transient rise in the concentration of auxin was observed in weeks 2 and 3 after excision, the sucrose concentration was maintained above approximately 15mg/g DW throughout the callus formation period. The transient increase in auxin concentration may stimulate assimilate mobilisation to the basal region of the cutting. Auxin has been demonstrated to promote the utilisation and transport of metabolites in stems of *Phaseolus vulgaris* (Patrick and Wareing, 1976). Therefore, auxin may provide the initial stimulus for callus initiation and assist in the mobilisation of metabolites initially, until cell division occurs and a sink for carbohydrates is created. Once a sink has been established, sugars in the phloem are utilised by the developing adventitious root system.

In general, the exogenous application of sucrose to the basal stem region of *P. radiata* cuttings did not influence the subsequent rooting percentage. Weiseman and Lavee (1995) demonstrated that sucrose applied to the basal stem region of olive cuttings stimulated the rooting percentage. Also, they demonstrated that the application of both sucrose and auxin (IBA) had a synergistic effect on promoting the rooting of cutting. The application of sucrose in combination with auxin to *P. radiata* cuttings did not influence the rooting percentage of cuttings collected from two year old stock plants at a time in the year when the rooting percentage was known to be low. This indicated that sucrose may not be limiting the rooting success of cuttings. However, there are inherent problems associated with the application of exogenous carbohydrates to cuttings. Firstly, cuttings take up and translocate the exogenous carbohydrate from solutions primarily via the xylem, unlike endogenous carbohydrates

which are primarily transported in the phloem (Haissig, 1986). Secondly, exogenously applied sugars can exert a strong osmotic effect, possibly even toxic effects on plant cells (Veierskov, 1988).

Although the concentration of carbohydrates at the time of excision did not relate well with the rooting percentage of cuttings, the change in the concentration of sucrose after excision did appear to be important. This suggested that the ability to change or maintain the concentration after excision may be more important than the absolute concentrations of carbohydrates at the time of excision. This result was similar to that observed in the PGR studies.

In general, the water potential of cuttings decreased in a linear fashion during the first four weeks following excision. Anderson (1986) stated that the rate of water loss from a cutting is mainly dependent on the water vapour pressure difference between the leaf and the surrounding air. After week 4, the water potential in cuttings which exhibited a high rooting percentage plateaued and was maintained at a relatively constant level during the remaining weeks of callus formation. This indicated that cuttings were either increasing their rate of water uptake or decreasing their rate of water loss. This increase in water potential was associated with the appearance of callus tissue at the base of the cuttings. This suggested that the callus tissue may possess the ability to increase the rate of water uptake from the rooting media.

In those cuttings which exhibited a low rooting percentage, the LWP generally decreased at a faster rate than that observed in cuttings which exhibited a high rooting percentage. This indicated that these cuttings may be more sensitive to water loss than those cuttings which exhibited a high rooting percentage. This higher rate of water loss may be attributed to thinner epidermal layer observed in cuttings collected from two year old stock plants on the 26/10/94 and 7/12/94. Whereas the higher rate of water loss observed in cuttings collected from mature stock plants may have been a result of the higher leaf surface area per cutting.

In those cuttings collected from two year old stock plants which exhibited a low rooting percentage, a rapid decline in the concentration of soluble carbohydrates was observed in the weeks prior to cuttings necrosis. This reduction in soluble carbohydrate content may have also resulted in an increased the osmotic potential of the cells within the cutting. In other plant species, similar findings have been reported previously (Meier *et al.*, 1992; Tschaplinski *et al.*, 1995). An increase in cell osmotic potential would result in the cutting not being able to 'hold onto its water as strongly. Therefore, this would result in a faster rate of water loss from plant tissue.

In monitoring the leaf water potential (LWP) of individual cuttings, the lowest LWP observed in those cuttings which rooted was -2.38MPa. However, in those cuttings which failed to survive, the LWP of cuttings in the week prior to cutting necrosis was observed to be lower than -2.50MPa. This indicated that critical LWP may exist, beyond which cutting necrosis may occur. This threshold LWP appeared to be approximately -2.50MPa. However, the absolute value may differ depending on the individual cutting. In other plant species, a similar conclusion has been reported (Hartmann and Kester, 1983). In those cuttings which rooted successfully, the water potential decreased in a linear fashion during the first four weeks following excision. However, after this the water potential was maintained between approximately -1.75 and -1.69MPa during the remaining weeks. In those cuttings which failed to survive, the water potential decreased in a linear fashion from week 5 onwards until cutting necrosis was observed.

The rooting percentage of cuttings collected from 3 year old stock plants, at a time in the year when the rooting percentage was known to be low, and set under glasshouse conditions was much higher than that observed in cuttings set under phytotron conditions. The cuttings set in the glasshouse were misted every 5-10 minutes. A higher LWP was observed in cuttings set in the glasshouse, compared with cuttings set under phytotron conditions. This indicated that a higher water vapour pressure may have existed in the glasshouse. The higher rooting percentage of cuttings set under glasshouse conditions, where a higher LWP was maintained in the cutting throughout the callus formation period, indicated that cutting water relations may be an important physiological factor responsible for determining the rooting percentage.

The water potential of the stock plant at the time of excision did not appear to influence the final rooting percentage of cuttings. However, the ability maintain the water potential above some critical threshold value appeared more important. This finding is in agreement with other findings in this thesis which suggested that the ability to react to excision was more important than the absolute level of a physiological factor at the time of excision.

In summary, the ability of a cutting to 'react' to excision, either through changing the concentration of auxin or maintaining the concentration of sucrose and the water potential above a critical threshold, appeared to be more important than the absolute level of these factors at the time of excision in determining the rooting success of *P. radiata* cuttings. Also, the interaction between these factors appeared to be important in determining the rooting success of *P. radiata* cuttings.

VI. Bibliography

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VII. Appendices

Stock Plant Age and Timing of Collection Experiment

The rooting percentage of cuttings collected from 1, 2, 4 and 8 year old stock plants at different times in the year and set under field conditions at Meadowbank, Tasmania

Age of Cutting Material	Sample Date						
	30-Jun	28-Jul	25-Aug	23-Sep	20-Oct	23-Nov	LSD (0.05)
Age 1 SE	97.03 0.47	94.74 1.22	86.2 4.25	42.01 10.5	16.8 7.49	35.18 7.47	15.89
Age 2 SE	96.85 1.08	97.11 1.17	91.07 2.54	66.8 5.74	31.24 6.88	28.63 6.07	15.89
Age 4 SE	90.34 2.85	89.37 2.92	83.57 4.38	44.79 4.72	30.25 4.94	28.46 5.86	15.89
Age 8 SE	56.43 7.15	50.45 6.06	45.18 9.55	31.65 5	21.76 7.33	16.02 3.81	15.89
LSD (0.05)	15.89	15.89	15.89	15.89	15.89	15.89	
Significant Differences	1+8 2+8 4+8	1+8 2+8 4+8	1+8 2+8 4+8	1+2 2+4 2+8	ns	ns	

Cuttings Collected from 1, 2, 4 and 8 Year Old Stock Plants on the 30th June 1993 and Set Under Field Conditions at Meadowbank

(a) Average Stage of Root Growth (0-6)

Sample Date Days after Setting	4/8/93 34	23/9/93 85	27/10/93 119	29/11/93 152	22/12/93 175	28/1/93 212	9/3/93 252
Age 1	0	0.29	1	1.771	3.333	5	5.542
SE	0	0.074	0	0.167	0.355	0.261	0.189
Age 2	0	0.0417	0.604	1.021	2.375	4.708	5.083
SE	0	0.0417	0.143	0.048	0.255	0.328	0.245
Age 4	0	0	0.111	0.778	1.722	3	4.636
SE	0	0	0.111	0.147	0.188	0.464	0.253
Age 8	0	0	0	0.688	1.375	2.25	3.625
SE	0	0	0	0.111	0.152	0.356	0.574
LSD (0.05)	ns	0.121	0.243 0.263 vs 4	0.345 0.373 vs 4	0.715 0.733 vs 4	0.959 1.036 vs 4	0.999 1.021 vs 4
Significant Differences		1 + 2 1 + 4 1 + 8	All	1 + 2 1 + 4 1 + 8	1 + 2 1 + 4 1 + 8 2 + 8	1 + 4 1 + 8 2 + 4 2 + 8	1 + 8 2 + 8

(b) Average Amount of Callus Growth (mm)

Sample Date Days after Setting	4/8/93 34	23/9/93 85	27/10/93 119	29/11/93 152	22/12/93 175	28/1/93 212	9/3/93 252
Age 1	0	0.244	1.288	2.811	3.833	5.101	4.415
SE	0	0.074	0.253	0.27	0.328	0.343	0.428
Age 2	0	0.021	0.45	1.852	4.249	4.974	4.653
SE	0	0.021	0.142	0.272	0.313	0.444	0.31
Age 4	0	0	0.128	0.913	2.827	3.414	4.49
SE	0	0	0.128	0.262	0.392	0.356	0.43
Age 8	0	0	0	1.088	3.036	3.299	3.836
SE	0	0	0	0.25	0.353	0.347	0.404
LSD (0.05)	ns	0.113 0.122 vs 4	0.452 0.488 vs 4	0.735 0.794 vs 4	0.952 1.028 vs 4	1.051 1.135 vs 4	ns
Significant Differences		1 + 2 1 + 4 1 + 8	1 + 2 1 + 4 1 + 8	1 + 2 1 + 4 1 + 8 2 + 4 2 + 8	2 + 4 2 + 8	1 + 4 1 + 8 2 + 4 2 + 8	

(c) Average Amount of Top Growth (mm)

Sample Date Days after Setting	4/8/93 34	23/9/93 85	27/10/93 119	29/11/93 152	22/12/93 175	28/1/93 212	9/3/93 252
Age 1	0	0	0	5.083	85.833	85.833	169.167
SE	0	0	0	0.892	4.599	6.509	8.502
Age 2	0	0	0	0	61.667	77.917	89.583
SE	0	0	0	0	4.975	8.082	6.498
Age 4	0	0	0	0	33.889	44.444	43.889
SE	0	0	0	0	6.603	8.141	2.86
Age 8	0	0	0	0	21.667	14.583	29.583
SE	0	0	0	0	4.781	4.542	5.239
LSD (0.05)	ns	ns	ns	1.319 1.425 vs 4	14.232 15.372 vs 4	18.974 20.494 vs 4	17.902 19.337 vs 4
Significant Differences				1 + 2 1 + 4 1 + 8	1 + 2 1 + 4 1 + 8 2 + 4 2 + 8	1 + 4 1 + 8 2 + 4 2 + 8 4 + 8	1 + 2 1 + 4 1 + 8 2 + 4 2 + 8

Cuttings Collected from 1, 2, 4 and 8 Year Old Stock Plants on the 28th July 1993 and Set Under Field Conditions at Meadowbank

(a) Average Stage of Root Growth (0-6)

Sample Date Days after Setting	23/9/93 57	27/10/93 91	29/11/93 124	22/12/93 147	28/1/93 184	9/3/93 222
Age 1	0	0.583	1.417	2.625	4.917	5.042
SE	0	0.099	0.12	0.262	0.363	0.242
Age 2	0	0.542	1.042	1.979	2.833	4.833
SE	0	0.126	0.042	0.219	0.366	0.207
Age 4	0	0.062	0.458	1.5	2.167	4.583
SE	0	0.045	0.114	0.213	0.328	0.43
Aage 8	0	0	0.208	0.875	2.458	4
SE	0	0	0.096	0.109	0.298	0.492
LSD (0.05)	ns	0.237	0.28	0.594	0.969	1.036
Significant Differences		1 + 4 1 + 8 2 + 4 2 + 8	1 + 2 1 + 4 1 + 8 2 + 4 2 + 8	1 + 2 1 + 4 1 + 8 2 + 8 4 + 8	1 + 2 1 + 4 1 + 8	1 + 8

(b) Average Amount of Callus Growth (mm)

Sample Date Days after Setting	23/9/93 57	27/10/93 91	29/11/93 124	22/12/93 147	28/1/93 184	9/3/93 222
Age 1	0	0.633	2.063	3.401	4.195	3.998
SE	0	0.127	0.302	0.223	0.46	0.305
Age 2	0	0.46	2.114	3.315	4.862	4.788
SE	0	0.156	0.29	0.183	0.347	0.574
Age 4	0	0.103	0.836	2.738	3.411	4.457
SE	0	0.079	0.249	0.365	0.318	0.438
Aage 8	0	0	0.472	2.363	3.289	3.263
SE	0	0	0.295	0.415	0.437	0.447
LSD (0.05)	ns	0.308	0.811	0.888	1.126	1.285
Significant Differences		1 + 4 1 + 8 2 + 4 2 + 8	1 + 4 1 + 8 2 + 4 2 + 8	1 + 8 2 + 8	2 + 4 2 + 8	2 + 8

(c) Average Amount of Top Growth (mm)

Sample Date Days after Setting	23/9/93 57	27/10/93 91	29/11/93 124	22/12/93 147	28/1/93 184	9/3/93 222
Age 1	0	0	0	58.667	84.167	110.417
SE	0	0	0	7.821	7.683	10.668
Age 2	0	0	0	47.375	63.333	101.25
SE	0	0	0	8.193	6.256	8.121
Age 4	0	0	0	44.583	78.75	74.583
SE	0	0	0	6.013	5.37	8.471
Aage 8	0	0	0	28.75	19.583	38.333
SE	0	0	0	9.557	7.137	7.265
LSD (0.05)	ns	ns	ns	22.794	19.011	24.862
Significant Differences				1 + 8	1 + 2 1 + 8 2 + 8 4 + 8	1 + 4 1 + 8 2 + 4 2 + 8 4 + 8

Cuttings Collected from 1, 2, 4 and 8 Year Old Stock Plants
on the 25th August 1993 and Set Under Field Conditions at Meadowbank

(a) Average Stage of Root Growth (0-6)

Sample Date Days after Setting	23/9/93 30	27/10/93 63	29/11/93 96	22/12/93 119	28/1/93 156	9/3/93 194
Age 1	0	0	0.875	1.542	4.042	4.083
SE	0	0	0.065	0.208	0.424	0.412
Age 2	0	0.062	0.667	1.646	2.125	3.208
SE	0	0.033	0.117	0.17	0.276	0.234
Age 4	0	0	0.208	1.083	1.125	2.708
SE	0	0	0.074	0.135	0.231	0.542
Age 8	0	0	0.042	0.875	0.833	1.667
SE	0	0	0.042	0.065	0.112	0.256
LSD (0.05)	ns	0.047	0.226	0.439	0.809	1.089
Significant Differences		1 +2 2 + 4 2 + 8	1 + 4 1 + 8 2 + 4 2 + 8	1 + 4 1 + 8 2 + 4 2 + 8	1 + 2 1 + 4 1 + 8 2 + 4 2 + 8	1 + 4 1 + 8 2 + 8

(b) Average Amount of Callus Growth (mm)

Sample Date Days after Setting	23/9/93 30	27/10/93 63	29/11/93 96	22/12/93 119	28/1/93 156	9/3/93 194
Age 1	0	0	1.538	2.464	3.492	3.628
SE	0	0	0.273	0.196	0.384	0.318
Age 2	0	0.015	1.158	2.549	3.958	4.302
SE	0	0.008	0.291	0.334	0.281	0.364
Age 4	0	0	0.369	1.421	3.094	5.342
SE	0	0	0.186	0.258	0.494	0.62
Age 8	0	0	0.083	2.368	2.089	3.532
SE	0	0	0.083	0.394	0.5	0.413
LSD (0.05)	ns	0.011	0.638	0.869	1.178	1.266
Significant Differences		1 + 2 2 + 4 2 + 8	1 + 4 1 + 8 2 + 4 2 + 8	1 + 4 2 + 4 4 + 8	1 + 8 2 + 8	1 + 4 4 + 8

(c) Average Amount of Top Growth (mm)

Sample Date Days after Setting	23/9/93 30	27/10/93 63	29/11/93 96	22/12/93 119	28/1/93 156	9/3/93 194
Age 1	0	0	0	28.75	48.333	54.583
SE	0	0	0	3.085	5.088	8.106
Age 2	0	0	0	27.5	51.667	51.667
SE	0	0	0	4.151	5.162	7.842
Age 4	0	0	0	14.167	10.833	39.583
SE	0	0	0	3.684	2.941	11.12
Age 8	0	0	0	4.583	5	12.917
SE	0	0	0	1.893	2.303	5.821
LSD (0.05)	ns	ns	ns	9.444	11.62	24.05
Significant Differences				1 + 4 1 + 8 2 + 4 2 + 8 4 + 8	1 + 4 1 + 8 2 + 4 2 + 8	1 + 8 2 + 8 4 + 8

Cuttings Collected from 1, 2, 4 and 8 Year Old Stock Plants
on the 23rd September 1993 and Set Under Field Conditions at Meadowbank

(a) Average Stage of Root Growth (0-6)

Sample Date Days after Setting	27/10/93 34	29/11/93 66	22/12/93 89	28/1/93 126	9/3/93 164
Age 1	0	0.646	1.292	2	4
SE	0	0.113	0.156	0.399	0.354
Age 2	0.104	0.125	0.771	1.458	3.167
SE	0.048	0.065	0.149	0.264	0.428
Age 4	0	0	0.458	1	1.625
SE	0	0	0.13	0.204	0.283
Age 8	0	0	0.542	0.708	1.75
SE	0	0	0.13	0.179	0.372
LSD (0.05)	0.069	0.186	0.404	0.784	1.034
Significant Differences	1 +2 2 + 4 2 + 8	1 + 2 1 + 4 1 + 8	1 + 2 1 + 4 1 + 8	1 + 4 1 + 8	1 + 4 1 + 8 2 + 4 2 + 8

(b) Average Amount of Callus Growth (mm)

Sample Date Days after Setting	27/10/93 34	29/11/93 66	22/12/93 89	28/1/93 126	9/3/93 164
Age 1	0	0.62	1.837	2.131	2.768
SE	0	0.157	0.344	0.393	0.316
Age 2	0.037	0.206	1.408	3.109	3.142
SE	0.016	0.111	0.318	0.318	0.51
Age 4	0	0	0.887	2.097	2.949
SE	0	0	0.245	0.361	0.527
Age 8	0	0	1.386	1.65	3.01
SE	0	0	0.376	0.379	0.407
LSD (0.05)	0.023	0.274	0.925	1.038	ns
Significant Differences	1 + 2 2 + 4 2 + 8	1 + 2 1 + 4 1 + 8	1 + 4	2 + 8	

(c) Average Amount of Top Growth (mm)

Sample Date Days after Setting	27/10/93 34	29/11/93 66	22/12/93 89	28/1/93 126	9/3/93 164
Age 1	0	0	8.333	5.833	31.667
SE	0	0	2.706	1.829	5.412
Age 2	0	0	0	14.167	20.417
SE	0	0	0	4.212	3.506
Age 4	0	0	4.583	5.833	7.083
SE	0	0	1.79	3.527	1.681
Age 8	0	0	0.833	1.667	5
SE	0	0	0.833	1.124	2.538
LSD (0.05)	ns	ns	4.774	8.406	10.163
Significant Differences			1 + 2 1 + 8	2 + 8	1 + 2 1 + 4 1 + 8 2 + 4 2 + 8

Cuttings Collected from 1, 2, 4 and 8 Year Old Stock Plants
on the 20th October 1993 and Set Under Field Conditions at Meadowbank

(a) Average Stage of Root Growth (0-6)

Sample Date Days after Setting	29/11/93 39	22/12/93 62	28/1/93 99	9/3/93 137
Age 1	0.062	0.312	0.875	2
SE	0.033	0.082	0.152	0.348
Age 2	0.083	0.625	0.917	1.625
SE	0.047	0.109	0.056	0.343
Age 4	0	0.167	0.542	0.583
SE	0	0.071	0.096	0.132
Age 8	0	0	0.333	0.875
SE	0	0	0.112	0.152
LSD (0.05)	0.082	0.219	0.313	0.754
Significant Differences	2 + 4 2 + 8	1 + 2 1 + 8 2 + 4 2 + 8	1 + 4 1 + 8 2 + 4 2 + 8	1 + 4 1 + 8 2 + 4

(b) Average Amount of Callus Growth (mm)

Sample Date Days after Setting	29/11/93 39	22/12/93 62	28/1/93 99	9/3/93 137
Age 1	0	0.398	1.208	2.285
SE	0	0.117	0.214	0.135
Age 2	0.094	0.958	2.117	2.22
SE	0.066	0.234	0.221	0.302
Age 4	0	0.182	0.714	1.067
SE	0	0.083	0.152	0.24
Age 8	0	0	0.521	1.957
SE	0	0	0.221	0.426
LSD (0.05)	ns	0.392	0.582	0.842
Significant Differences		1 + 2 1 + 8 2 + 4 2 + 8	1 + 2 1 + 8 2 + 4 2 + 8	1 + 4 2 + 4 4 + 8

(c) Average Amount of Top Growth (mm)

Sample Date Days after Setting	29/11/93 39	22/12/93 62	28/1/93 99	9/3/93 137
Age 1	0	0	0	20.417
SE	0	0	0	4.746
Age 2	0	1.667	1.667	15
SE	0	1.667	1.124	4.395
Age 4	0	0	5.583	12.917
SE	0	0	3.625	4.864
Age 8	0	0	2.083	3.333
SE	0	0	2.083	2.247
LSD (0.05)	ns	ns	ns	11.971
Significant Differences				1 + 8

Cuttings Collected from 1, 2, 4 and 8 Year Old Stock Plants on the 23rd November 1993 and Set Under Field Conditions at Meadowbank

(a) Average Stage of Root Growth (0-6)

Sample Date Days after Setting	22/12/93 23	28/1/93 60	9/3/93 98
Age 1	0.062	0.417	1.208
SE	0.033	0.135	0.356
Age 2	0.146	0.833	0.917
SE	0.037	0.155	0.183
Age 4	0	0.042	0.417
SE	0	0.042	0.104
Age 8	0	0.125	0.542
SE	0	0.09	0.176
LSD (0.05)	0.07	0.325	0.641
Significant Differences	1 + 2 2 + 4 2 + 8	1 + 2 1 + 4 2 + 4 2 + 8	1 + 4 1 + 8

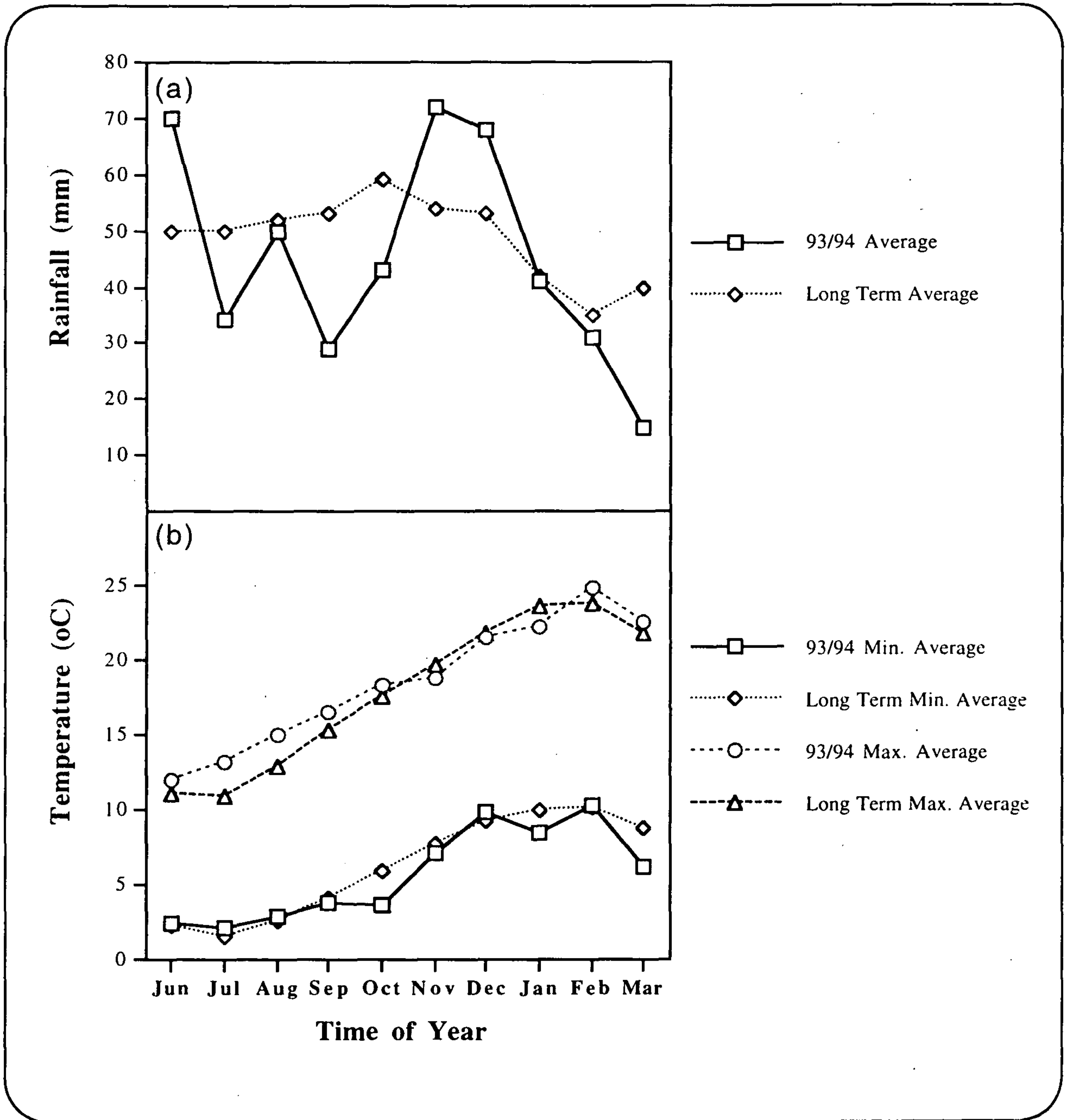
(b) Average Amount of Callus Growth (mm)

Sample Date Days after Setting	22/12/93 23	28/1/93 60	9/3/93 98
Age 1	0	0.699	1.368
SE	0	0.216	0.278
Age 2	0	0.946	1.516
SE	0	0.257	0.272
Age 4	0	0.081	0.884
SE	0	0.081	0.233
Age 8	0	0.147	0.949
SE	0	0.099	0.302
LSD (0.05)	ns	0.512	ns
Significant Differences		1 + 4 1 + 8 2 + 4 2 + 8	

(c) Average Amount of Top Growth (mm)

Sample Date Days after Setting	22/12/93 23	28/1/93 60	9/3/93 98
Age 1	0	0	2.083
SE	0	0	1.145
Age 2	0	0	0.833
SE	0	0	0.833
Age 4	0	0	1.667
SE	0	0	1.124
Age 8	0	0	3.333
SE	0	0	2.562
LSD (0.05)	ns	ns	ns
Significant Differences			

Meadowbank Environmental Data 93/94 and Long Term
Average Rainfall (mm) and Minimum and Maximum Temperatures (°C)



Appendix 9

Stock Plant Age and Timing of Collection Experiment

The Concentration of IAA and ABA in the Basal Stem Region of Cuttings Collected at Different Times in the Year

(a) Concentration of IAA (ng/g DW)

Physiological Age of Cutting Material	Jun-30	Sample Aug-25	Date Oct-20	Dec-22	LSD (0.05)
Age 1	154.77	358.64	344.55	487.37	112.99
se	46.90	28.42	50.48	61.53	
Age 2	239.07	434.75	255.63	763.05	112.99
se	26.05	39.54	23.19	88.17	
Age 4	269.11	284.55	223.93	289.11	112.99
se	37.78	18.62	26.86	30.32	
Age 8	286.54	201.76	216.07	275.80	112.99
se	35.11	16.32	37.66	10.69	
LSD (0.05)	112.99	112.99	112.99	112.99	
Significant Differences	1 + 4	1 + 8	1 + 4	1 + 2	
	1 + 8	2 + 4	1 + 8	1 + 4	
		2 + 8		1 + 8	
				2 + 4	
				2 + 8	

(b) Concentration of ABA (ng/g DW)

Physiological Age of Cutting Material	Jun-30	Sample Aug-25	Date Oct-20	Dec-22	LSD (0.05)
Age 1	106.03	115.82	204.24	177.11	23.46
se	10.86	5.35	10.29	7.73	
Age 2	141.07	158.78	159.61	165.90	23.46
se	8.30	10.12	9.16	6.61	
Age 4	166.66	171.12	167.51	159.21	23.46
se	12.93	8.05	5.48	6.71	
Age 8	132.64	185.78	171.50	152.71	23.46
se	5.84	6.38	4.24	8.50	
LSD (0.05)	23.46	23.46	23.46	23.46	
Significant Differences	1 + 2	1 + 2	1 + 2	1 + 8	
	1 + 4	1 + 4	1 + 4		
	1 + 8	1 + 8	1 + 8		
	2 +4	2 + 8			
	4 + 8				

Appendix 10

Stock Plant Age and Timing of Collection Experiment

The Concentration of GA and ZR in the Basal Stem Region of Cuttings Collected at Different Times in the Year

(a) Concentration ofGA (ng/g DW)

Physiological Age of Cutting Material	Jun-30	Sample Aug-25	Date Oct-20	Dec-22	LSD (0.05)
Age 1	33.20	17.97	28.91	15.42	8.68
se	2.85	0.96	2.11	0.95	
Age 2	33.70	31.78	26.23	19.16	8.68
se	5.45	3.95	3.76	1.09	
Age 4	14.98	37.62	19.58	32.50	8.68
se	0.86	2.09	1.53	1.91	
Age 8	25.18	62.67	29.07	29.54	8.68
se	0.86	7.70	1.20	1.69	
LSD (0.05)	8.68	8.68	8.68	8.68	
Significant Differences	1 + 2	1 + 2	1 + 4	1 + 4	
	1 + 4	1 + 4	4 + 8	1 + 8	
	2 + 4	1 + 8		2 + 4	
	2 + 8	2 + 8		2 + 8	
	4 + 8	4 + 8			

(b) Concentration of ZR (ng/g DW)

Physiological Age of Cutting Material	Jun-30	Sample Aug-25	Date Oct-20	Dec-22	LSD (0.05)
Age 1	45.45	62.24	11.67	35.98	17.00
se	8.23	7.22	1.85	9.22	
Age 2	52.95	57.13	62.16	44.11	17.00
se	6.39	7.31	8.13	4.75	
Age 4	63.48	40.01	39.64	43.74	17.00
se	7.11	3.10	5.55	7.36	
Age 8	24.35	24.25	10.06	9.55	17.00
se	2.75	6.49	1.95	1.02	
LSD (0.05)	17.00	17.00	17.00	17.00	
Significant Differences	1 + 4	1 + 4	1 + 2	1 + 8	
	1 + 8	1 + 8	1 + 4	2 + 8	
	2 + 8	2 + 4	2 + 4	4 + 8	
	4 + 8	2 + 8	2 + 8		
			4 + 8		

Appendix 11

Stock Plant Age and Timing of Collection Experiment

The Concentration of DHZR in the Basal Stem Region of Cuttings Collected at Different Times in the Year

(a) Concentration of DHZR (ng/g DW)

Physiological Age of Cutting Material	Jun-30	Sample Aug-25	Date Oct-20	Dec-22	LSD (0.05)
Age 1 **se**	44.29 8.83	50.51 7.35	9.30 1.35	37.65 8.71	19.55
Age 2 **se**	27.28 2.89	60.32 10.02	71.81 8.27	66.39 5.56	19.55
Age 4 **se**	57.00 12.20	73.22 8.77	23.71 5.46	39.64 6.19	19.55
Age 8 **se**	38.44 5.58	24.56 4.30	17.23 3.50	37.36 5.94	19.55
LSD (0.05)	19.55	19.55	19.55	19.55	
Significant Differences	2 + 4	1 + 4 1 + 8 2 + 8 4 + 8	1 + 2 2 + 4 2 + 8	1 + 2 2 + 4 2 + 8	

Controlled Environment Experiment

Cutting Material Collected from One Year Old Stock Plants and Set Under Constant Environmental Conditions in the Phytotron

(a) The Rooting Percentage of Cuttings Collected From 1 Year Old Stock Plants at Different Times in The year

Cutting	Sample		Date				
Material Age	30-Jun	28-Jul	25-Aug	23-Sep	20-Oct	23-Nov	LSD (0.05)
Age 1	92.50	83.13	73.75	58.75	0.00	13.13	8.46
se	1.77	3.44	3.61	4.27	0.00	2.13	

(b) Average Stage of Root Growth (0-6)

Collection		Sample		Date					
Date		5/8/93	7/9/93	23/9/93	27/10/93	29/11/93	22/12/93	28/1/94	9/3/94
	Days After Set	35	69	85	119	152	175	212	252
Jun-30	Average	0.08	1.48	1.92	3.13	4.5	5.42	6	6
	se	0.056	0.369	0.363	0.619	0.634	0.281	0	0
	Days After Set		41	57	91	124	147	184	224
Jul-28	Average		0.63	0.79	1.67	3.5	4.04	5.54	5.88
	se		0.139	0.13	0.307	0.738	0.52	0.415	0.125
	Days After Set			29	63	96	119	156	196
Aug-25	Average			0.21	0.47	1.11	2.21	4.67	5.17
	se			0.074	0.167	0.26	0.441	0.722	0.534
	Days After Set				34	66	89	126	166
Sep-23	Average				0.48	1.1	2.08	3.96	4.58
	se				0.121	0.482	0.393	0.638	0.503
	Days After Set								
Oct-20	Average				NO		RESULTS		
	se								
	Days After Set						29	66	106
Nov-23	Average						0.06	0.17	2.33
	se						0.044	0.071	0.217

(c) Average Amount of Callus Growth (mm)

Collection		Sample		Date					
Date		5/8/93	7/9/93	23/9/93	27/10/93	29/11/93	22/12/93	28/1/94	9/3/94
	Days After Set	35	69	85	119	152	175	212	252
Jun-30	Average	0	2.13	2.45	3.09	3.57	3.78	4.33	3.99
	se	0	0.492	0.523	0.509	0.439	0.285	0.415	0.56
	Days After Set		41	57	91	124	147	184	224
Jul-28	Average		0.61	0.93	1.45	3.9	3.89	4.15	3.37
	se		0.192	0.196	0.372	0.795	0.316	0.744	0.255
	Days After Set			29	63	96	119	156	196
Aug-25	Average			0.12	0.37	1.78	2.4	3.19	3.28
	se			0.057	0.181	0.403	0.306	0.677	0.378
	Days After Set				34	66	89	126	166
Sep-23	Average				0.63	0.93	2.49	2.89	2.13
	se				0.267	0.621	0.236	0.416	0.317
	Days After Set								
Oct-20	Average				NO		RESULTS		
	se								
	Days After Set						29	66	106
Nov-23	Average						0.04	0.26	1.43
	se						0.078	0.216	0.258

(d) Average Amount of Top Growth (mm)

Collection		Sample		Date					
Date		5/8/93	7/9/93	23/9/93	27/10/93	29/11/93	22/12/93	28/1/94	9/3/94
	Days After Set	35	69	85	119	152	175	212	252
Jun-30	Average	0	0	0	4	38.33	37.08	37.92	85.42
	se	0	0	0	3.075	9.631	9.563	0.468	5.017
	Days After Set		41	57	91	124	147	184	224
Jul-28	Average		0	0	5.83	15.4	24.08	31.67	67.08
	se		0	0	3.27	3.887	4.76	6.405	3.767
	Days After Set			29	63	96	119	156	196
Aug-25	Average			0	0	2.22	7.33	18.89	41.67
	se			0	0	2.22	1.597	5.341	5.528
	Days After Set				34	66	89	126	166
Sep-23	Average				0	0	3.33	12.5	36.67
	se				0	0	1.421	3.398	6.376
	Days After Set								
Oct-20	Average				NO		RESULTS		
	se								
	Days After Set						29	66	106
Nov-23	Average						0	0	0
	se						0	0	0

Appendix 13

Length and Diameter Experiment

The Effect of Cutting Length and Diameter on the Rooting and Growth of Cuttings as of the 9/3/94

(The Average and Standard Errors Displayed)

Cutting Length (cm)	Cutting Diameter (mm)		Rooting Percentage	Callus Diameter (mm)	Callus Growth (mm)	Top Growth (cm)	Root Number	Root DW (g)	Top Growth DW (g)
5	2-4	Average	73.41	7.84	4.36	10.56	2.44	0.37	1.87
		Std Error	5.93	0.55	0.51	0.96	0.38	0.07	0.20
5	4-6	Average	65.87	8.63	3.71	11.61	5.67	0.60	2.52
		Std Error	5.93	0.30	0.24	1.06	0.90	0.07	0.22
5	6-8	Average	63.89	9.70	3.46	12.67	6.22	0.68	3.12
		Std Error	5.16	0.40	0.37	1.29	1.20	0.12	0.31
10	2-4	Average	95.24	9.72	5.55	12.22	6.22	0.90	3.18
		Std Error	2.06	0.34	0.36	1.64	0.86	0.09	0.32
10	4-6	Average	96.43	10.91	5.53	11.11	7.89	0.99	3.70
		Std Error	1.82	0.41	0.36	0.68	0.99	0.18	0.44
10	6-8	Average	96.03	12.41	5.46	14.61	12.22	1.01	5.75
		Std Error	1.73	0.60	0.44	1.27	1.39	0.17	0.77
15	2-4	Average	100.00	11.25	5.88	17.56	8.00	1.17	4.43
		Std Error	0.00	0.27	0.30	2.19	0.91	0.13	0.59
15	4-6	Average	99.60	12.59	6.02	15.06	10.89	1.13	5.58
		Std Error	0.40	0.49	0.40	2.07	0.93	0.12	0.94
15	6-8	Average	99.60	13.65	5.96	18.39	13.00	1.65	7.55
		Std Error	0.40	0.48	0.41	1.65	1.20	0.28	0.90
		LSD (0.05)	6.35		0.62	2.39	1.70	0.26	0.97

The Rooting Percentage of Cuttings Collected from 2 and 9 Year Old Stock Plants

(a) Rooting Percentage of Cuttings Collected from 2 Year Old Stock Plants at Different Times in the Year

Rep. No.	Collection Date			
	3/8/94	14/9/94	26/10/94	7/12/94
1	90.00	75.00	0.00	5.00
2	95.00	65.00	0.00	10.00
3	85.00	85.00	0.00	15.00
4	90.00	80.00	0.00	25.00
5	95.00	75.00	0.00	15.00
Average	**91.00**	**76.00**	**0.00**	**14.00**
Std Error	**1.87**	**3.32**	**0.00**	**3.32**

LSD(0.05) **7.88**

(b) Rooting Percentage of Cuttings Collected From 2 and 9 Year Old Stock Plants on the 3/8/94

Rep. No.	Age 2	Age 9
1	90.00	20.00
2	95.00	40.00
3	85.00	30.00
4	90.00	35.00
5	95.00	30.00
Average	**91.00**	**31.00**
Std Error	**1.87**	**3.32**

LSD(0.05) **8.78**

(a) Formalin-Acetic-Alcohol (FAA)

50% Ethanol	90ml
Glacial acetic acid	5ml
Formalin	5ml

(b) Mayer's Adhesive

White of an egg	20ml
Glycerine	20ml
Sodium salicylate	1gm

(c) Safranin Stain

Safranin Stock Solution	1% safranin in 95% EtOH
Working Solution	Dilute safranin stock solution (50:50 v/v) with distilled water

(d) Acidified 70% Ethanol

70% EtOH	995ml
Concentrated HCl	5ml

(e) Fast Green Stain

Fast Green Stain	0.5% fast green in a solution of clove oil and 50% EtOH (50:50 v/v)

(f) Differentiation Solution

clove oil	50%
100% EtOH	25%
100% xylene	25%

Changes in the Concentration (ng/g DW) of Indole-3-Acetic Acid in the Basal Stem Region of P. radiata Cuttings during the Callus Formation Period

Collection Date + Age	Week No. 0	Week No. 1	Week No. 2	Week No. 3	Week No. 4	Week No. 5	Week No. 6	Week No. 7	Week No. 8	Week No. 9	Week No. 10	Week No. 11
3/8/94	2115.15	1455.78	5354.93	5865.21	761.22	769.30	349.69	682.29	796.55	415.01	80.66	204.79
Age 2	584.17	1204.96	5857.44	4137.32	524.21	291.77	589.57	604.82	639.22	1321.25	960.57	849.90
	1902.47	651.67	2104.55	5856.11	374.49	327.36	947.52	649.90	1063.17	1531.79	637.11	304.92
	861.05	414.71	2889.91	196.37	524.99	494.31	697.12	362.04	509.23	895.68	661.16	1005.24
	1741.64	261.87	4399.20	2107.07	122.85	668.45	629.60		413.28		720.15	977.83
Average	**1440.90**	**797.80**	**4121.21**	**3632.42**	**461.55**	**510.24**	**642.70**	**574.76**	**684.29**	**1040.93**	**611.93**	**668.54**
Std Error	**302.35**	**229.57**	**714.03**	**1102.68**	**104.89**	**93.16**	**96.11**	**72.66**	**114.52**	**247.05**	**144.67**	**171.64**
3/8/94	415.95	123.69	169.45	1113.05	220.61	366.78	205.65			408.88	115.99	
Age 9	305.01		787.28	1455.43	310.76	135.40	161.36	186.48	91.41	339.51	81.47	
	280.74	1267.34	1238.20	1975.44	1111.00	71.67	660.89	365.08		83.73	206.54	
	635.34	2549.21	3399.38	418.98	745.91	466.01	239.89	319.21	108.46	25.07	117.01	
	1254.44	1079.12	370.59	289.37	331.98	112.80	554.54	228.62	92.98	39.69	220.36	
Average	**578.30**	**1254.84**	**1192.98**	**1050.45**	**544.05**	**230.53**	**364.47**	**274.85**	**97.62**	**179.38**	**148.27**	
Std Error	**180.28**	**498.83**	**581.33**	**316.35**	**168.25**	**78.15**	**101.49**	**40.88**	**5.44**	**80.87**	**27.45**	
14/9/94	972.31	1067.73	2870.44	2080.81	2047.85	1175.65	954.07	1469.25	1869.28	1396.66	1812.95	1583.72
Age 2	1274.24	1024.77	2459.65	2201.70	1358.59	1553.95	1282.05	1450.15	1624.39	1978.05	2099.91	1273.11
	1707.95	789.18	2517.25	3490.51	1336.69	1719.07	1499.56	1265.32	2085.07	1404.71	1727.15	1248.40
	1398.89	1386.81	2208.55	3108.49	2314.61	1226.29	1700.48	1354.62	1434.76	1480.73	1954.85	1305.62
	1169.05	1392.71	3618.09	2716.44	2513.59	1144.96	1651.12	1901.89	1552.63	1128.70	1149.54	1661.79
Average	**1304.49**	**1132.24**	**2734.80**	**2719.59**	**1914.27**	**1363.98**	**1417.46**	**1488.25**	**1713.23**	**1477.77**	**1748.88**	**1414.53**
Std Error	**122.74**	**115.34**	**244.80**	**266.63**	**242.87**	**115.02**	**136.84**	**109.65**	**117.01**	**138.57**	**162.70**	**86.38**
26/10/94	660.04	1922.69	2023.85	730.52								
Age 2	1233.19	914.13	1618.64	184.73								
	1168.90	192.93	623.90	623.89								
		225.25	1150.18	458.67								
	956.81	328.32	1593.85	1477.77								
Average	**1004.74**	**716.66**	**1402.08**	**695.12**								
Std Error	**129.18**	**328.64**	**238.67**	**216.25**								
7/12/94	2477.65	1498.23	1800.77	1733.85	1907.07	2345.38	3108.68					
Age 2	2982.27	1375.35	1898.10	1877.75	2594.91	2570.03	4693.77					
	2284.12	1319.17	2061.29	2214.30	1653.40	3647.77	3258.82					
	2072.71	1454.44	2266.74	1491.28	1510.94	2963.40	2728.64					
	3132.81	1159.37	2109.69	1853.13	1744.40	2355.12	2638.25					
Average	**2589.91**	**1361.31**	**2027.32**	**1834.06**	**1882.14**	**2776.34**	**3285.63**					
Std Error	**202.77**	**59.23**	**81.63**	**117.13**	**189.44**	**244.96**	**370.42**					

Appendix 17

Changes in the Concentration (ng/g DW) of Abscisic Acid in the Basal Stem Region of P. radiata Cuttings during the Callus Formation Period

Collection Date + Age	Week No. 0	Week 1 1	Week 2 2	Week 3 3	Week 4 4	Week 5 5	Week 6 6	Week 7 7	Week 8 8	Week 9 9	Week 10 10	week 11 11
3/8/94	98.24	195.41	105.96	152.04	91.96	93.23	100.81	114.24	95.67	61.98	111.56	62.67
Age 2	95.44	170.47	91.18	100.69	93.34	156.13	64.21	84.09	112.79	30.23	78.00	64.28
	102.47	170.30	74.81	111.10	91.04	89.73	60.02	102.63	54.20	31.70	48.33	62.10
	94.53	178.72	77.78	67.46	110.67	54.49	54.42	60.47	71.22	32.18	79.43	72.31
	120.78	176.93	104.63	85.09	117.46	90.40	64.87	44.20	49.22		48.14	63.78
Average	102.29	178.36	90.87	103.27	100.90	96.80	68.87	81.13	76.62	39.02	73.09	65.03
Std Error	4.82	4.58	6.50	14.25	5.50	16.45	8.20	12.96	12.15	7.66	11.79	1.86
3/8/94	110.92	190.77	68.01	103.63	130.75	92.75	75.10	34.54	30.53	45.84	43.61	
Age 9	77.01		87.77	125.14	76.15	57.25	43.65	45.72	51.56	37.89	54.09	
	110.74	131.02	78.04	129.37	87.13	31.70	83.31	55.22	43.39	61.32	43.18	
	118.23	100.70	60.26	101.06	112.03	104.56	77.12	91.85	70.68	60.93	58.02	
	112.24	96.12	58.41	113.51	109.38	106.24	96.43	61.02	87.39	59.19	77.79	
Average	105.83	129.65	70.50	114.54	103.09	78.50	75.12	57.67	56.71	53.03	55.34	
Std Error	7.33	19.49	5.53	5.63	9.66	14.65	8.70	9.65	10.06	4.74	6.32	
14/9/94	117.89	205.78	122.65	138.08	142.69	96.25	125.58	96.54	77.64	53.99	86.35	112.98
Age 2	124.46	180.64	108.95	122.77	129.96	111.02	109.46	83.84	99.84	80.34	99.83	101.78
	130.98	191.28	111.88	129.95	134.89	128.83	138.67	101.74	104.43	66.72	73.23	80.43
	112.69	175.33	127.81	130.22	130.90	116.84	107.75	85.94	82.68	90.22	84.46	118.94
	105.35	201.12	124.00	126.08	127.74	89.92	131.22	90.06	88.48	59.83	81.09	98.25
Average	118.27	190.83	119.06	129.42	133.23	108.57	122.54	91.62	90.61	70.22	84.99	102.48
Std Error	4.46	5.80	3.66	2.56	2.63	7.02	6.06	3.33	5.06	7.44	4.34	6.66
26/10/94	133.73	188.76	127.83	121.12								
Age 2	139.83	165.35	145.78	131.69								
	132.76	195.37	133.23	108.64								
	128.87	146.73	156.33	167.84								
	124.94	209.83	134.79	132.98								
Average	132.03	181.21	139.59	132.45								
Std Error	2.49	11.22	5.10	9.87								
7/12/94	109.74	218.51	188.44	86.75	87.49	146.83	176.44					
Age 2	132.23	128.94	177.63	98.48	95.66	109.68	109.42					
	128.42	188.67	168.35	143.89	137.77	97.27	86.84					
	120.05	125.48	148.79	129.93	122.97	145.62	144.64					
	118.87	146.30	122.07	100.63	99.46	109.94	133.67					
Average	121.86	161.58	161.06	111.93	108.67	121.87	130.20					
Std Error	3.94	18.13	11.72	10.70	9.36	10.21	15.28					

Changes in the Concentration (ng/g DW) of Zeatin Riboside in the Basal Stem Region of P. radiata Cuttings during the Callus Formation Period

Collection Date + Age	Week No. 0	Week No. 1	Week No. 2	Week No. 3	Week No. 4	Week No. 5	Week No. 6	Week No. 7	Week No. 8	Week No. 9	Week No. 10	Week No. 11
3/8/94	44.35	26.73	28.04	29.81	41.31	18.11	14.62	23.07	11.09	4.42	3.78	33.25
Age 2	51.37	14.44	11.16	46.34	24.65	13.97	18.63	18.90	12.86	7.35	3.44	26.51
	60.12	22.04	23.91	23.80	16.88	9.67	18.35	34.58	19.11	4.85	3.18	21.63
	108.82	28.40	34.81	27.18	23.58	37.42	10.48	14.05	35.23	9.51	2.53	
	90.96	30.47	29.20	18.88	17.55	22.44	10.35	11.85	18.48		5.36	
Average	**71.12**	**24.42**	**25.42**	**29.20**	**24.79**	**20.32**	**14.49**	**20.49**	**19.35**	**6.53**	**3.66**	**27.13**
Std Error	**12.33**	**2.85**	**3.97**	**4.66**	**4.41**	**4.77**	**1.81**	**4.02**	**4.26**	**1.18**	**0.47**	**3.37**
3/8/94	4.37	40.81	13.95	12.26	9.94	7.21	7.01	8.22	7.32	5.83	4.86	
Age 9	15.19	4.74	24.06	8.64	4.64	9.87	10.07	7.80	13.19	3.59	6.17	
	12.86	5.22	12.10	9.90	9.08	8.32	6.72	8.51	3.26	2.72	4.23	
	15.69	13.97	14.33	8.06	9.71	6.11	4.79	8.25	2.76	3.72	11.01	
	14.34	9.83	26.67	8.31	7.21	10.03	4.23	7.04	6.52	4.60	9.07	
Average	**12.49**	**14.91**	**18.22**	**9.43**	**8.12**	**8.31**	**6.56**	**7.96**	**6.61**	**4.09**	**7.07**	
Std Error	**2.09**	**6.69**	**2.97**	**0.77**	**0.99**	**0.76**	**1.03**	**0.26**	**1.87**	**0.53**	**1.29**	
14/9/94	76.17	27.09	14.04	41.23	4.74	27.37	36.79	36.64	22.62	59.11	30.95	38.46
Age 2	59.18	39.21	37.37	12.27	24.82	29.52	34.96	64.44	67.46	25.29	32.08	64.60
	82.29	33.08	36.15	21.10	17.66	49.47	29.09	28.71	53.31	41.58	26.67	53.49
	78.83	43.32	39.97	9.94	31.99	45.11	33.64	15.20	38.25	29.97	20.06	41.32
	89.38	18.71	28.86	12.25	38.51	46.21	29.97	19.85	88.14	27.45	38.33	32.25
Average	**77.17**	**32.28**	**31.28**	**19.36**	**23.54**	**39.54**	**32.89**	**32.97**	**53.96**	**36.68**	**29.62**	**46.02**
Std Error	**5.01**	**4.37**	**4.69**	**5.79**	**5.85**	**4.60**	**1.47**	**8.69**	**11.36**	**6.27**	**3.03**	**5.79**
26/10/94	62.73	37.00	20.19	28.77								
Age 2	57.37	35.89	27.41	39.85								
	49.42	29.73	26.35	28.64								
	41.05	40.50	37.62	21.59								
	36.00	20.84	50.17	27.48								
Average	**49.31**	**32.79**	**32.35**	**29.27**								
Std Error	**4.95**	**3.46**	**5.26**	**2.96**								
7/12/94	32.80	24.15	8.15	8.38	17.68	31.64	45.58					
Age 2	35.33	50.60	14.77	17.40	22.26	25.10	36.83					
	38.89	23.48	17.73	15.39	15.18	42.13	27.34					
	26.81	25.71	32.99	26.11	34.58	26.11	23.08					
	27.78	26.45	17.35	22.29	40.64	25.20	27.94					
Average	**32.32**	**30.08**	**18.20**	**17.91**	**26.07**	**30.04**	**32.15**					
Std Error	**2.27**	**5.16**	**4.08**	**3.03**	**4.94**	**3.26**	**4.03**					

Changes in the Concentration (ng/g DW) of Di-Hydro Zeatin Riboside in the Basal Stem Region of P. radiata Cuttings during the Callus Formation Period

Collection Date + Age	Week No. 0	Week No. 1	Week No. 2	Week No. 3	Week No. 4	Week No. 5	Week No. 6	Week No. 7	Week No. 8	Week No. 9	Week No. 10	Week No. 11
3/8/94	21.87	26.38	22.21	31.88	37.61	28.21	21.51	25.25	31.79	12.18	2.84	21.56
Age 2	24.77	18.74	34.99	41.27	28.53	32.87	46.75	24.86	37.01	11.60	10.46	14.72
	120.04	15.72	23.95	26.10	22.33	19.62	35.40	36.32	46.72	8.53	11.23	27.40
	25.71	36.79	29.76	24.36	26.31	32.68	24.11	29.02	53.31	9.56	68.50	10.82
	37.59	22.68	38.74	26.58	21.52	21.37	28.47	36.10	35.81		9.24	12.29
Average	**46.00**	**24.06**	**29.93**	**30.04**	**27.26**	**26.95**	**31.25**	**30.31**	**40.93**	**10.47**	**20.45**	**17.36**
Std Error	**18.70**	**3.66**	**3.15**	**3.08**	**2.89**	**2.78**	**4.53**	**2.52**	**3.95**	**0.86**	**12.10**	**3.11**
3/8/94	8.15	19.57	24.84	19.56	14.11	13.69	19.25	29.00	28.41	8.95	7.23	
Age 9	12.29	10.16	30.38	12.88	13.46	20.54	21.40	23.11	25.57	10.80	8.92	
	26.44	16.77	14.41	13.06	17.10	17.24	14.67	22.84	14.15	6.85	5.35	
	16.66	30.06	21.48	13.43	21.63	17.08	19.84	18.78	12.09	6.13	3.25	
	16.64	11.41	23.62	13.55	18.33	29.41	18.51	27.19	22.81	3.69	17.82	
Average	**16.04**	**17.59**	**22.95**	**14.50**	**16.93**	**19.59**	**18.73**	**24.18**	**20.61**	**7.28**	**8.51**	
Std Error	**3.04**	**3.56**	**2.59**	**1.27**	**1.48**	**2.68**	**1.12**	**1.79**	**3.20**	**1.22**	**2.51**	
14/9/94	23.29	29.60	29.31	55.96	26.18	56.16	57.19	41.28	32.56	51.06	40.18	40.13
Age 2	25.55	31.29	35.59	31.36	43.40	41.12	33.30	89.31	55.45	49.59	51.06	70.13
	27.84	29.28	25.95	34.12	27.73	49.33	32.15	42.60	53.31	39.87	41.95	57.54
	27.48	38.41	35.70	27.06	37.71	50.92	29.33	24.09	40.98	43.87	44.58	31.40
	22.40	30.36	28.38	17.51	23.08	52.11	20.83	36.82	102.51	32.67	62.88	49.52
Average	**25.31**	**31.79**	**30.99**	**33.20**	**31.62**	**49.93**	**34.56**	**46.82**	**56.96**	**43.41**	**48.13**	**49.74**
Std Error	**1.09**	**1.69**	**1.98**	**6.35**	**3.83**	**2.48**	**6.06**	**11.11**	**12.13**	**3.35**	**4.12**	**6.73**
26/10/94	44.39	36.61	30.19	30.57								
Age 2	37.34	30.84		33.60								
	30.00	31.43	25.12	29.55								
	25.34	50.25	34.35	33.27								
	16.91	29.24	43.06	27.23								
Average	**30.80**	**35.67**	**33.18**	**30.84**								
Std Error	**4.75**	**3.85**	**3.80**	**1.19**								
7/12/94	23.38	31.25	34.32	28.73	38.71	59.58	41.57					
Age 2	27.94	27.77	36.11	35.44	42.09	41.84	34.71					
	22.24	28.79	27.67	30.92	31.64	54.91	27.16					
	21.78	26.58	49.24	51.39	44.48	42.02	29.14					
	22.51	29.97	40.10	59.98	54.03	40.73	29.37					
Average	**23.57**	**28.87**	**37.49**	**41.29**	**42.19**	**47.82**	**32.39**					
Std Error	**1.12**	**0.82**	**3.56**	**6.13**	**3.67**	**3.93**	**2.61**					

Changes in the Carbohydrate Content of Pinus radiata Cuttings Collected From 2 Year Old Stock Plants on the 3rd of August 1994 During Callus Formation

Soluble Carbohydrate	Week No. 0	Week No. 1	Week No. 2	Week No. 3	Week No. 4	Week No. 5	Week No. 6	Week No. 7	Week No. 8	Week No. 9	Week No. 10	Week No. 11	LSD (0.05)
Sucrose	26.457	22.978	22.332	25.039	21.470	19.521	16.463	19.364	15.168	19.003	29.299	27.265	4.035
Std Error	1.229	0.795	1.401	0.223	2.551	0.387	0.802	0.912	2.522	2.140	0.643	2.344	
Glucose	10.422	14.690	17.248	17.702	15.363	10.544	7.969	8.615	6.955	6.205	4.860	3.890	3.06
Std Error	0.812	0.538	1.972	1.311	2.227	1.406	0.866	1.346	0.894	0.594	0.446	0.176	
Fructose	3.959	4.343	5.498	5.684	3.752	2.412	2.873	2.632	2.326	2.009	2.275	1.702	0.998
Std Error	0.426	0.258	0.870	0.331	0.619	0.161	0.306	0.353	0.209	0.131	0.264	0.088	
Pinitol	6.463	6.609	6.858	8.357	8.094	7.921	9.318	8.885	9.757	8.714	9.209	9.362	1.559
Std Error	0.358	0.336	0.303	0.758	0.437	0.734	0.807	0.295	0.709	0.186	1.018	0.849	
Sequoitol	1.661	1.267	0.482	0.492	0.298	0.211	0.238	0.200	0.183	0.191	0.253	0.231	0.198
Std Error	0.156	0.150	0.053	0.069	0.014	0.034	0.007	0.014	0.003	0.013	0.020	0.020	
myo-Inositol	0.670	0.373	0.183	0.150	0.119	0.109	0.139	0.137	0.111	0.154	0.191	0.180	0.04
Std Error	0.030	0.015	0.015	0.010	0.011	0.004	0.010	0.008	0.012	0.020	0.009	0.028	
Pinpollitol	0.233	0.218	0.208	0.207	0.217	0.222	0.341	0.385	0.354	0.388	0.370	0.416	0.07
Std Error	0.014	0.022	0.031	0.020	0.028	0.027	0.023	0.023	0.020	0.040	0.039	0.031	
Total Soluble	49.864	50.478	52.809	57.631	49.312	40.940	37.341	40.218	34.853	36.665	46.457	43.046	6.172
Std Error	1.611	1.026	4.332	1.707	4.833	1.892	0.936	1.732	2.395	1.620	1.255	2.917	
Stem Starch	10.293	6.335	6.561	7.530	6.873	7.034	6.713	7.117	6.445	7.727	8.983	11.301	1.138
Std Error	0.875	0.248	0.721	0.154	0.408	0.352	0.119	0.294	0.260	0.342	1.113	1.269	
Total Basal Stem Carbohydrate	60.157	56.813	59.370	65.161	56.185	47.974	44.054	47.335	41.298	44.392	55.440	54.347	
Leaf Starch	10.600	8.119											
Std Error	0.303	0.163											

Appendix 21

Changes in the Carbohydrate Content of Pinus radiata Cuttings Collected From 9 Year Old Stock Plants on the 3rd of August 1994 During Callus Formation

Soluble Carbohydrate	Week No. 0	Week No. 1	Week No. 2	Week No. 3	Week No. 4	Week No. 5	Week No. 6	Week No. 7	Week No. 8	Week No. 9	Week No. 10	Week No. 11	LSD (0.05)
Stem Sucrose	22.783	17.484	15.902	20.263	12.066	8.749	11.231	8.177	10.112	11.660			3.686
Std Error	1.706	0.938	1.490	0.760	1.860	0.252	0.834	1.513	1.068	1.428			
Stem Glucose	10.758	10.175	10.364	10.940	9.038	7.305	7.283	5.141	5.483	3.665			2.595
Std Error	0.635	0.619	0.794	0.956	0.351	0.440	0.654	0.167	0.467	0.654			
Stem Fructose	2.736	3.493	4.380	3.886	2.419	1.630	2.762	1.825	1.729	1.298			0.625
Std Error	0.150	0.205	0.481	0.391	0.164	0.076	0.176	0.075	0.101	0.253			
Stem Pinitol	4.281	4.283	4.520	4.710	4.014	4.553	5.401	5.445	5.896	5.676			0.795
Std Error	0.152	0.113	0.386	0.254	0.124	0.277	0.184	0.203	0.289	0.606			
Stem Sequoitol	0.582	0.349	0.232	0.120	0.118	0.104	0.130	0.130	0.131	0.143			0.155
Std Error	0.037	0.029	0.049	0.007	0.009	0.014	0.009	0.007	0.008	0.008			
Stem myo-Inositol	0.249	0.145	0.110	0.109	0.089	0.098	0.106	0.106	0.088	0.088			0.040
Std Error	0.022	0.007	0.007	0.004	0.008	0.010	0.005	0.008	0.005	0.012			
Stem Pinpollitol	0.262	0.242	0.207	0.212	0.185	0.227	0.290	0.356	0.367	0.344			0.069
Std Error	0.010	0.020	0.018	0.025	0.020	0.010	0.023	0.031	0.049	0.039			
Total Stem Soluble	41.651	36.172	35.715	40.240	27.929	22.668	27.204	21.180	23.806	22.874			5.587
Std Error	2.494	1.781	2.003	1.990	1.921	0.749	1.455	1.227	0.890	2.551			
Stem Starch	7.388	7.751	6.316	6.237	5.917	6.067	5.718	5.811	5.992	5.907			1.138
Std Error	0.312	1.019	0.124	0.216	0.035	0.021	0.221	0.144	0.138	0.177			
Total Basal Stem Carbohydrate	49.039	43.923	42.031	46.477	33.846	28.735	32.922	26.991	29.798	28.781			
Basal Leaf Starch	11.461	9.167											
Std Error	0.446	0.243											

Changes in the Carbohydrate Content of Pinus radiata Cuttings Collected From 2 Year Old Stock Plants on the 14th of September 1994 During Callus Formation

Soluble Carbohydrate	Week No. 0	Week No. 1	Week No. 2	Week No. 3	Week No. 4	Week No. 5	Week No. 6	Week No. 7	Week No. 8	Week No. 9	Week No. 10	Week No. 11	LSD (0.05)
Stem Sucrose	27.034	21.537	22.228	20.386	19.047	16.920	15.488	15.995	25.574	24.835	19.628	20.134	4.743
Std Error	1.662	1.579	0.896	1.186	0.984	0.572	2.193	1.893	2.279	1.506	2.797	2.000	
Stem Glucose	12.131	30.516	21.416	16.209	12.718	9.750	7.793	6.058	5.584	4.210	3.347	3.579	3.894
Std Error	0.612	1.449	2.854	2.246	1.798	1.355	1.188	0.735	0.696	0.363	0.324	0.478	
Stem Fructose	6.257	12.963	7.512	4.497	2.906	2.124	1.853	1.985	1.926	1.367	1.21	1.251	1.439
Std Error	0.208	0.866	1.101	0.897	0.439	0.180	0.194	0.113	0.178	0.092	0.111	0.212	
Stem Pinitol	7.563	8.576	9.988	9.417	10.931	10.653	11.117	10.050	8.718	9.028	8.775	9.923	1.427
Std Error	0.399	0.615	1.148	0.702	0.920	0.899	1.716	0.777	0.554	0.616	0.607	0.944	
Stem Sequoitol	1.892	2.417	2.028	1.276	0.802	0.684	0.304	0.322	0.217	0.183	0.161	0.175	0.283
Std Error	0.053	0.109	0.275	0.138	0.046	0.140	0.017	0.037	0.011	0.021	0.01	0.007	
Stem myo-Inositol	1.021	0.724	0.622	0.354	0.226	0.189	0.141	0.137	0.166	0.150	0.112	0.123	0.081
Std Error	0.034	0.032	0.080	0.043	0.023	0.016	0.013	0.004	0.014	0.011	0.009	0.023	
Stem Pinpollitol	0.193	0.189	0.224	0.204	0.259	0.269	0.335	0.332	0.275	0.319	0.308	0.341	0.057
Std Error	0.014	0.008	0.024	0.020	0.019	0.028	0.061	0.031	0.028	0.033	0.029	0.051	
Total Stem Soluble	56.091	76.922	64.018	52.343	46.888	40.589	37.031	34.880	42.459	40.112	33.542	35.526	7.217
Std Error	2.494	2.398	5.099	3.570	2.024	2.548	1.595	1.514	2.380	0.926	3.001	1.822	
Stem Starch	42.370	5.497	7.234	5.630	6.124	6.171	6.259	7.222	11.266	10.196	7.884	6.951	2.264
Std Error	1.120	0.240	0.704	0.327	0.539	0.460	0.270	0.186	1.757	0.935	0.417	0.932	
Total Basal Stem Carbohydrate	98.461	82.419	71.252	57.973	53.012	46.760	43.290	42.102	53.725	50.308	41.426	42.477	
Basal Leaf Starch	55.659	8.509											
Std Error	3.643	0.184											

Changes in the Carbohydrate Content of Pinus radiata Cuttings Collected From 2 Year Old Stock Plants on the 26th of October 1994 During Callus Formation

Soluble Carbohydrate	Week No. 0	Week No. 1	Week No. 2	Week No. 3	Week No. 4	Week No. 5	Week No. 6	Week No. 7	Week No. 8	Week No. 9	Week No. 10	Week No. 11	LSD (0.05)
Stem Sucrose	20.914	10.728	4.043	1.810									2.286
Std Error	0.522	0.812	0.800	0.501									
Stem Glucose	62.787	21.659	10.327	6.521									7.137
Std Error	4.396	1.262	1.060	0.529									
Stem Fructose	16.796	5.843	2.935	1.785									3.892
Std Error	2.767	0.651	0.456	0.296									
Stem Pinitol	6.228	6.983	8.322	9.692									0.959
Std Error	0.513	0.410	0.357	0.591									
Stem Sequoitol	4.706	4.321	3.686	2.926									0.864
Std Error	0.268	0.238	0.210	0.353									
Stem myo-Inositol	2.765	1.283	0.568	0.286									0.269
Std Error	0.187	0.055	0.066	0.051									
Stem Pinpollitol	0.232	0.199	0.258	0.309									0.044
Std Error	0.012	0.012	0.027	0.017									
Total Stem Soluble	114.428	51.016	30.139	23.328									10.807
Std Error	6.087	2.282	2.143	0.725									
Stem Starch	23.216	5.394	5.218	5.149									2.344
Std Error	1.320	0.053	0.095	0.035									
Total Basal Stem Carbohydrate	137.644	56.410	35.357	28.477									
Basal Leaf Starch	9.663	7.173											
Std Error	0.304	0.081											

Appendix 24

Changes in the Carbohydrate Content of Pinus radiata Cuttings Collected From 2 Year Old Stock Plants on the 7th of December 1994 During Callus Formation

Soluble Carbohydrate	Week No. 0	Week No. 1	Week No. 2	Week No. 3	Week No. 4	Week No. 5	Week No. 6	Week No. 7	Week No. 8	Week No. 9	Week No. 10	Week No. 11	LSD (0.05)
Stem Sucrose	16.057	12.631	5.273	7.446	9.104	0.110	1.041						2.784
Std Error	0.977	1.173	1.321	2.195	0.204	0.043	0.349						
Stem Glucose	45.212	19.285	11.624	9.956	10.860	11.943	12.855						3.492
Std Error	1.519	1.290	1.102	1.360	0.879	2.304	0.943						
Stem Fructose	7.281	3.616	2.842	3.447	4.123	5.110	5.443						1.785
Std Error	1.043	0.533	0.304	0.580	0.287	1.161	0.573						
Stem Pinitol	7.142	7.109	8.250	10.582	13.412	10.885	17.651						3.245
Std Error	0.290	0.219	0.218	1.857	1.592	2.439	2.635						
Stem Sequoitol	5.901	5.430	4.348	5.296	4.164	2.458	3.246						1.022
Std Error	0.216	0.199	0.311	0.970	0.344	0.385	0.320						
Stem myo-Inositol	2.556	1.872	0.826	0.667	0.590	0.354	0.310						0.314
Std Error	0.210	0.172	0.099	0.095	0.091	0.075	0.053						
Stem Pinpollitol	0.222	0.194	0.246	0.287	0.322	0.308	0.436						0.058
Std Error	0.012	0.011	0.020	0.046	0.022	0.024	0.029						
Total Stem Soluble	84.370	50.137	33.410	37.680	42.575	31.167	40.982						8.264
Std Error	2.145	2.974	2.056	2.903	2.404	5.672	3.967						
Stem Starch	12.090	5.552	5.549	5.360	5.861	5.829	5.568						1.446
Std Error	1.146	0.210	0.075	0.161	0.299	0.048	0.055						
Total Basal Stem Carbohydrate	96.460	55.689	38.959	43.040	48.436	36.996	46.550						
Basal Leaf Starch	8.762	7.263											
Std Error	0.166	0.162											

Average Leaf Water Potential (MPa) of P. radiata Cuttings during the Callus Formation Period.
Cuttings Collected from 2 Year Old Stock Plants on the 3/8/94, 14/9/94, 26/10/94 and 7/12/94, and from 9 year old stock plants on the 3/8/94.

	Collection Date + Age	Week No. 0	After Transport	Week No. 1	Week No. 2	Week No. 3	Week No. 4	Week No. 5	Week No. 6	Week No. 7	Week No. 8	Week No. 9	Week No. 10	Week No. 11
Average	3/8/94	0.000	-0.025	-0.173	-0.170	-0.468	-0.763	-0.628	-2.365	-2.458	-2.278	-2.018	-1.873	-1.853
Std Error	Age 2	0.000	0.025	0.068	0.059	0.135	0.168	0.071	0.134	0.115	0.112	0.123	0.081	0.123
Average	3/8/94	-0.945	-1.338	-1.308	-2.273	-2.650	-2.198	-2.258	-2.253	-2.115	-2.000	-2.003	-1.910	
Std Error	Age 9	0.113	0.137	0.168	0.166	0.121	0.111	0.154	0.156	0.089	0.121	0.133	0.118	
Average	14/9/94	-0.965	-2.003	-2.198	-1.688	-1.695	-1.658	-1.975	-2.065	-2.259	-2.073	-1.850	-1.805	-1.900
Std Error	Age 2	0.176	0.070	0.098	0.051	0.048	0.074	0.088	0.11	0.088	0.135	0.073	0.065	0.066
Average	26/10/94	-0.635	-1.278	-1.873	-2.143	-2.623								
Std Error	Age 2	0.030	0.092	0.067	0.122	0.055								
Average	7/12/94	-1.325	-1.408	-1.725	-1.675	-1.958	-2.440	-2.413	-2.548					
Std Error	Age 2	0.033	0.090	0.059	0.077	0.061	0.093	0.070	0.066					

Change in the rate of carbon dioxide exchange, water potential and stomatal resistance in cuttings collected from 3 year old stock plants on the 2/5/96 and set in the phytotron

	Time of Excision	1 Day After Excision	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12
Rate of CO_2 Exchange (μmol.m-2s-1)	4.49	-0.38	-0.13	-0.07	-0.03	0.03	-0.06	0.06	-0.02	0.01	0.10	0.06	0.10	0.10
se	0.58	0.10	0.06	0.04	0.03	0.05	0.11	0.03	0.04	0.07	0.04	0.02	0.02	0.01
Leaf Water Potential	-0.92	-1.46	-1.45	-1.44	-1.53	-1.67	-1.81	-1.77	-1.73	-1.95	-1.88	-1.75	-1.81	-1.78
se	0.04	0.04	0.06	0.04	0.03	0.06	0.07	0.07	0.10	0.09	0.08	0.13	0.10	0.05
Stomatal Resistance (s.cm-1)	6.42	113.18										10.54		
se	0.03	0.59										0.05		

Change in Cutting Assimilation Rate During a 30 minute Period Following Excision Cuttings Collected From 3 Year Old Stock Plants on the 2/5/96

Time After Excision (min)	Temperature (oC)	PFD (μmol.m-2.s-1)	Assimilation Rate (μmol.m-2.s-1)
0	15.6	450	4.49
5	16.6	770	6.32
10	17.8	790	7.59
15	17.8	880	7.49
20	17.0	550	5.55
25	16.5	1400	8.83
30	16.5	680	5.67

Light Saturation Curve Data
Experiment Conducted on Healthy Well Watered Seedling

Temperature (oC)	PFD (µmol.m-2.s-1)	Assimilation Rate (µmol.m-2.s-1)
20.0	1250	5.043
20.0	700	5.003
20.0	555	4.661
20.0	330	3.978
20.0	215	3.235
20.0	127	2.230
20.0	86	1.467
20.0	42	0.563
20.0	11	0.020
20.0	Dark	-0.201

Changes in Leaf Water Potential (MPa) During Callus Formation

Date	Week No.	Cutting No. 1	Cutting No. 2	Cutting No. 3	Cutting No. 4	Cutting No. 5	Cutting No. 6	Cutting No. 7	Cutting No. 8	Cutting No. 9	Cutting No. 10	Cutting No. 11	Cutting No. 12	Cutting No. 13
24/4/96	0	-1.400	-1.200	-1.250	-1.100	-1.350	-1.575	-1.450	-1.425	-1.375	-1.475	-1.300	-1.2250	-1.3500
1/5/96	1	-1.550	-1.425	-1.350	-1.450	-1.550	-1.775	-1.400	-1.475	-1.400	-1.425	-1.475	-1.4500	-1.4250
8/5/96	2	-1.875	-1.675	-1.400	-1.625	-1.675	-1.850	-1.425	-1.625	-1.450	-1.475	-1.525	-1.7250	-1.6000
15/5/96	3	-1.950	-1.775	-1.425	-1.800	-1.625	-2.075	-1.625	-1.875	-1.450	-1.575	-1.675	-1.8250	-1.7000
22/5/96	4	-2.100	-1.800	-1.400	-1.875	-1.775	-2.375	-1.775	-2.000	-1.550	-1.725	-1.725	-1.9500	-1.8750
29/5/96	5	-2.200	-1.850	-1.450	-1.950	-1.650	-2.650	-1.625	-1.775	-1.425	-1.700	-2.050	-2.0000	-1.8750
5/6/96	6	-2.225	-1.875	-1.450	-2.025	-1.575	died	-1.550	-1.875	-1.525	-1.825	-2.000	-2.0000	-1.8750
12/6/96	7	-2.325	-1.900	-1.625	-1.975	-1.550		-1.425	-2.125	-1.400	-1.550	-1.775	-2.0000	-2.0250
19/6/96	8	-2.525	-1.875	-1.300	-1.725	-1.575		-1.375	-2.475	-1.350	-1.550	-1.525	-1.9750	-2.0500
26/6/96	9	-2.575	-1.875	-1.425	-1.650	-1.725		-1.425	-2.400	-1.550	-1.575	-1.550	-1.9250	-2.2250
3/7/96	10	-2.625	-1.875	-1.500	-1.525	-1.925		-1.500	-2.350	-1.775	-1.625	-1.600	-1.8250	-2.4000
10/7/96	11	-2.875	-1.925	-1.500	-1.475	-1.875		-1.425	-2.625	-1.625	-1.800	-1.725	-1.7250	-2.5000
17/7/96	12	-2.875	-1.950	-1.475	-1.450	-1.875		-1.450	-2.750	-1.650	-1.775	-1.700	-1.6750	-2.6250
		died							died					died

Date	Week No.	Cutting No. 14	Cutting No. 15	Cutting No. 16	Cutting No. 17	Cutting No. 18	Cutting No. 19	Cutting No. 20	Total Average	TA Standard Error	Rooted Average	RA Standard Error	Dead Average	DA Standard Error
24/4/96	0	-1.225	-1.425	-1.750	-1.575	-1.350	-1.650	-1.700	-1.4075	0.0389	-1.3983	0.0509	-1.4350	0.0376
1/5/96	1	-1.250	-1.550	-1.775	-1.575	-1.600	-1.550	-1.700	-1.5075	0.0301	-1.4917	0.0350	-1.5550	0.0599
8/5/96	2	-1.325	-1.625	-1.825	-1.750	-1.975	-1.550	-1.725	-1.6350	0.0385	-1.6083	0.0462	-1.7150	0.0605
15/5/96	3	-1.475	-1.750	-1.875	-1.875	-2.225	-1.675	-1.900	-1.7575	0.0457	-1.7200	0.0543	-1.8700	0.0677
22/5/96	4	-1.650	-1.825	-2.050	-1.975	-2.250	-2.000	-2.175	-1.8925	0.0528	-1.8450	0.0590	-2.0350	0.0977
29/5/96	5	-1.650	-1.775	-2.075	-1.950	-2.375	-2.000	-2.075	-1.9050	0.0667	-1.8550	0.0681	-2.0550	0.1680
5/6/96	6	-1.500	-1.725	-1.975	-1.875	-2.325	-1.975	-1.950	-1.8487	0.0556	-1.8283	0.0651	-1.9250	0.1061
12/6/96	7	-1.450	-1.825	-1.950	-1.950	-2.275	-2.000	-1.950	-1.8461	0.0636	-1.7850	0.0687	-2.0750	0.1041
19/6/96	8	-1.375	-1.950	-1.950	-1.950	-2.075	-1.825	-1.925	-1.8079	0.0810	-1.6900	0.0690	-2.2500	0.1461
26/6/96	9	-1.575	-2.125	-1.900	-1.925	-2.050	-1.775	-1.950	-1.8526	0.0738	-1.7250	0.0527	-2.3313	0.0992
3/7/96	10	-1.775	-2.250	-1.850	-1.875	-2.050	-1.725	-1.975	-1.8961	0.0731	-1.7600	0.0454	-2.4063	0.0793
10/7/96	11	-2.125	-2.500	-1.850	-1.550	-1.900	-1.625	-2.000	-1.9276	0.0961	-1.7417	0.0534	-2.6250	0.0884
17/7/96	12	-2.100	-2.675	-1.900	-1.625	-1.900	-1.600	-2.125	-1.9566	0.1047	-1.7500	0.0567	-2.7313	0.0554
			died											

The Percentage Rooting Data for the TIBA Experiment
Cuttings Collected from 2 year old Stock Plants on the 21/7/95

Rep. No.	Control	Lanolin Control	TIBA	Apex Removed
1	75.00	77.78	28.57	50.00
2	70.00	61.11	31.58	40.00
3	95.00	78.95	21.05	55.00
4	70.00	66.67	42.86	45.00
5	65.00	70.59	30.43	45.00
Average	**75.00**	**71.02**	**30.90**	**47.00**
Std Error	**5.24**	**3.36**	**3.51**	**2.55**

LSD (0.05) 11.35

The concentration of IAA (ng/g DW) in the apical and basal stem region of Pinus radiata cuttings collected from 2 year old stock plants on the 21/7/95

Date	Control Apex	Control Basal Region	TIBA Apex	TIBA Basal Region
21/7/95	1319.20	2521.20	1319.20	2521.20
Week 0	1767.20	2561.50	1767.20	2561.50
	1350.70	2625.50	1350.70	2625.50
	1347.50	1438.50	1347.50	1438.50
	1722.80	2051.80	1722.80	2051.80
Average	**1501.48**	**2239.70**	**1501.48**	**2239.70**
Std Error	**99.81**	**224.60**	**99.81**	**224.60**
28/7/95	1470.50	1943.20	1637.70	1624.10
Week 1	1677.30	1854.60	1645.50	1369.70
	1483.40	2349.50	1540.90	2033.90
	1396.10	2292.10	1518.20	2191.00
	1150.50	2562.40	1687.90	1930.20
Average	**1435.56**	**2200.36**	**1606.04**	**1829.78**
Std Error	**85.08**	**131.80**	**32.57**	**147.68**
4/8/95	1462.80	2531.60	1512.00	1391.20
Week 2	1280.20	2568.20	1432.00	2385.40
	2326.10	2734.40	1848.60	1951.80
	1700.50	2645.00	1662.70	1986.70
	1925.80	2668.50	1445.00	1967.30
Average	**1739.08**	**2629.54**	**1580.06**	**1936.48**
Std Error	**182.74**	**36.13**	**78.66**	**158.52**
11/8/95	1869.40	2324.60	2433.50	1415.20
Week 3	1750.70	2665.00	1179.20	1332.30
	1727.90	2290.80	1586.00	1103.90
	1718.60	2209.40	1759.90	1428.70
	1601.20	2238.30	2039.20	1115.10
Average	**1733.56**	**2345.62**	**1799.56**	**1279.04**
Std Error	**42.73**	**82.32**	**211.11**	**71.18**
18/8/95	1128.50	1029.10	1158.40	1711.40
Week 4	1045.30	1374.90	1215.20	1492.00
	1328.20	1664.00	1467.00	1360.70
	1438.50	1626.00	1444.90	1375.70
	1824.10	1681.20	1966.60	1269.30
Average	**1352.92**	**1475.04**	**1450.42**	**1441.82**
Std Error	**136.92**	**124.48**	**142.71**	**76.13**

Appendix 32

Auxin Concentration and Timing of Application Experiment

The Rooting Percentage Data for Cuttings Set in the Phytotron
Cuttings Collected From 3 Year Old Stock Plants on the 14/12/95

		Rep. No.	0 (Talc)	3000 ppm	8000 ppm	Average+Se
	T0	1	20.00	20.00	0.00	
		2	35.00	35.00	0.00	**15.42**
		3	20.00	25.00	10.00	**3.61**
		4	10.00	10.00	0.00	
	Average		21.25	22.50	2.50	
	Std Error		5.15	5.20	2.50	
Auxin Application Time (Week)	T2	1	35.00	25.00	45.00	
		2	30.00	15.00	35.00	**33.75**
		3	25.00	35.00	40.00	**2.55**
		4	40.00	35.00	45.00	
	Average		32.50	27.50	41.25	
	Std Error		3.23	4.79	2.39	
	T4	1	15.00	10.00	0.00	
		2	0.00	20.00	5.00	**8.75**
		3	10.00	5.00	5.00	**2.14**
		4	20.00	0.00	15.00	
	Average		11.25	8.75	6.25	
	Std Error		4.27	4.27	3.15	
	Average		**21.67**	**19.58**	**16.67**	
	Std Error		**3.45**	**3.45**	**5.45**	

LSD (0.05) 12.43

Auxin Concentration and Timing of Application Experiment

The Rooting Percentage Data for Cuttings Set in the Glasshouse
Cuttings Collected From 3 Year Old Stock Plants on the 14/12/95

Auxin Application Time (Weeks)		Rep. No.	0 (Talc)	3000 ppm	8000 ppm	Average+se
	T0	1	85.00	65.00	80.00	
		2	75.00	60.00	60.00	73.33
		3	90.00	70.00	75.00	3.54
	Average		83.33	65.00	71.67	
	Std Error		3.82	2.50	5.20	
	T2	1	85.00	65.00	65.00	
		2	70.00	80.00	50.00	68.33
		3	70.00	70.00	60.00	3.44
	Average		75.00	71.67	58.33	
	Std Error		4.33	3.82	3.82	
	T4	1	85.00	80.00	40.00	
		2	80.00	90.00	80.00	73.33
		3	40.00	70.00	95.00	6.72
	Average		68.33	80.00	71.67	
	Std Error		12.33	5.00	14.22	
	Average		75.56	72.22	67.22	
	Std Error		5.03	3.13	5.66	

Auxin and Sucrose Application Experiment

The Rooting Percentage Data for Cuttings Set in the Phytotron
Cuttings Collected From 3 Year Old Stock Plants on the 14/12/95

		Rep. No.	Talc	Sucrose	Average + se
Timing of Auxin Application 3000ppm (Weeks)	Control	1	25.00	10.00	
		2	25.00	20.00	**20.63**
		3	30.00	20.00	**2.20**
		4	20.00	15.00	
	Average		25.00	16.25	
	Std Error		2.04	2.39	
	T0	1	15.00	15.00	
		2	10.00	20.00	**13.13**
		3	15.00	10.00	**1.32**
		4	10.00	10.00	
	Average		12.50	13.75	
	Std Error		1.44	2.39	
	T2	1	25.00	15.00	
		2	40.00	20.00	**21.88**
		3	20.00	10.00	**3.13**
		4	25.00	20.00	
	Average		27.50	16.25	
	Std Error		4.33	2.39	
	T4	1	15.00	45.00	
		2	30.00	25.00	**25.00**
		3	20.00	25.00	**3.27**
		4	20.00	20.00	
	Average		21.25	28.75	
	Std Error		3.15	5.54	
	Average		**21.56**	**18.75**	
	Std Error		**1.97**	**2.17**	

LSD (0.05) 9.17

Appendix 35

Auxin and Sucrose Application Experiment

The Rooting Percentage Data for Cuttings Set in the Glasshouse
Cuttings Collected From 3 Year Old Stock Plants on the 14/12/95

Timing of Auxin Application 3000ppm (Weeks)		Rep. No.	Talc	Sucrose	Average + se
	Control	1	80.00	65.00	
		2	80.00	90.00	71.67
		3	65.00	50.00	5.87
	Average		75.00	68.33	
	Std Error		5.00	11.67	
	T0	1	85.00	90.00	
		2	65.00	85.00	78.33
		3	90.00	55.00	6.01
	Average		80.00	76.67	
	Std Error		7.64	10.93	
	T2	1	95.00	50.00	
		2	80.00	90.00	69.17
		3	75.00	25.00	10.91
	Average		83.33	55.00	
	Std Error		6.01	18.93	
	T4	1	85.00	75.00	
		2	90.00	90.00	86.67
		3	95.00	85.00	2.79
	Average		90.00	83.33	
	Std Error		2.89	4.41	
	Average		**82.08**	**70.83**	
	Std Error		**2.92**	**6.24**	

Lifting Experiment

Rooting Percentage Data for Cuttings Set Under Both Phytotron and Glasshouse Conditions
Cuttings Collected from 3 Year Old Stock Plants on the 14/12/95

(a) Phytotron

Rep. No.	Control (0 Weeks)	Lifted (2 Weeks)	Lifted (4 Weeks)
1	30.00	45.00	40.00
2	35.00	30.00	20.00
3	35.00	35.00	30.00
Average	**33.33**	**36.67**	**30.00**
Std Error	**1.67**	**4.41**	**5.77**

(b) Glasshouse

Rep. No.	Control (0 Weeks)	Lifted (2 Weeks)	Lifted (4 Weeks)
1	85.00	50.00	85.00
2	90.91	53.33	92.31
3	85.00	65.00	75.00
Average	**86.97**	**56.11**	**84.10**
Std Error	**1.97**	**4.55**	**5.02**

The change in leaf water potential (MPa) of cuttings propagated in the phytotron and glasshouse during a 6 week period after excision

	14/12/95 Week 0	21/12/95 Week 1	28/12/95 Week 2	4/1/96 Week 3	11/1/96 Week 4	18/1/96 Week 5	25/1/96 Week 6
Phytotron	0.500	1.100	1.575	1.800	1.750	2.225	2.275
	0.550	1.225	1.450	1.825	1.675	2.075	2.050
	0.525	1.075	1.775	1.925	1.850	2.350	1.975
	0.550	1.475	1.625	1.775	2.275	1.975	2.325
	0.525	1.350	1.550	2.125	2.075	1.875	2.325
Average	**0.530**	**1.245**	**1.595**	**1.890**	**1.925**	**2.100**	**2.190**
Std Error	**0.009**	**0.076**	**0.053**	**0.064**	**0.110**	**0.085**	**0.074**
Glasshouse	0.500	0.975	0.825	0.725	1.550	1.650	1.750
	0.550	0.700	0.750	0.975	1.325	1.725	1.425
	0.525	0.550	0.950	0.850	1.625	1.475	1.550
	0.550	0.825	0.725	0.875	1.450	1.575	1.525
	0.525	0.750	0.875	1.225	1.525	1.525	1.600
Average	**0.530**	**0.760**	**0.825**	**0.930**	**1.495**	**1.590**	**1.570**
Std Error	**0.009**	**0.070**	**0.041**	**0.084**	**0.051**	**0.044**	**0.053**

Glucose and Fructose Application Experiment

Rooting Percentage Data for Cuttings Set in the Phytotron
Cuttings Collected From 3 Year Old Stock Plants on the 23/4/96

Rep. No.	Talc Control	Glucose Dip	Fructose Dip	Glucose and Fructose Dip
1	75.00	50.00	45.00	40.00
2	65.00	40.00	40.00	20.00
3	80.00	35.00	50.00	25.00
4	75.00	45.00	25.00	45.00
5	70.00	30.00	30.00	35.00
Average	**73.00**	**40.00**	**38.00**	**33.00**
Std Error	**2.55**	**3.54**	**4.64**	**4.64**

LSD (0.05) 11.75