A STUDY OF ENVIRONMENTAL, CULTURAL, AND PHYSIOLOGICAL FACTORS INFLUENCING THE VEGETATIVE PROPAGATION OF *EUCALYPTUS GUNNII*.

by

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ABSTRACT

Environmental, cultural and physiological factors affecting the propagation of Eucalyptus gunnii by cuttings were studied.

Environmental factors studied included water (misting and the relative water content of the cutting); light (intensity, duration, colour and photoperiod); temperature (of air and substratum); and season. Cultural factors included rooting media and control of infection. Physiological factors first tested included treatment with varied concentrations of exogenous auxins, gibberellins, a cytokinin, organic nitrogenous compounds, vitamins, sugars, chlorogenic acid, and growth retardants (B-9, Cycocel and Phosphon). Various methods of application were tested, and all findings were tested statistically. Rooting was a rare occurrence: endogenous causes for failure were therefore investigated.

Endogenous growth promoting substances (auxins, gibberellins, cytokinins) were found, by extraction chromatographic separation and various bioassays, to be present in leaf extracts of adult and of rooted and unrooted juvenile Eucalyptus gunnii and of Myrtus communis, a related species. Concentrations of each substance found were similar to those found in other rooted species. Thus deficiency of these growth promoting substances probably could not explain failure to root.

Alternatively, the various endogenous growth inhibitors found to be present in all leaf extracts might explain the inhibition of rooting. The hypothesis that the extractable inhibitor abscisic acid was primarily responsible for failure to root was eliminated

both by lack of correlation between endogenous content and effect and by direct experimental tests. Other endogenous inhibitors chromatographing together at another Rf region, 0.85-1.0, strongly inhibited survival and rooting. Among these inhibitors, xanthoxin-like substances were found which were strongly inhibitory to Eucalyptus and eight other species and may therefore have more general biological importance than previously realised. Other distinguishable components such as farnesol and leptospermone may also be present and active.

The concentrations of these inhibitors were, however, similar in leaf extracts of rooted and unrooted Eucalyptus. Even higher inhibitor concentrations were found in leaf extracts of the easily rooted Myrtus communis. Hence mere presence of such inhibitors cannot fully explain lack of rooting in Eucalyptus. Hence sites of root initiation may be accessible to such inhibitors in Eucalyptus gunnii, but not in Myrtus communis. In agreement with this idea, the Myrtus inhibitors when applied exogenously to Myrtus cuttings inhibited their own rooting. Thus, degree of access of growth inhibitors to sites of root initiation, as well as mere presence of extractable inhibitors, is a new factor to be considered in all future work on rooting of cuttings.

DECLARATION

I hereby declare that the following thesis is based on the record of work done by me, that the thesis is my own composition, and that it has not previously been presented for a Higher Degree.

The Research was carried out in the Department of Botany and the University Botanic Garden, University of St. Andrews, under the direction of Dr. D. C. Weeks.

CERTIFICATE

I certify that Robert J. Mitchell has spent eight terms of research work under my supervision, and that he has fulfilled the conditions of Ordinance No. 51 (St. Andrews), and that he is qualified to submit the accompanying thesis in application for the Degree of Master of Science.

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CHAPTER I

INTRODUCTION

Eucalyptus trees vary considerably within the species and it is impossible at present to select special clones for a variety of important economic requirements. Eucalyptus species are being regularly planted throughout the world as a quick-return timber tree with a number of commercial merits: these include their value in paper making; for their aromatic oils; for their tannins; for use in arid regions to stabilise soil against erosion; and for visual effect in the landscape, (World Eucalyptus Conference, 1961). It is therefore all the more desirable to find a technique for selecting clones with valuable qualities such as the production of elite timber, freedom from twisting, hardiness or drought resistance, or increased yields of the aromatic oils.

At present, propagation by seed provides the only method of producing large numbers of plants, but, as the species of Eucalyptus freely hybridise one with another leading to variations in the genotype, a standard clone cannot be produced in this way. Eucalyptus hybrids occur in the wild, and there is evidence to suggest that there is a natural preference for outbreeding. Selfing is decreased in some species by a gene-controlled incompatibility system (Pryor, 1961): thus a homozygous plant is rarely produced. It is therefore necessary to find a method which would produce a great number of plants of a known standard phenotype. This must be done by vegetative means to ensure that the clonal stock is retained.

Vegetative Propagation of Plants in General

Vegetative propagation, adopted in commerce for a very wide range of plant species, as a means of raising large numbers of plants which have a known phenotype, can be achieved in three main ways:-

(a) Grafting, which involves removing shoots from the stock plant and attaching them to seedling rootstocks grown specifically for the purpose. Success is achieved by ensuring that the cambium of the scion is in strict juxta-position to that of the stock. With care, these will unite and produce the desired plant,

(b) (1) Layering, which involves making a cut or removing a portion of the bark to expose the cambium and pegging the shoot down to the ground. This portion is covered with a suitable moisture-retaining medium and left for at least a year before being detached from the parent plant. In the case of Eucalyptus, this is not practical, so

(2) Air-layering is used. This is a form of layering above ground level, usually after wounding the stem. A suitable rooting medium is placed round the wounded shoot and maintained in a moist condition, and

(c) Cuttings, where any severed part of a plant, when given the proper conditions, will root to produce a new plant.

ii Vegetative Propagation of Eucalyptus

The fact that Eucalyptus is difficult to propagate by vegetative means has been known for many decades. Grafting has proved a very variable means of shoot propagation in Eucalyptus. Some methods have proved more satisfactory than others, but none provides root systems of the desirable clone.

Approach Grafting. Using a seedling "stock" grown especially for the purpose and approach grafting the desired branch to it, has proved very effective. When the cambium has united to form an effective union, the shoot is severed, converted to a bottle graft for a further period and treated carefully in nursery conditions. After several months, the union is fully developed and the plant is then ready for planting out in field conditions.

Bottle Grafting. This involves removing a shoot from the desired clonal stock and grafting it to the seedling stock. The clonal material is supported by having its base in a bottle of water.

Tip Grafting. This is similar to an inverted saddle graft, and has been used in some cases with variable results. This method involves decapitating young seedlings some inches above the cotyledons, splitting the top and inserting a wedge-shaped shoot apex into the split end.

Side Grafting. This has been less reliable. Side grafting makes full use of the head of the stock. A slice of bark is removed from the side of the stock, and a shallow slicing cut is made at the bottom of this wound to produce a small tongue. The scion shoot containing buds and leaves has a matching long cut and short basal cut and is fitted to the stock with the base resting on the short tongue. It is then bound tightly. On uniting, the top of the stock is removed.

Grafting does not, of course, require the need for roots to form. The callus tissue of stock and scion must unite and form joint vascular tissue to prove successful.

Pryor (1957) reports successful air-layering of adult shoot growth on six year old trees of E. linearis. Similar tests conducted on 1-2 year old seedlings of E. fastigiata, E. saligna and E. botryoides also provided successful root formation on air layers at about the 12th node above the cotyledons. However, trials on three year old and more mature trees of a large number of species failed to produce the earlier successful results (Pryor and Willing 1963).

In the following table, details of the propagation of Eucalyptus are given.

Propagation Method	Results			
	Very reliable	Good	Variable with species	Nil
Approach Grafting	x			
Bottle Grafting (Scion with buds and leaves			x	
Bottle Grafting (without leaves)		x		
Budding			x	
Side Grafting			x	
Tip Grafting (some species)		x		
Tip Grafting (all species)			x	
Cutting (Young seedling and lignotuber)	x			
Cutting (Mature material)				x
Air-layering			x	

After Pryor and Willing (1963).

Natural air-layering is reported in Hawaii in plantations of E. robusta where, due to the high humidity, aerial roots formed 30 feet above the ground, (Jacobs 1955). This does not happen in Australia due to the less favourable climatic conditions. There also appears to be a considerable difference in response within a plantation of a single species to natural air-layering, for this is not a regular and common occurrence.

Grafting and layering are labour-intensive methods and therefore possible only on a very small scale.

The cutting method alone is therefore suitable for mass vegetative production of the desired plant as a whole, i.e. root and shoot. Vegetative propagation, using detached cuttings, is possible from young seedlings in the juvenile stage of all Eucalyptus systematic groups (Pryor and Willing 1963) but the cuttings must be taken before the juvenile seedlings reach the fifteenth leaf pair stage. Since cuttings must include the terminal bud (stem apex), this means that only two plants are possible from one seed sown. Branching can, however, increase the number of potential apical cuttings per seed. The quality of the phenotype is, however, unknown at this juvenile stage. Forest trees often do not show their capabilities for 50-60 or more years. In Eucalyptus species the elite timber aspect may not be evident for 20 or more years, even though they are quick growing trees. With seed-grown plants a very wide variation occurs, not only in tree habit and stem twisting, but in all other factors as well. It is only when trees reach mature

proportions that the true phenotype is known. Thus propagation from mature phenotypes of proven quality is required. However, cuttings from adult growth have so far failed completely to provide any reliable positive rooting despite widespread attempts.

Lignotuber shoots are produced in some species when the Eucalyptus is a very young tree or when it is cut over at ground level. The shoots are never produced in quantity, so again this method of propagating large quantities of trees is of very limited value to the theme of this work.

The object of this work is therefore to find methods of mass propagation, by cuttings, of specially selected clonal material from mature Eucalyptus shoots.

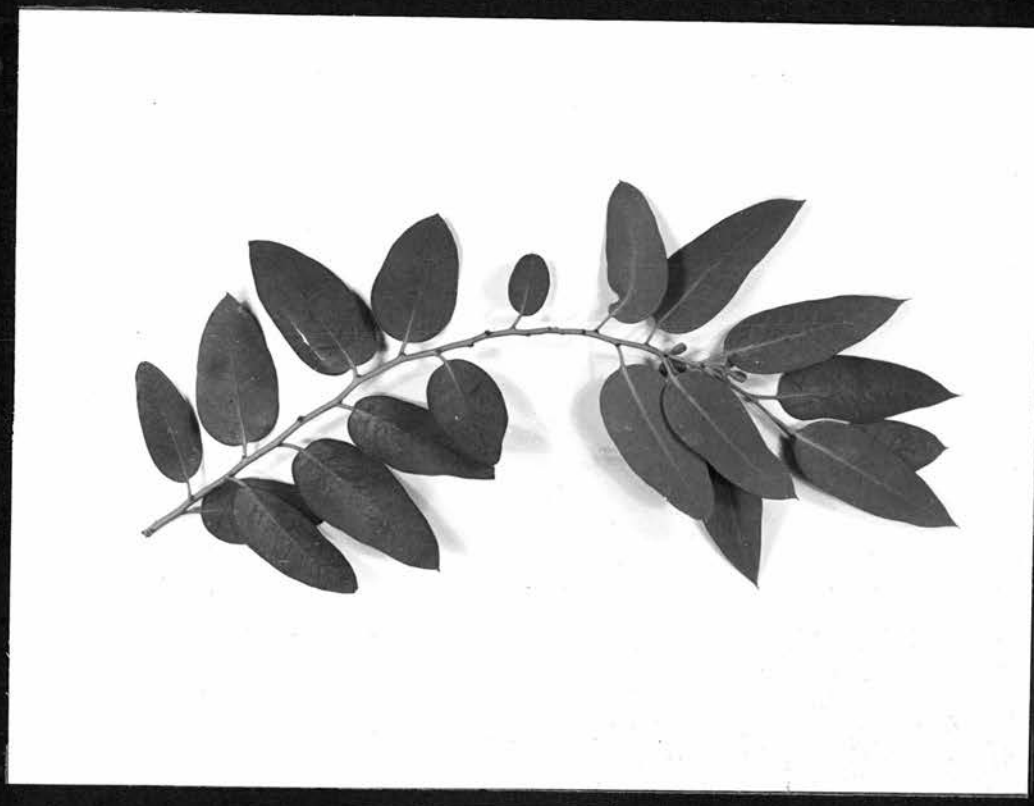
iii Heteroblastic Stages of Growth in Plants

Many trees and shrubs pass through stages of growth before reaching their adult growth phase. This phase-change causes changes in form and physiology while passing through juvenile growth to the adult growth stage. These stages are an ontogenetic development of the plant. Juvenile stages may look so different from their mature forms that it can be difficult to identify the two stages as belonging to the same species.

It should be understood that juvenile and adult stages of growth are different from young and old shoots since the meristematic regions of the plant remain young throughout the life of the plant. Thus juvenile and adult meristems are both young. Plates 1 and 2 illustrate juvenile and adult Eucalyptus gunnii shoots.

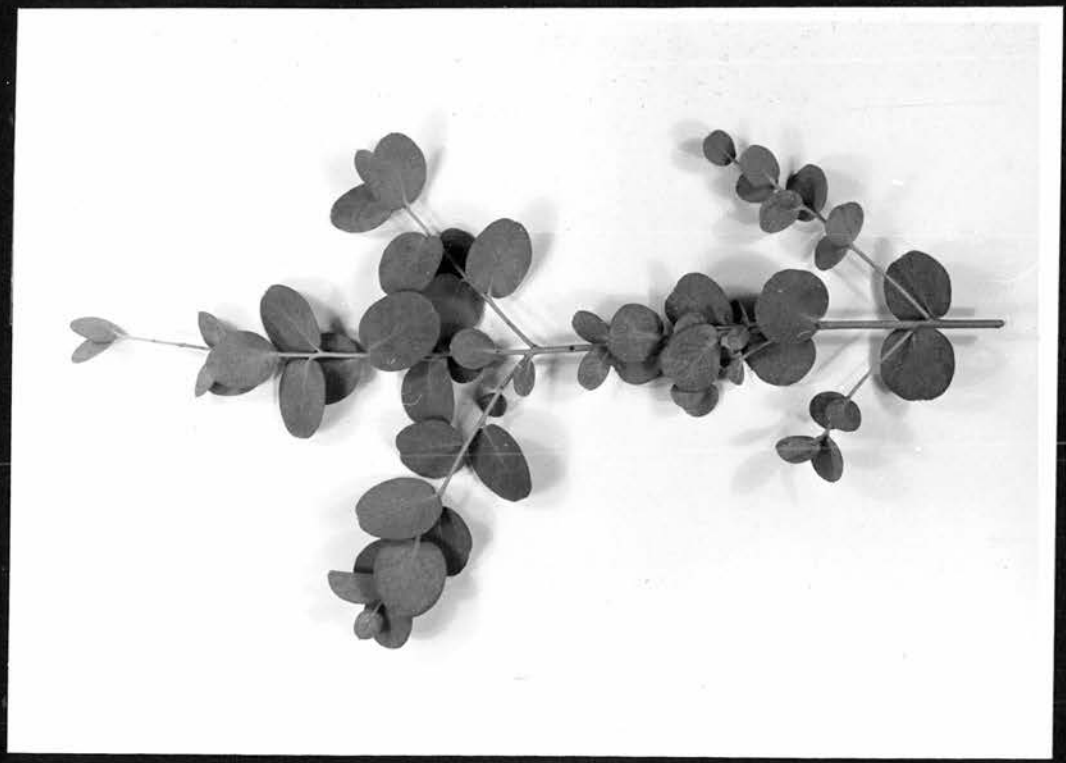
Heteroblastic stages of growth in Eucalyptus gunnii.

Plate 1



Adult stage.

Plate 2



Juvenile stage.

Juvenility of the plant, then, does not necessarily mean chronological age, for, while some of the Gymnosperms pass through the juvenile stage in less than a year, and birch in 5-10 years, it may take oak and beech 30-40 years to reach the adult growth stage (Wareing 1959).

Eucalyptus species are included among the plants which have juvenile and adult stages of growth. This phenomenon is known in many of the plants from Australia and New Zealand, and is common in other floras. Hedera helix is an excellent European example, (Muckadell 1959).

Ontogenetic aging consistently results in a decreased rooting ability in cuttings of many species, (Kozlowski 1971).

Thimann and Delisle (1939) recognised that plants which proved difficult to propagate rooted better when taken from seedlings or young plants showing the juvenile growth phase. They give details of Pinus strobus and Quercus borealis rooting only from young plants showing the juvenile stages of growth. Commercial crops such as Hevea, Mangifera, Olea and Psidium also show this characteristic. Hedera helix is a plant which roots with ease from cuttings taken from the juvenile phase of growth, and with difficulty from the adult stage. It exhibits marked morphological differences between juvenile and adult growth and these are similar to those of many other genera including Eucalyptus. In Eucalyptus, a similar rooting pattern to that of Hedera helix occurs. All species of Eucalyptus can be propagated by cuttings taken from seedlings between cotyledons and the fifteenth node. Thereafter the rooting

capacity of cuttings diminishes rapidly. So far, cuttings from the adult stage of all species of Eucalyptus (except E. deglupta) have failed to root, (Pryor and Willing 1963).

The process of development from juvenile to adult is reversible in some species. In Hedera helix, juvenility is induced by exposing new adult shoots to temperatures of -10°C for a few hours (Frank and Renner 1956). Robbins (1960), also working with Hedera helix, induced reversion to juvenility by spraying with gibberellic acid over a period of 19 weeks. Similar applications of gibberellic acid do not affect the Eucalyptus in the same way. In this case, the leaves are transformed from typically juvenile to mature adult - a hastening of the ontogenetic development of the plant.

However, regular pruning of Eucalyptus trees retains the juvenile growth stage if pruning commences from an early stage (Waldron 1968). Lopping or cutting back the crown of 60 feet high Eucalyptus cladocalyx resulted in a change of leaf shape which we can take to be a temporary reversion to juvenility, (Maggs and Alexander 1967). It might therefore be possible to propagate Eucalyptus from such induced juvenile shoots.

iv Anatomy of Rooting

The process of rooting usually involves two completely independent stages - wound healing and root initiation. Wound healing with periderm formation is necessary in cuttings before rooting will take place. In a severed shoot, liquid water is lost only as far as the first xylem perforation plate. A suberin layer is formed over the position of the unruptured cells at the base of the cuttings. Kemp (1948) states that the thin suberin surface layer is formed by

the oxidizing of the fatty materials which appear at the cut surface. Later, a phellogen layer is formed inside the suberized cell layer producing a water resistant phellem. If the protective fatty substances formed at the wound, made by taking the cuttings, are leached out and no healing takes place, the cuttings then blacken and die from the base upwards. These fatty substances, which include cutin and perhaps suberin, hinder microbial attack probably by preventing ingress of water-soluble degradative exo-enzymes from bacteria and fungi. It is therefore of the utmost importance that wound healing or sealing with plastic film (e.g. "S600") should take place quickly. The likelihood of root initiation or elongation might then be increased.

Mahlstede and Haber (1957) recognise two types of roots produced by the cutting: (a) 'morphological' roots, i.e. preformed root initials already present in whole plant stems but which develop into roots only after excision of stem cuttings, and (b) wound roots.

(a) 'Morphological' Roots - Root initials are frequently already present in stems of plants and await favourable conditions for growth and emergence. All the easily rooted genera have these preformed root initials. They include Ficus, Forsythia, Jasminum, Populus, Ribes and Salix, etc. These preformed root initials are found in similar positions to other adventitious roots in stems, and develop when the stem is detached from the plant. Callus tissue may, or may not, develop.

(b) Wound Roots - The formation of root initials of wound roots takes place after the cutting has been detached from the parent plant. Wound root development in stem cuttings can be divided into three stages:-

(i) the initiation of groups of meristematic cells - the root initials - either at the cambium or in wound callus tissue, which then become a prerequisite for rooting,

(ii) the differentiation of the root initials into recognisable root primordia, and

(iii) the development and emergence of new roots and the formation of connecting vascular tissue.

In cuttings of woody plants, adventitious wound roots are commonly formed by the vascular cambium next to the central core of xylem tissue (Hartmann and Kester, 1968). In Conifers, roots appear from various tissues such as procambium, parenchyma and callus. Cuttings of most species of Conifers difficult to root produce roots mainly or only from the callus tissue (Satoo 1956). Abelia, Abies, Acanthopanax, Picea, Pinus and Thujopsis all produce roots originating in the callus. Clematis, Cryptomeria, Cupressus and Thuja produce roots from vascular cambium, while Hedera, Ligustrum, Ribes, Rosa, Sambucus, Tamarix and Ulmus originate wound roots from parenchyma cells.

However, prior to this development, callus tissue is formed over the cut end of the cuttings. It develops mainly through the activity of the cambium, and the degree of activity is related to the age and hence the lignification of the cutting. In slightly lignified (soft) cuttings of Myrtus, cambial activity develops and spreads out over the entire base of the cuttings - from the vascular cambium and also phloem, cortex and pith; while in heavily lignified shoots, callus is said to develop only from the vascular cambium and then spread outwards, (Komissarov 1969).

Cormack (1965) stated that the pH of the rooting medium had an influence on the type and amount of callus produced. When the medium was slightly acid (pH 6.0 is given), callus cells in Populus balsamifera were large, irregular in shape, and soft. With increasing alkalinity, up to pH 11.0, callus cells were smaller and more compact. Root outgrowth was also greatly reduced down to nil at high pH values although, on sectioning, root primordia were still well formed.

From this evidence we can deduce that the structural and micro-environmental state of the callus often correlates with, and may thus control, the actual emergence of roots.

The idea that a compact callus acts as a mechanical barrier in alkaline media preventing root emergence has been suggested by some authors, but others cannot find any relationship between density of callus or its sclerenchyma fibre content and root emergence. They favour more the idea of ease of formation of root initials. This suggests that the callus itself may hinder formation of root initials because it forms a barrier hindering either the diffusive inflow of water, air and solutes, or the outflow of CO_2 and inhibitory metabolites; or both. However, these may be only a few of the various possible causes of non-emergence.

v Environmental Factors in the Rooting of Cuttings

Plant propagation involves many factors used in such a way as to produce the desired result. It is well known that plants vary in their requirements. Chadwick (1951) considers that control of certain major factors is the best basis for success. This success is dependant on the careful manipulation of external factors to fit the internal conditions.

The external factors can be considered under two headings - environmental and cultural. The internal factors, which are of great importance, can also be divided into two groups - anatomical and physiological. The latter are not independent factors, but rather are different viewpoints for practical convenience.

The environmental conditions include the supply of water, light, temperature and oxygen, and the cultural factors concern the choice of the rooting medium, treatment of the cutting, and use of plant growth regulators or hormones and fungicides. The cutting's ability to heal the wound and the presence of root initials, or the ability to form them, are included in the anatomical factors; while food supply, hormones or endogenous growth regulators, and water in the plant are the main physiological factors.

1. Water

Softwood and leafy cuttings must be kept in a fully turgid condition if successful rooting is to occur. Leaves, even those on detached cutting material, still transpire even though their normal source of water is removed. Water loss from the cutting is dependent on several factors, the greatest of which is the humidity of the atmosphere. To reduce water loss to a minimum, the air can be saturated with moisture and if the water vapour pressure in the leaf is equal to that outwith the leaf, no water loss will ensue (Hess, 1954).

The reducing of the transpiration rate of cuttings by water application can be achieved by two methods - humidification of the atmosphere or the moistening of the leaf surfaces. Humidification

increases the relative humidity around the cuttings by dispersing fine particles of water into the atmosphere but does not generally deposit moisture on the leaves. Humidification on its own will reduce the transpiration rate.

Higher humidity above the cutting bed can be achieved in two ways. The older method using closed frames and involving manual application of water regularly throughout the day to produce a humid microclimate is successful but very costly (Balfour, 1913). The closed frame also encourages a high temperature in the enclosed space.

More recently, electrically operated mist forming systems have been used providing greater reliability and effectiveness. Labour costs are negligible. This is controlled by an electronic sensor placed in the cutting bed. The gap between the sensors has a high resistance to electric current flow when dry; when wet the current flowing through the wet film switches the spray valves off.

Mist systems provide a certain degree of humidity but their main aim is to keep the leaf surface covered with a film of water at all times thus cooling the leaf and minimising the outward vapour pressure gradient from within the leaf, again reducing the rate of transpiration (Snyder & Hess, 1955). A further advantage of the mist propagation technique arises from the increased permitted light intensity which promotes full photosynthetic activity.

Hess (1962) proved that cuttings of Prunus serrulata and Cornus florida var. rubra had a higher rooting rate under mist than in a closed case. The leaf temperature of the cuttings was lower under mist. Photosynthesis was increased in the light mist system and the carbohydrate increase under mist was striking.

Mist applications continually wash fungal spores from the leaves into the rooting medium thereby reducing the possibility of infection, while the highly humid still conditions of the closed case favour fungal spread.

In Eucalyptus propagation, Ivashenko (1939) obtained moderate success using a high humidity by regular hand watering, while Giordano (1961); Fazio (1964); Gonderman & Martin (1970); all used the intermittent mist propagation technique, with varied success, on shoots with juvenile foliage. Pryor & Willing (1963) and Paton and others (1970) carried this a stage further by rooting seedling cuttings of Eucalyptus deglupta with their bases standing in water: an extreme case. This is the only species of Eucalyptus which has proved possible to propagate vegetatively from cuttings with consistently high rooting rates.

Natural layering after flooding (Jacobs, 1955); air-layering (Pryor, 1957); and the appearance of aerial roots on Eucalyptus robusta growing in a plantation in Hawaii due to the very high humidity of the climate (Pryor & Willing, 1963); showed that some species of Eucalyptus can, and will, produce aerial roots even from mature trees.

Conclusion

If flooding and moist air layer both induce rooting through a common cause such as correct water balance, then mist propagation of detached cuttings should optimise the rooting of cuttings. Regrettably low or nil rooting results are obtained in my work, suggesting that poor water balance is not the major cause of failure of cuttings to root.

2. Light

Light is essential for photosynthesis in all plants and this is also true for cuttings detached from the parent plant and placed in a rooting medium. Lowering the light intensity reduces the rate of photosynthesis in cuttings (Hess, 1962). There is strong evidence from many reports concerning the essential need for abundant carbohydrates if rooting is to occur, and Snyder & Hess (1955) found rooting to be positively related to the content of endogenous carbohydrates as regulated by light intensity and temperature. Hess (1962) gives details of the accumulation of carbohydrates in cuttings under mist being eight times more than those in shaded, closed frames. Thus light and temperature are inter-related.

Light itself is responsible for growth in various ways. It is an important factor in propagation, for if the entire cutting is exposed to light, root initiation is generally inhibited (Briggs, 1966; Pierak, 1969). Root growth is also inhibited by light once initiation has taken place. This is why cuttings are always pushed into a dark rooting medium while the upper part of the cutting is exposed to light, allowing rooting to occur (Stoutemyer & Close, 1946).

There are three basic light factors involved in the growing of plants and all interact one with another - light intensity, light duration and light quality. All again are interconnected with other environmental factors, the most important being temperature (Waxman, 1970).

(a) Light intensity is important in propagation and many plants differ in their requirements to light (Macdonald, 1969).

Plants which demand a high light intensity for vegetative growth, such as Myrtus capensis, Myrtus communis, Rosmarinus officinalis, Quercus suber, Pinus sylvestris and Larix sibirica, also root better in high light intensities i.e. 5000-6000 lux. In shaded daylight conditions i.e. 600-900 lux the same cuttings root poorly. However, cuttings of high light requiring plants will not root in complete darkness, (Komissarov, 1969).

Shade demanding plants such as Azalea, root with excellent results in a light intensity of 3000 lux, (Stoutemyer, 1961). It is reported that with increased light intensity many cuttings increase their ability to root. Some shade tolerant plants such as Euonymus japonica, Gardenia, and Aucuba japonica root equally well (100%) in intense and poor light conditions. They will also root in complete darkness if there are sufficient food reserves present. Buxus sempervirens and Taxus baccata, two shade tolerant species, fail to root entirely in the dark.

Since rooting involves growth, what then are the light requirements for growth of Eucalyptus? Peterson (1959) gives the minimal light intensity for growing Eucalyptus (probably globulus) from seed as a commercial pot plant as 500-2000 lux. Cameron (1969) states that the juvenile stages of Eucalyptus fastigiata can grow in low light intensities i.e. 1600 lux, whereas the intermediate and adult stages must have higher light intensities for efficient photosynthesis.

Conclusion

Thus no general correlation can be drawn between the light intensity required for good vegetative growth and that required for optimal rooting success. Successful rooting in light demanding species favours the higher light intensities. It could also be possible that the correct combination of light intensity and temperature would provide the ideal rooting conditions.

(b) Photoperiod

Daylength is an important factor whether it is applied to the stock plant or to the cutting in the rooting bed, and has a distinct effect on rooting. Photoperiods most favourable for rooting vary from plant to plant, with some rooting best under long days and others under short photoperiods (Leopold, 1955).

Lanphear and Meahl (1961) give statistical evidence supporting long day treatments of cuttings of Juniperus horizontalis 'Plumosa', Ilex opaca and Rhododendron mucronulatum. Taken in the autumn, all produced higher percentage rooting in long days than in short days. Juniperus horizontalis 'Plumosa' is a plant which continues to grow in both long and short days, while Rhododendron mucronulatum stops growing during short day treatment. Peringer (1961) reports heavier rooting of Ilex crenata under long day photoperiods.

Photoperiodic treatments of 9, 18 and 24 hours of cuttings of Cornus florida var. rubra grown under mist conditions produced 100% rooting in all cases. However,

differences did arise in the size of the root systems. Cuttings with the long photoperiods had over three times as many roots as those under 8 hour illumination (Waxman, 1965).

Ilex crenata 'Hetzi' responded to photoperiodic treatment of both stockplant and cutting material. In this case, cuttings taken from plants receiving the greatest number of short days rooted best. The total root length was greater, more roots were initiated, length of roots was increased, and numbers of secondary roots were doubled compared with cuttings taken from stockplants receiving long days. However, cuttings rooted under long days produced more total root length and a greater number of secondary roots than those receiving short days, but otherwise the photoperiod received by the cuttings did not affect the number of adventitious roots produced (Kelley, 1965). It would therefore appear that in the case of Ilex crenata 'Hetzi', the requirements for the best rooting are short day treatments for the stockplant followed by long day treatments for the cuttings.

Most reports link photoperiodic treatments with seasonal daylength thus confirming the underlying seasonal influence, and these we treat under this subheading.

Cuttings of Juniperus horizontalis 'Plumosa' and Taxus cuspidata 'Nana' rooted best during the winter months. Photoperiod had little effect on Juniperus. However, long day treatments in the winter months caused inhibition in the rooting of Taxus cuspidata 'Nana' (Lanphear, 1963). Thus seasonal adaptation can override photoperiodic control.

Scurfield (1961) found that given a temperature adequate for normal growth (16-19°C night time, 20-25°C day time), increasing daylength from 8 hours to 18 hours produced an increasing growth response in seven Eucalyptus species tested. These were E. bicostata, blakelyi, rubida (Macrantherae Sect.), E. niphophila, pauciflora, stellulata (Renantherae Sect.), and E. polyanthemos (Terminales Sec.). He postulates that the Eucalyptus species would therefore belong to vegetative Class C of Nitsch (1957) which means that they continue to grow in long or short days but more rapidly under long day treatment.

Conclusion

From the references cited and the general information available, each plant has its optimum photoperiod for growth, flowering, etc. This same factor is often likewise critical for the rooting of cuttings and it is only by experimentation that the correct photoperiod can be determined. We know that long photoperiods favour the incremental growth of Eucalyptus shoots but we are not sure of the photoperiod which favours rooting. 'Longer photoperiod' does not distinguish between prolonged photosynthesis and longer daylength effects.

(c) Wavelength

In normal conditions all wavelengths play their part in the growth of the plant and it is only when plants are studied in artificial conditions in the laboratory that the effect of the various wavelengths alone and in combination can be determined. The use of differing wavelengths in physiological research in laboratory conditions is fairly extensive and growth patterns can

be determined. Their use in propagation on a commercial scale is very limited and little is known about the reaction of specific plants or groups of plants to the various wavelengths.

The three most important wavelength regions, from the plant growth and rooting aspect, are blue, red and far red (around 450, 660 and 730 nm. respectively).

Blue Region

In plant growth the wavelengths from the blue region assist in phototropism causing the plant to grow usually towards the light due to the movement of the auxin radially within the plant away from the source of light (Waxman, 1970). The influence of uniform blue light produces short stocky plants when used on Lycopersicon esculentum and Mirabilis jalapa. High blue light intensity retards the growth of many plants (Van der Veen, 1958).

In propagation, cuttings from stock plants of Gordonia axillaris treated with blue fluorescent tubes produced the heaviest rooting and lightest callus compared with daylight tube, blue and pink tube, and normal daylight (Stoutemyer and Close, 1947). However, Stoutemyer and Close (1946) found that blue lighting treatment on cuttings of Forsythia ovata, Spiraea and Ligustrum ovalifolium (all plants which prove easy to root) produced an inhibiting effect on root formation.

Root formation on kidney bean cuttings is also strongly inhibited by blue light treatment of the cuttings (Herman, 1967).

Red Light

Plant growth responses to red light appear to vary with the species. Growth of Lycopersicon esculentum increased moderately while shoots of Mirabilis jalapa increased significantly. However, red light treatment on Salvia resulted in a short plant, and inhibited the growth of potato sprouts when very small quantities were used.

Red light is required to bring about the expansion of leaves in beans and peas (Van der Veen, 1958).

Stoutemyer and Close (1946) found that red light is effective in promoting rooting in Cinchona ledgeriana where either the stock plants or the cuttings are treated. Turetskeya (1961) found red light increased rooting in plants which root easily in diffused light. However, in the case of kidney bean, root formation was slightly inhibited by red light (Herman, 1967).

The level of Abscisic acid in tomatoes is increased by red light treatment (Khudairi and Joglekar, 1971).

Far Red Light

Far red light strongly inhibits the germination of lettuce seeds and the expansion of bean leaves. Both are promoted by red light (Wareing and Phillips, 1970). Far red light causes considerable stem elongation (Van der Veen, 1958). High far red radiation increases the temperature of leaves of Phaseolus but does not significantly increase growth in relation to low far red radiation (Pallas and Michel, 1971).

Active phytochrome produced by far red light promotes shoot growth, cell division and D.N.A. and R.N.A. synthesis (Khudairi and Johnnykutty, 1971).

Mixed Wavelength

Wavelengths in differing combinations produce various effects on plant growth. Blue and red light together produce short, stocky plants when used on tomato. When far red is added to blue, vigorous stem elongation occurs. This latter effect is also true for Salvia when high light intensities of far red are required to increase shoot growth. In Mirabilis, combined or solo red and blue wavelengths produce short plants, and with the addition of far red light to blue there is only a slight elongation (Van der Veen, 1958).

A combination of blue and far red light inhibited nodule formation in Phaseolus vulgaris to a greater extent than a combination of red and far red light. Both treatments, however, suppressed the production of lateral roots (Grobbelaar et al, 1971).

In propagation Ritson (1971) found that fluorescent light containing 46% red light and 23% blue light reduced losses and speeded up rooting when used on Geranium cuttings compared with daylight. Losses were reduced from 6% to 4% and throughput was increased from 4 weeks (controls) to 7-8 days. Certainly light intensity and photoperiod also play a part.

Mechanisms of Light Effect in Plants

Van der Veen (1958) postulates that inhibiting substances or effects are produced in both blue and red light. The "blue" inhibitor (dominant in Mirabilis) is slowly but irreversibly

destroyed by small amounts of far red, whereas the "red" inhibitor (dominant in tomato) is reversibly inactivated by far red light. Many workers have shown that reversibility of red and far red light effects is mediated by the phytochrome system. They show that phytochrome P730 (or sometimes simply called P_{FR}) produced by red light, is transformed to phytochrome P660 (or P_R) by far red illumination. Darkness causes a slow reversion to P660 (P_R) but considerable amounts are destroyed.

Conclusion

From the restricted number of references available, it would appear that blue light treatment of the cuttings produces an inhibiting effect on rooting. Treatment of the stock plant has, however, a stimulating effect on rooting but as this is impractical with forest trees its use has been discarded in my own work.

Red and far red light offers distinct possibilities with regard to rooting and it might be worth testing as a suitable treatment for Eucalyptus cuttings.

3. Temperature

Temperature and light are very closely linked together in their interaction or effect on the propagation of plants. Air temperatures and substrate temperatures are also interdependent and together or singly can determine the success or failure of the rooting of cuttings which are more difficult to propagate.

Plants vary in their optimum air temperatures and it is therefore important to find the correct temperature range. Day temperatures are important, for too high temperatures can bring

about excessively rapid respiration of carbohydrate reserves and, if very high, can even cause biochemical and structural damage. It is generally found that when temperature has risen, the light intensity should also be raised to counterbalance by increased photosynthesis any faster respiration of food reserves.

Hess and Snyder (1955), and many others, have proved that if the surface temperature of the cutting can be reduced, the unnecessary wastage of food reserves is also stopped due to the reduction in respiration. It is not only a relationship of light and temperature but also of water balance. Indeed, the temperature of the leaf surface under mist propagation can be 3-5°C lower than those cuttings under double glass frames (Hess, 1954; Hess and Snyder, 1955) thus decreasing both water and carbohydrate losses.

Air temperatures of 21-27°C during the day are normally used in most establishments with a nightly drop to 15°C. Minimal night temperatures can be achieved with thermostatically controlled heaters but maximal day temperatures, particularly with a large influx of solar radiation, are less easily controlled without the aid of expensive cooling and ventilating equipment. Hartmann & Kester (1968) point out that excessively high temperatures can prove lethal to cuttings, and Kester (1970) warns of the danger of temperatures which fluctuate considerably.

Excessively high air temperatures tend to promote shoot growth to the detriment of the rooting processes. This is why substrate heating is used on cuttings which are difficult to root. The ideal situation is where the air temperature is cooler than the substrate

to allow more cellular activity at the base of the cutting. The optimum substrate temperature for rooting varies considerably from plant to plant. Komissarov (1969) in his review of Russian research on propagation quotes that a substrate temperature of 20°C suited Hydrangea but that root formation in Malus 'Springdale Variety' was possible only at temperatures as high as 34-38°C. Camellia japonica, known to require high temperatures for root formation, will root best in the temperature range of 35-37°C.

Suitable substrate temperatures fall in general into the range 20-27°C and Rowe-Dutton (1959) gives the optimum general temperature as 25°C for the propagation of the majority of cool temperate and hardy plants. It has long been the practice at the Royal Botanic Garden, Edinburgh, where experimentation on propagation has been carried out for over sixty years, that the air temperature for such plants has been 10-18°C with the substrate temperature slightly higher at 24°C.

Soil warming with mist propagation is now more or less taken for granted for all plants. In 1959 when Rowe-Dutton reviewed mist propagation equipment, very few trials had been carried out but it was assumed that substrate heating was necessary for the propagation of all plants. However, Nelson (1966) carried out extensive trials to establish the value of soil warming using 200 taxa. He summarises his results stating that though soil warming is not essential in the majority of cases, it is necessary for cuttings which are difficult to root. It helps to speed up rooting and increases the rooting percentage in all taxa.

In Eucalyptus propagation, soil warming has been used in most cases. Fazio (1964) used substrate temperatures of 21°C, while Gonderman and Martin (1970) used 24°C. Air temperature is only mentioned by Ivashenko (1939) who gives 20-22°C as the day temperature, falling to 8°C at night.

Conclusion

Two main temperature ranges emerge: for the cool temperate and hardy plants an air temperature of 10-18°C with substrate temperature maintained at 24°C; and for the warm temperate and tropical plants an air temperature of 21-27°C with substrate temperature slightly higher. Eucalyptus gunnii is a sub-alpine plant from Tasmania and little is known of its temperature requirements in rooting.

4. Season

Among the plants difficult to root there is a definite season when each plant will root best even under optimal conditions such as mist propagation and soil warming. Timing is then the critical factor with these plants. Each plant has its own particular season, however short, and by missing this period, rooting can be greatly diminished or completely absent.

As far back as 1934, the Royal Botanic Garden, Edinburgh, through the work of L. B. Stewart and his associates, published in the Trans. Bot. Soc. Ed. XXXL pp. 457-9, a Plant Propagation Calendar. This gave the optimum months of the year in which to prepare softwood cuttings of 208 taxa.

Thimann and Behnke-Rogers (1950) summarised 291 papers dealing with the propagation of plants and their treatments. They gave the dates of the taking of cuttings of about 3,380 plants with their rooting percentages. It seems certain that in many cases the timing for the taking of leafy cuttings is critical.

Komissarov (1969) lists eleven groups of plants according to their rooting potential. Some will root throughout the year while others have a definite rooting period. Evans (1971) gives the relationship of the date of taking cuttings with the rooting response of softwood cuttings of seventy-five taxa during July and August. His findings show the variability of the plants' responses. Nineteen taxa were unaffected by the date; twenty-seven rooted best when taken in July; while twenty-two showed a preference for August. Cuttings of twelve taxa failed to root within the sixty day period allowed for rooting in the tests.

Deciduous Plants

Tyce (1957) found that there was a seasonal rooting response in cuttings of Salix fragilis under constant cultural conditions. The high peaks of rooting were found to be January and February.

Vieitez and Pena (1968) found that there was a seasonal rhythm of rooting in Salix atrocinerea cuttings. From January to April, rooting was particularly good with strong, vigorous roots appearing. A second rooting peak occurred in August, but here the roots were small and less vigorous.

Siebenthaler (1956) and Kirkpatrick (1956) both state that the timing for Syringa cuttings must be confined to a very short period just after growth begins in the springtime.

Congdon (1965) gives the season for Syringa and Kolkwitzia to be no more than three weeks.

Evergreen Plants

Many coniferous plants have proved difficult to root. These include Pinus, Tsuga, Larix and Picea. Some species, however, root more readily than others and some at different times of the year. For instance, Picea abies roots best from mid-July to October but Picea pungens roots best in February-March (Meahl, 1957).

The winter months have been the normal time to take most of the coniferous cuttings, and experiments with Chamaecyparis, Juniperus and Thuja by Nelson (1959) confirm this to be the best time although bottom heat and auxin treatments were necessary to give good results.

The Umbrella Pine (Sciadopitys) roots best from February to early April and thereafter the rooting response is very low (Waxman, 1960).

Working on Juniperus horizontalis 'Plumosa' and Taxus cuspidata 'Nana', Lanphear (1963) found that both rooted best in the winter months.

Doede (1969) found that Juniperus and Taxus produced more roots in early winter. In Taxus, rooting was better when the cuttings were taken in January and February but only when treated with auxin. In Juniperus, where auxin was also necessary, rooting was best in cuttings taken in December.

Stack (1971) states that Pinus roots best in mid-December but that the rooting response is very variable and generally low.

Wells (1955) states that the timing of softwood cuttings of Ilex opaca is extremely critical at the end of August or beginning of September but that during the winter months rooting does occur but with a lesser success rate. Childers and Snyder (1958) also found

that the timing of propagation was critical for Ilex opaca but that the choice of cultivar material used was also important since some root more easily than others. In the one cultivar which proved difficult to root, the main rooting period, within the August-November trial period, was restricted to early September - a very narrow time span. The other two cultivars had their peak rooting at that same time but the rooting percentage was spread over a much longer period.

In the northern hemisphere Eucalyptus cuttings have generally been taken in the spring. Ivashenko (1939) took cuttings in February but his research was carried out in sub-tropical conditions where growth would be early. Fazio (1964) found that March cuttings produced the best results, although limited rooting was found in July, September and November. Giordano (1961) successfully rooted E. camaldulensis when cuttings were taken in March and April, but Gonderman and Martin (1970) produced good results with cuttings taken in July and August.

Conclusion

There is variety in seasonal responses of different species. In deciduous plants the optimal time for rooting appears to be when the plants are actively growing, except with conifers where winter-time is generally (but not always) better. From the very limited information on Eucalyptus, it would also appear that cuttings taken from actively growing plants generally root better.

5. Rooting Medium

The rooting medium is a very important aspect of propagation and the success of the whole process can depend on it. It is the means of supporting the cutting during its rooting period; it supplies water to the cutting and should also retain sufficient supplies of oxygen which is required in the initiation of roots.

Various types of media have been used:

Sand on its own is cheap and easily obtainable and can be sterilized for re-use. It varies considerably in particle size and lime content. The ideal sand to use is a sharp, gritty, quartz sand which allows a free exchange of air due to its quick draining properties, while retaining sufficient moisture for the cuttings.

Sand and peat mixtures are generally used in horticultural practice. This is also one of the main media used in scientifically orientated experiments. They are sufficiently aerated yet hold a large amount of water.

Perlite is a volcanic rock expanded by heating to 1800°F and is a sterile medium which does not decay. It has a high water-holding capacity and is sufficiently coarsely grained and angular to allow free oxygen exchange when used as a rooting medium. It's pH is 7.0 to 7.5 and contains a high proportion of sodium, which is available to the rooted plants.

Peat and Perlite mixtures are used frequently, usually in equal proportions. The peat gives this medium a high water-holding capacity and also a lower pH value which is of value to calcifuge plants.

Vermiculite is mentioned as being suitable for pot plant culture and propagation, but under mist conditions it deteriorates rapidly and compresses causing poor aeration, thus making it of very limited value as a rooting medium.

Pumice, a granulated volcanic ash, is used in propagation frames with species which are difficult to root. It absorbs a considerable amount of water, yet leaves sufficient air spaces to allow free exchange of air to the roots. It is very expensive to purchase.

Coconut fibre was also used in pre-mist propagation times but is now little used because it breaks down rapidly in wet conditions.

Baystrat, a polyurethane foam, available in large sheets for propagation purposes, provides a sterile medium. It has a high water-holding capacity. The material is extremely light and is easily handled but is expensive. It was first marketed in 1971 and has proved successful with general commercial plant material which is easy to root.

A considerable amount of literature has been written about rooting media, (Hitchcock, 1928; Wells, 1955; Rowe-Dutton, 1959; Vermuelen, 1965; Reisch, 1967; etc. etc.). Reviews of rooting media very frequently come to different conclusions.

Rowe-Dutton (1959), in her review of mist propagation, lists 36 different materials from her study of the literature. Most materials were obtained locally and are therefore of little value in general propagation, and the remainder are used on a commercial scale.

According to Decker (1933), the water/air relationship is of the utmost importance and he states that 19-21% moisture, expressed as a dry weight of sand, was required for root initiation and development. This air/water ratio is dependent on the particle size of the rooting medium. Coarse sand will retain very little water, while fine sand will hold too much and the oxygen balance is depleted.

Sand and pumice can have the same particle size yet they have totally different water-holding powers. Pumice absorbs a very high amount of water while sand retains it only by surface tension between grains.

Mahlstede (1953) states that particle size has some bearing on the root size and root formation. Large particle size produces thin fibrous roots. Sand gives a root system which is brittle, heavy and sparsely branched in comparison with the peat medium which produces a slender, well-branched root system. He concludes by suggesting that a more fibrous root system may be obtained by compacting the sand thereby reducing the air content.

Kemp (1948), however, makes a further point in the need for an air/water balance. Fatty substances are exuded from the wound and when oxygen is present these oxidize and dry on the plant surface. However, when too much water is present this oxidation does not take place; the fatty substances leach away, no healing takes place and the cutting ultimately dies. It is therefore important that the correct air/water balance be found to achieve success. Oxygen provides metabolic energy from respiration, and inhibits anaerobic metabolism with toxic end products.

The pH value is one of the factors which may limit the use of a rooting medium. The hydrogen ion concentration can vary considerably over a very wide range of values. Sphagnum peat can have a reading as low as Ph 3.6; vermiculite varies from 6.5 to 7.2 although from African sources it can be as high as pH 9.6 Perlite has a pH range of 7.0 to 7.5. Quartz sand is variable tending to be on the acid side (about pH 6.5). Beach sand is not used as it contains a high proportion of shells containing lime. There are many species which demand a low pH. The Ericaceae, to quote only one example, will not tolerate lime.

Hitchcock and Zimmerman (1926) found that cuttings of Azalea amoena rooted best in very acid media. They give the optimum ranges as pH 3.70 to 4.68. At the other extreme, Carnations, Coleus and Chrysanthemums prefer a lime-rich medium in the pH range 7 to 8 (Parker and Kamp, 1959).

Plants obviously vary in their pH requirements, for Rauch (1972) states that the low pH of a peat moss medium inhibited the rooting of two clones of Euonymus fortunei. Thuja occidentalis rooted better as the pH was raised from 5.1 to 7.1, the optimal, while above this there was a slight decrease in rooting (Bruckel and Johnson, 1970).

Callus formation for many species is inhibited in low pH media (below pH 4) with the exception of calcifuge plants (Mahlstede, 1953). Rooting therefore requires a suitable air/water balance and pH value for each type of plant. The range of tolerance of each factor and their interaction should also be considered. Moreover, the uptake of auxin (IAA) is sensitive to pH being nearly three times faster at pH 4.5 than at pH 7.5 (Steward, 1972).

Successful propagation of juvenile Eucalyptus cuttings has been achieved with various media. Ivashenko (1939) used sand, Giordano (1961) experimented with a medium of sand and agrilit - a very porous substance derived from trachytic rocks; Fazio (1964) used various media which included perlite, vermiculite, sawdust, sand and combinations of these. Gonderman and Martin (1970) used a mixture of peat and perlite, while Paton, Willing, Nichols and Pryor (1970) rooted seedling E. grandis in a sand/peat (2:1) mixture. Paton and Pryor (1971) found that E. deglupta would easily root in water: this is, however, an exceptional case.

Conclusion

It could therefore be stated that overall there is no single best rooting medium, as long as the conditions necessary for root growth are provided.

6. Infection of Cuttings

Mist propagation certainly reduces the incidence of fungal attack compared with the closed case method (Wells, 1955; Snyder & Hess, 1955; Rowe-Dutton, 1959). This is attributed to the continual washing down of the leaves so that airborne spores are washed into the rooting medium. Here soil-inhabiting organisms in unsterilized media destroy most of these spores (Camp, 1956). Movement of air around the cuttings also gives a good measure of control. The high humidity technique of moisture retention thus favours fungal infection, while mist propagation does not. However, diseases can build up in the rooting medium and, due to the high humidity there, they can quickly spread, especially in sterilized media.

Infection

Plant diseases enter cuttings through wounds, stomata or directly through the cut end of the plant tissue. In order to reduce infection from a soil-borne infestation, the rooting medium should be changed regularly (Snyder & Hess, 1955).

Hygiene

Cleanliness is necessary not just on the cutting and rooting media. The whole process from the time of taking the cutting material from the stock plant to the time it is inserted in the medium should be as aseptic as possible. This, then, involves regular daily cleaning of all working surfaces. The propagation house should be cleaned down regularly and the floors and working tops daily sprayed with fungicides (Osborne, 1961).

Prevention of diseases spreading through the rooting medium can be brought about in several ways. Glasshouse sanitation plays a major part in overcoming disease (Wells, 1971). All dead leaves and other debris should be removed daily.

Kubo (1962) and Coate (1969) took the utmost precaution against diseases by disinfecting all tools, bench surfaces and floors daily.

Treatment of Cuttings

Fungicidal treatment of cuttings, particularly those which are difficult to root, is now done as a normal routine. This is usually carried out in combination with auxin treatment. It can be applied either in powder or liquid form. Such treatments give protection to the cutting during the vulnerable stages, to provide better survival conditions. Root quality is improved, indicating that even limited infection adversely affects the endogenous metabolic control of growth.

Snyder (1966) summarises research carried out on the hormone/fungicide treatment of cuttings, and his conclusions are that most species root better when both are present, although in some cases the fungicide alone was sufficient to stimulate rooting. He reiterates the words of many researchers by stating that fungicides should not replace good management. This includes sanitation and cleanliness.

The widespread use of fungicides has, however, led to delays in rooting. In some instances inhibition of rooting occurs, (Hill, 1958).

Application of Fungicides

Fungicides can be applied in several ways. As already stated, sanitation which involves immersing whole cuttings in a bath of fungicide produces a fungal-free cutting at the outset (Hill, 1958; Rowe-Dutton, 1959; Coate, 1969). Various fungicides were used.

As a treatment to the cuttings, base only, Captan is widely used as it has a residual effect. Most papers give reports of improved rooting quality when it is used alone or in association with auxin treatment. Thus, Wells (1963) found healthier rooting although the percentage rooting of Rhododendron cuttings was not affected.

Lanphear (1963) found that soil drenches of a proprietary fungicide on cuttings of Juniperus reduced basal rot by 24-40%, thus improving the percentage rooting.

Working on Pinus taeda, Grigsby (1965) found that Captan stimulated rooting, producing better quality of rooting. He postulated a synergistic effect between the Captan and the auxin (usually IBA) treatments.

Benomyl is one of a new type of fungicides now becoming available. It is systemic in action. It has been used to control a wide range of fungal pathogens in field trials. Tests with cuttings have so far been relatively few.

Monthly drenches of various fungicides, including Benomyl, proved to be excellent in controlling a wide variety of soil-inhabiting organisms (Hoitink, 1968). Raabe (1968) found that by incorporating a mixture of Benomyl and gypsum into the rooting medium excellent results were obtained against root rot on potted Chrysanthemums caused by Pythium Pringsh. and Rhizoctonia DC ex Fr.

Tichnor (1971) used Captan and Benomyl in association with IBA on Pinus contorta and found Captan to be the better fungicide, Benomyl giving a poor rooting response. However, Carville (1971) found Benomyl to be superior to Captan when using it in association with IBA on the rooting of Rhododendron cuttings.

Summary of Reported Infections

Various pathogens have been cited in literature as causing losses during propagation. While no indication is given as to whether these are primary or secondary infections, it would appear that these are the most probable causes of the death of the cuttings. Species of the following genera are involved:-

Rhizoctonia DC ex Fr. appears regularly on propagation benches (Wells, 1955; Davis, 1960; Heiningen, 1960; Weidner, 1960; Osborne, 1961; Reisch, 1963; Raabe, 1968; Hoitink, 1968; Edgington and Snel, 1968; Coate, 1969; Raabe, 1968; Anon, 1970).

Fusarium Link. ex Fr. prevalent in Carnations also appears to be a problem in woody plant material (Davis, 1960; Osborne, 1961; Reisch, 1963; Edgington and Snel, 1968; Anon, 1970).

Verticillium Nees ex Wallr., another of the vascular invading fungi, is also mentioned (Davis, 1960; Osborne, 1961; Reisch, 1963; Raabe, 1968; Upstone, 1973).

Phytophthora de Bary (Davis, 1960; Weidner, 1960; Reisch, 1963; Hoitink, 1968; Edgington and Snel, 1968; Coate, 1969; Lert et al, 1970; Upstone, 1973).

Phomopsis Sacc. (Davis, 1960; Snyder, 1966).

Pythium Pringsh. (Weidner, 1960; Reisch, 1963; Hoitink, 1968; Edgington and Snel, 1968; Raabe, 1968; Lert, 1970).

Cylindrocladium Morgan (Hoitink, 1968; Anon, 1970).

Cochliobolus Drechsler (Edgington and Snel, 1968).

Thielaviopsis Went (Reisch, 1963).

Botrytis Pers. ex Fr. (Raabe, 1968).

Pestalotia de Not. (Anon, 1970).

Botryosphaeria ribis Grossenb & Duggar (Anon, 1970).

Most of these directly rot living tissues of the cutting.

The roles of bacteria and/or viruses as primary infections or as major causes of cutting death have received little attention. Most cuttings die as a result of fungal attack (Davis, 1960).

Conclusion

Any condition which will aid fungal spread should be removed and it is obvious, particularly with cuttings which take months to root, that application of fungicides either to the cuttings or as drenches is desirable.

vi Physiological Factors involved in Propagation

1. Endogenous Plant Regulators

Plant growth regulators have been isolated from plant tissue and have been identified as belonging to two main groups. The hormones, which comprise the auxins, gibberellins and cytokinins, all promote growth. Each one has its own function to perform in the plant although they all interact one with another. In addition to these, there are the inhibitors among which abscisic acid and the gas ethylene have been identified (Wareing and Phillips, 1970).

These growth regulators (except ethylene) are translocated about the plant and are responsible for the control of growth and differentiation of the developing tissues often far from the place of biosynthesis. They are active and present at natural endogenous concentrations of about 10^{-7} to 10^{-6}M.

Auxins are formed in the shoot apex and leaves and move polarly downward in the plant. They tend to build up at the base of the cutting where they are responsible for root primordium initiation. Root growth, however, is not promoted. Auxins activate cambial cells and promote root initiation by differentiation of the meristematic cells. They also promote cell elongation, (Audus, 1959; Steward, 1972).

In Eucalyptus, endogenous auxin initiates cambial activity and stimulates cell extension of the young shoot. However, it retards cell extension in young roots but stimulates the initiation of roots in juvenile cuttings (Jacobs, 1955).

Gibberellins are produced mainly in the growing leaves, but also in fruits and roots, and move both acropetally and in the same basipetal polar fashion as auxins (Jacobs, 1972). They stimulate plant growth especially stem elongation (Phillips, 1971) and this may explain why root initiation is markedly inhibited. Gibberellins stimulate the production and effects of auxins, and together they activate cambial division and cell differentiation.

Cytokinins are synthesized in the roots and transported by the phloem and xylem to the shoots, whereas exogenous application of cytokinin does not move far from the place of application. They promote cell division and the formation of nucleic acids and protein synthesis. They keep the leaves green and healthy by delaying abscission. This is why they have been termed 'maintenance hormones' (van Overbeek, 1966). Cytokinins promote bud formation and cell division (Skoog and Miller, 1957) and are necessary for cell growth and differentiation (van Overbeek, 1966). However, in combination with auxins, and depending on the balance of the two, they control cell growth and lateral root formation (Hume, 1967).

Auxins and cytokinins are both necessary for good rooting of cuttings but this depends on the ratio between the two. High auxin to cytokinin favours the initiation of root primordia, while high cytokinin to auxin produces more buds and fewer roots, (Leopold, 1955; Thimann, 1972).

Temperature also influences this relationship. High temperatures, 27°C, inhibit bud formation and oppose the stimulatory effect of cytokinin on bud formation as well as the suppressing effect

of cytokinin on root formation. Auxins, however, stimulate at this temperature, but less so at 15°C.

Phenolic Growth Stimulants

Phenolic constituents are found to be widespread in the plant. This is a very large group of compounds and the various phenolic constituents affect the growth of higher plants. There is an extensive literature on this field e.g. Ribereau-Gayon (1972). Their role varies very widely from synergistic effects with plant growth hormones to inhibition of growth.

Chlorogenic Acid

Some of them specifically affect auxin metabolism which in turn controls growth and, in particular, adventitious root formation. Chlorogenic, caffeic, ferulic and other such phenolic acids have been shown to inhibit auxin oxidase thus maintaining the endogenous IAA level in the tissue, and maintaining the growth responses to this IAA. Such compounds might therefore stimulate rooting in response to IAA. In fact Osterejko (1969) applied chlorogenic acid to excised cherry embryos at 10^{-5} and 10^{-6}M and found that this stimulated root formation in young seedlings which developed. Hence chlorogenic acid has been tested as a rooting stimulant in this work.

Chlorogenic acid is known to be widely distributed in higher plants. Its effects were first detected in coffee in 1837, Leopold (1955). Recent experimentation on its effects on growth has been carried out mainly with *in vitro* cultures.

Chlorogenic acid is more concentrated in growing than in dormant tissues, in young than in old tissues, and in juvenile than in adult tissues. It is found naturally in the plant in all tissues tested. Its concentration in the plant fluctuates and increases as a result of attacks by plant pathogens or of mechanical injury (Farkes and Kiraly, 1962). Moreover, concentrations of chlorogenic acid in Theobroma cacao decreased in amount during growth and disappeared at maturity (Griffiths, 1958).

Since all plant parts of the juvenile growth phase contained higher total concentrations of these phenols, including chlorogenic acid, than those of the mature growth phase, this could thus be a cause of the known easier rooting capability of the juvenile phase plants.

However, this cannot be the only cause for more successful rooting of juvenile phase cuttings. Hess (1965) using "Wilson's White" variety of Hibiscus rosa-sinensis which proves difficult to root, found that although chlorogenic acid was present, rooting co-factor 4 was absent. This fraction 4 contains mainly oxygenated terpenoid compounds which therefore are also essential in this variety at least. However, it is also conceivable that rooting of adult phases is actively inhibited by some other inhibitor compounds present as well.

Endogenous Rooting Co-factors

Hess in 1959 found that by mixing auxin with an extract from juvenile leaves of ivy it produced a very large increase in rooting, while the same auxin treatment with adult leaf extract caused little or no rooting increase. He therefore came to the conclusion that

there was a rooting co-factor present in the juvenile growths. By 1961 Hess had isolated four co-factors in the easy to root juvenile form of Hedera helix and the equally easy red flowering form of Hibiscus rosa-sinensis.

Identification of the rooting co-factors has so far produced the following information. No active component of rooting co-factor 1 was identified. Co-factor 2 contained chlorogenic acid at 5×10^{-5} to 10^{-4}M. Co-factor 3 has at least three components - chlorogenic, isochlorogenic acids and an unknown promoter P-257. Co-factor 4 belongs to a group of oxygenated terpenoids with high levels of chlorogenic and isochlorogenic acids in all tissues, (Girouard, 1969).

Heuser and Hess (1972) found three lipid root-initiating substances in co-factor 4 in juvenile shoot tissue of Hedera helix. These were isolated and two were identified as mandelonitrile and p-hydroxymandelonitrile (both unstable compounds).

Rooting Inhibitors

Many plants have proved difficult to root from cuttings even at the more accepted times of the year, and with exogenous application of growth promoting substances.

Spiegel (1955) found an inhibitor was present in cuttings difficult to root, and, when treating cuttings, which had hitherto been easy to root, with an extract from the difficult to root cuttings, the former showed a marked reluctance to root. This fact resulted in a series of experiments using easy and difficult to root plants to determine the substances involved.

Fadl and Hartmann (1967) found that buds on the difficult to root Pear variety 'Bartlett' did not produce a rooting co-factor but produced a strong rooting inhibitor.

Lee (1969) isolated four co-factors and an inhibitor from three cultivars of Rhododendron. These were chosen because of their variability in rooting. The inhibitor was present in all cases but the rooting co-factors varied. It is interesting to note that the highest levels of rooting co-factors were found in the easier to root varieties, diminishing to the lowest level in the difficult to root varieties.

Among the endogenous growth inhibitors, ethylene, abscisic acid (ABA) and certain phenols are so far accepted.

Ethylene is found as an endogenously synthesized gas and can both correlate with, and cause when applied, various growth effects. It inhibits elongation of stem and root growth but stimulates the cells to grow transversely (Wareing and Phillips, 1970). Ethylene promotes the emergence of lateral roots where the root primordia are already formed (Mullins, 1972). It also promotes leaf abscission. Auxin transport is inhibited and auxin levels are reduced in the tissue. It is an anti-auxin. However, it is known that high auxin concentration in the cutting stimulates ethylene production (Wareing and Phillips, 1970). When the ethylene to auxin ratio is low, rooting is permitted (Mullins, 1972).

Abscisic Acid

ABA is known to occur in a large number of plants and its prime effect is shoot growth inhibition. It induces the cessation of growth and imposes dormancy on plants growing in long days (Cathey, 1972). It is known to be an inhibitor of seed germination. Germination and root growth of Nemophila insignis are similarly inhibited.

Abscisic acid appears to be transported in stem and petiole tissue at the rate of 20-30 mm. per hour and there is a slight tendency for a basipetal movement (Steward, 1972). ABA is synthesized in leaves and then translocated in the phloem to stem apices. This is consistent with the isolation of ABA from seeds and buds etc.

Steward (1972) in his review of the plant growth hormones states that ABA now begins to appear to be a general inhibitor. This inhibition effect may be due primarily to the inhibition of hydrolytic enzymes which are themselves essential for many of the plants' responses.

Early experiments suggest antagonism between ABA and other growth hormones - particularly GA and cytokinin. Recent evidence quoted by Addicott (1972) suggests that while ABA is still strongly inhibitory on plant growth, there is a biochemical non-competitive interaction with the growth promoting hormones.

Little is known of the effect of ABA in propagation. Inhibition of growth of radicles and root sections is quoted in the review by Addicott and Lyon (1969).

Barlow (1961), Coyama (1962) and Vieitez (1966) support the view that all cuttings have the ability to root but when they do not, this ability is prevented by the presence of endogenous inhibitors (Vieitez, 1968).

Barlow (1961) isolated a rooting inhibitor from plum cuttings and this was identified by Milborrow (1967) as abscisin II. This has subsequently been called abscisic acid.

However, conflicting evidence is available from Ting-Yun Chin et al (1969) who claim that ABA stimulates rooting of stem cuttings of Phaseolus aureus (Mung bean) and Hedera helix var. baltica. These are both plants which root very easily in normal circumstances. Nevertheless, concentrations of ABA up to 25 ug/ml were significantly better when used on Mung bean than the water control.

In this thesis the working hypothesis is tested that ABA, or some similarly acting endogenous substance, is responsible for the lack of rooting in Eucalyptus.

Phenolic Inhibitors

It is at present generally agreed that the phenolic inhibitors fall into three main chemical groups - phenolic acids, lactones and flavonoids - and many of the substances contained in these general groups inhibit coleoptile and stem growth, and seed germination.

Inhibitors in Eucalyptus

Paton and others (1970) found that of all the species of Eucalyptus only E. deglupta was easily rooted from cuttings. This then provided a useful test for extracts of other Eucalyptus species whose cuttings do not root. By this means an inhibitor was found and isolated at an Rf value of 0.9 from an extract of Eucalyptus grandis.

During the course of this work, Nichols, Crow and Paton (1972) have further fractionated this inhibitor into three compounds - G1, G2 and G3 - and although these have not been identified, G3 closely resembles leptospermone.

Conclusion

Endogenous inhibitors may prevent rooting of E. gunnii cuttings older than fifteen nodes.

2. Exogenous Growth Substances

Since auxins were first discovered, their primary role in root formation has been the subject of much experimentation. The review by Thimann and Behnke-Rogers in 1950 lists 291 papers and covers 1,240 plant references.

Audus (1959) covers the whole problem of the rooting of cuttings. He gives the main responses of suitably applied concentrations of auxins as a quicker rate of initiation of root primordia and hence quicker rooting of cuttings. Van Overbeek (1966) states that auxins are required for cell elongation as well as cell proliferation. They promote root formation in cuttings but not root growth.

Steward (1972) states that when high concentrations of auxins are applied to cuttings, rooting may not necessarily be limited to the base of the cutting.

Auxins will produce a response only if the other factors in the cutting are correct. In some plants a response is obtained, while in others it is not. In the case of Hevea, Coffea and Mangifera, responses were obtained only from cuttings taken from young trees (Skoog, 1951). (Leafy cuttings were necessary to produce the endogenous auxin effect.)

Leopold (1955) states that the plant response is controlled not only by auxins but by the addition of adenine and the correct auxin/adenine ratio is necessary for rooting. Through the work of van Overbeek, Gordon and Gregory (1946) the relationship between auxin and the endogenous substances produced in the leaves, was also found important. These effects could be produced by the application of sugars and nitrogenous substances, together with auxins, to the cutting.

Auxins are therefore a major factor in a complex of other factors such as environment, nutrition, age of the plant etc. etc., and all must be effective in order to promote root initiation.

Application of Growth Regulators

Various methods of application have been used for research purposes.

Powder preparations, using talc as the carrier, are available as proprietary preparations and are easy to apply. The cuttings are simply dipped into the talc after they have been excised and before being pushed into the rooting medium. Moisture exuded by the cut end allows sufficient growth substance in the talc to adhere to the cutting. Frequently fungicides are also added to increase survival of cuttings which may then root.

Liquid preparations provide a more workable approach from the research point of view. Solutions can be easily formulated and give a range of controlled concentrations. The quick dip method using high concentrations is generally used, for as the cuttings are only in the solution for less than one minute there is no delay in inserting them into the rooting medium. The prolonged overnight soak is now less common, for the cuttings must be placed in a weak solution for up to 24 hours, and this is both time- and space-consuming.

Distilled water and/or alcohol are the main solvents used, although more recently DMSO*has been used with some good results.

Terminal dips have been used by a few researchers and this simply involves dipping the growing point and leaves in the solution. It is normally a quick dip treatment (McGuire, 1966).

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*DMSO is the abbreviation for dimethylsulphoxide.

Overhead sprays are a development of this and are much easier and less time-consuming than other methods. The in situ cuttings are simply sprayed over with the growth regulator solution (Whatley. 1966).

No matter which means of application is used, the growth substances do not lose their efficiency when used under mist and do not appear to be leached out (Rowe-Dutton, 1959).

It is obvious that growth regulator application is empirical and is judged by its success in rooting and ease of large scale application. The relationship between externally applied concentration and resulting internal, still less intracellular, concentration is almost always neither known nor even considered. The rates and proportions of regulator uptake may be crucial to understanding control of rooting where endogenous inhibitors are not mainly controlling rooting.

3. Effects of Exogenous Application of Growth Substances

(a) Auxins

Long before Went in 1934 discovered the enhanced rooting effect of applied auxins, Dutch workers achieved increased rooting by embedding wheat seeds into cuttings. Seeds produce auxins and also gibberellic acid, therefore the Dutch were using this same treatment although they did not realise it.

Auxins have been used continually since 1934 as an aid to rooting, particularly with the plants which prove more difficult to root. For example, Thimann and Delisle (1939) give details of the convincing effects of auxins on cuttings of Tsuga canadensis with 63% rooting against no rooting at all without auxin treatment.

Audus (1959) gives responses of cuttings to auxin treatment. IAA, which is known to be present naturally in the plant, was used frequently to promote rooting where no rooting occurred under optimal conditions. Excellent rooting was achieved with IAA when used singly on Aleurites fordii, Chodanthus splendens, Erythrina corallodendron, Hibiscus syriacus, Impatiens, Ixora incarnata, while only slight or no stimulation was achieved when used on Aleurites moluccana, Crataegus, Fraxinus americana and Pittosporum.

It is not uncommon that IAA will not promote rooting, and other synthetic auxins have proved to be more efficient in aiding rooting. IBA and NAA have both been found to be more active than IAA in inducing rooting. IAA is destroyed enzymically within plants, and also by strong sunlight and by a widely distributed species of Azotobacter. IBA is more light-stable and is not affected by Azotobacter. NAA and 2-4D are entirely light-stable and are not destroyed enzymically, and therefore maintain their effectiveness over a longer period (Hartmann and Kester, 1968).

Phenoxy acids have been found to be highly active in Rhododendron species where IAA, IBA and NAA had little effect on rooting (Audus, 1959). Phenoxy acids have a disadvantage in rooting because they produce short, thick roots which prove brittle when handled. 2-4D when used at very low concentrations (at high levels it is a herbicide) aids rooting but impairs shoot growth. Of all the auxins mentioned, IBA and NAA are recommended for their highly active effect in rooting and their general lack of side effects. They are used extensively in commerce.

In cases where rooting fails to occur with one auxin on its own, two auxins can be used simultaneously. IBA and NAA have proved to be very effective in increasing rooting in many plants.

Lavender and Zaerr (1967) report that Pseudotsuga douglasii is particularly unresponsive to various growth substances. Erratic response is achieved with auxin treatment. IBA alone or in combination with NAA did, however, induce a small percentage rooting of cuttings. When used on hardwood cuttings of gooseberry, IBA and NAA with or without 2, 4, 5-T.P. gave the best results in comparison with single auxin treatment and various other combinations (Whalley, 1969).

Audus (1959) states that not all cuttings respond to auxin treatment. In fact, many of the "shy" rooting plants remain unresponsive towards any auxin treatment.

Auxins have failed to stimulate rooting in several plants. Thimann and Behnke-Rogers (1950) review the use of auxins in the rooting of woody cuttings and many genera regularly failed to root. These include:-

Acacia
Atraphaxis
Chimonanthus
Cornus macrophylla
" nuttallii
" racemosa
Cotoneaster serotina
Cupressus arizonica glauca
Cyrilla racemiflora
Cytissus batandieri
Elaeagnus angustifolia
Emmenopterys henryi
Eucalyptus
Eucryphia glutinosa
Fabiana imbricata
Feijoa
Fothergilla monticola
Gordonia
Grevillea
Hamamelis
Hoheria
Indigofera
Leptospermum
Liriodendron
Magnolia
Meliosma
Nothofagus
Nyssa
Pomaderris
Purshia
Quercus
Sinowilsonia

Kochba and Spiegel-Roy (1972) have been working on clonal propagation of Prunus amygdalis (Almond) with no success at all. This genus is naturally cross-pollinated, therefore standard clones from trees of proven high quality cannot be produced. This is a problem similar to that of Eucalyptus - the theme of this thesis. In trying to estimate the effects of plant growth substances on Almond seedlings, applications of auxins (IAA, IBA and NAA) were carried out by wick feeding to intact shoots. Rooting occurred along the stem. It was only possible to remove one cutting from each seedling. However, such propagation could give rise to an estimated 16 genetically identical plants in a year. It is interesting to note that IBA and NAA caused root formation, with IBA having the better effect: IAA had no effect at all. It is presumed that there was a breakdown of IAA in the plant tissues. So far, detached cuttings have shown no response to rooting.

Endogenous inhibitors are known to occur in many plants. Abscisic acid or similar compound has been identified as present in some cases. Seasonal responses have been proved by several authors to be the result of a high ratio of unknown rooting inhibitor to auxin. It is also suggested that the same effect is produced in Eucalyptus where adult shoot cuttings have so far failed completely to root. There is a similar effect with juvenile and adult Hedera helix and here rooting inhibitors have been found.

Auxins have been applied to Eucalyptus. Fazio (1964) applied NAA and IBA in 45% ethyl alcohol to lignotuber cuttings of Eucalyptus rostrata and E. polyanthemos with a maximum percentage take of 65%

but we do not know of the number of cuttings treated. Gonderman and Martin (1970) found that IBA treatment on juvenile cuttings of Eucalyptus ficifolia produced much better results than E. gunnii, where very low rooting percentages were recorded.

Conclusion

While exogenous auxins are helpful, and sometimes essential, in aiding rooting of cuttings, there are obviously other factors involved. The ratio of auxin to endogenous inhibitors is important as are other physiological factors. It is therefore necessary to have the factors, both internal and external, in the correct balance for plants, including Eucalyptus, which prove extremely difficult to root.

(b) Gibberellic Acid

Roots of mature plants do not show any growth response to gibberellic acid at low application rates, although there is a slight tendency to retard root growth at high concentrations (Steward, 1972). The main effects of gibberellic acid are to increase shoot growth, break dormancy, enhance germination, hasten senescence and flowering.

Wareing and Phillips (1970) and Phillips (1971) state that due to the application of gibberellic acid, the endogenous auxin level is increased. Examples of the work of Kuraishi and Muir (1964), where auxin levels in Hyoscyamus plants were increased by 43 times those of control plants after treatment with GA_3, and Nitsch (1963), who found auxin levels in bean seedlings to be 200 times greater than the control after a similar gibberellic acid treatment, are given by Steward (1972).

Two hypotheses are presently advanced to explain this -

(1) that gibberellic acid inhibits the activity of auxin oxidase, and/or

(2) that gibberellic acid itself stimulates the formation and/or effects of auxins (e.g. Steward, 1972).

Despite the enhanced auxin levels in the plant, gibberellic acid not only inhibits the root initiation in cuttings (Phillips, 1971) but counteracts the stimulation of rooting as a result of auxin treatment (Stowe and Yamaki, 1959). There appears to be some auxin/gibberellic interaction. For example, working on easily rooted Forsythia and Ligustrum, Fleming (1966) found that gibberellic acid on its own delayed rooting although, when IBA was added, maximal growth, similar to that in the control, was achieved. He concludes that in the cases of Forsythia 'Spring Glory' and Ligustrum ovalifolium there are no beneficial rooting responses as a result of gibberellic acid treatment.

Conclusion

Increased shoot growth due to gibberellic acid application could therefore be a reason why root initiation is sometimes inhibited.

(c) Kinetin

Kinetin plays a very important role in plant propagation. While this area of experimentation has been little reported, its effects on cell and callus growth regulation in in vitro culture has been studied extensively. So it is under these conditions of growth that we can understand the influence of kinetin in propagation.

Kinetin is a derivative of adenine which at concentrations up to 50 mg/litre when added to callus tissue, produces a striking formation of shoot buds. These buds may develop several leaves but unless root formation occurs there is no further development or growth. Combined treatments with auxins (which promote root initiation) produce increased cell division, but the results vary with the changing ratio of auxin to kinetin. High auxin concentrations promote root formation and prevent the formation of buds, while a high kinetin ratio promotes bud formation and decreases the formation of roots (Skoog and Tsui, 1951; Skoog and Miller, 1957).

Bachelard and Stowe (1963) found that isolated root tips of Eucalyptus camaldulensis grown in Bonner's culture medium required kinetin (present in coconut milk) to promote growth. Aneja and Atal (1969), using Murashige's culture, supplemented with sucrose, vitamins, auxins and coconut water, produced callus tissue with Eucalyptus citriodora. Subsequent subculturing of each bud-like segment of callus tissue in the basic culture with NAA transformed this into a green aerial bud which later developed into a plantlet.

It must therefore be obvious at this stage that kinetin is necessary in the complex growth requirements of cuttings, first of all to assist in the differentiation of cell tissue to form callus and then later in diminished concentration to allow auxins to initiate rooting.

Conclusion

We therefore postulate that a high kinetin/low auxin ratio is necessary for callus tissue to be produced, and a low kinetin/high auxin ratio for root initiation to take place.

(d) Phenolic Compounds

Exogenous application of some phenols, including chlorogenic and caffeic acids, promote the elongation of isolated Avena coleoptile sections. This suggests that these phenols might interact synergistically with exogenously supplied IAA by the inhibition of IAA oxidase (Sondheimer, 1964). Several workers have confirmed this hypothesis. For example, Gorter (1969) found that both chlorogenic acid and caffeic acid, at concentrations of 10^{-3}M, were active inhibitors of IAA oxidase (at phenolic concentrations of the magnitude found naturally). Moreover, in association with tryptophane, a precursor for IAA biosynthesis, both chlorogenic and caffeic acid at 10^{-3}M stimulated rooting of cuttings of Phaseolus vulgaris and produced the greatest number of roots when compared with four other similar phenols tested.

Girouard (1969) has verified the synergistic effect of chlorogenic acid and related compounds (catechol and pyrogallol) with IAA on the rooting of Mung bean cuttings.

Conclusion

It is known that under laboratory conditions chlorogenic acid as well as other phenolic compounds stimulate rooting of Phaseolus vulgaris and Phaseolus mungo. We know that the effect of plant extracts containing chlorogenic acid with additional auxin is to increase the rooting rate. It is therefore conceivable that such a treatment on Eucalyptus gunnii would enhance the rooting ability of cuttings.

(e) Nitrogenous Substances

Nitrogenous substances have been found to be necessary for the successful rooting of some species. Inorganic nitrogen has been applied as an overhead spray with the normal misting technique. Commercially it has been used as a "nutrient" mist on cuttings which were left *in situ* in the rooting medium long after rooting had taken place, and was invaluable for providing nutrients for the developing plants. The drawback in this method of application is the encouragement of mosses and algal growth on the surface of the medium.

With the early removal of rooting cuttings from the propagation bench, nutrient mist is not necessary, but applications of nitrogenous compounds to the cutting prior to insertion in the rooting medium are considered feasible.

Casein hydrolysate is used as a convenient standard organic nitrogen source. It is a preformed mixture of protein amino acids ready for immediate metabolic utilization. All the published experiments using casein hydrolysate have been carried out by means of *in vitro* tissue, organ and cell cultures.

Skoog and Miller (1957) clearly demonstrated the need for casein hydrolysate in culture solutions containing tobacco callus tissue. Casein promoted growth but only in the presence of IAA and kinetin. They state: "In the presence of high auxin concentrations, root development was favoured; the inhibiting action of IAA on root elongation was counteracted. In the presence of high levels of kinetin, bud development was encouraged and again the inhibiting action of IAA was counteracted: a buffering effect, in essence."

Since then, casein hydrolysate has been included in most of the organic tissue and cell cultures requiring root and bud development and has involved many plant species from a variety of families.

The ultimate development of this kind of work was by Steward et al (1964) who produced flowering carrot plants from a suspension of free, single carrot phloem cells by the manipulation of auxin and kinetin levels in a modified White's basal medium supplemented with casein hydrolysate and coconut milk.

Conclusion

Since casein

(a) has promoted root formation and subsequent root growth, and was almost essential in cell cultures;

(b) is a preformed mixture of the protein amino acids which are necessary for growth, and aids the supply if endogenous amino acids are limited in quantity;

(c) has some buffering effect on the action of growth hormones, extending their range of positive growth effects;

its potential value in the rooting of cuttings cannot be overlooked; this was therefore tested in Eucalyptus.

(f) Vitamins

Vitamins of the B group, such as nicotinic acid, pyridoxin and thiamin, are co-factors of enzymes. Thiamin is synthesized in the leaves and is required for root metabolism and growth. Its value in rooting is therefore important and its use justified.

Vitamin B_1 is known to be essential for root growth and has proved valuable in propagation in some cases.

According to Thimann and Behnke-Rogers (1950), Vitamin B_1 has proved necessary or has increased the rooting percentage of the

following plants when auxins were also used. Frequently the Vitamin B_1 treatment was given one week after the auxin treatment.

Chamaecyparis lawsoniana 'Allumii'

Chamaelaucium ciliatum

Citrus limonia var. Eureka

" medica var. Meyer's Lemon

" sinensis

" hybrid Limequats

Picea omorika

Pinus caribaea

" palustris

Sciadopitys verticillata

Thea japonica 'Pink Perfection'

" " 'Red Camellia'

" sinensis

" " var. macrophylla

It had no effect, or else a deleterious effect, on

Cinchona ledgeriana

Olea europaea

Vitamin B_6 proved beneficial to a number of plants and again auxin must also be present. The following plants responded to this treatment:-

Aristolochia sp.

Buxus sempervirens 'Hardsworthii'

Citrus sinensis

Cyrilla racemiflora

Deutzia sp.

Lonicera nitida

Nathusia sp.

Phillyrea angustifolia

Pyracantha coccinea 'Lalandii'

Symplocos paniculata

It was absolutely necessary with cuttings of *Chionanthus retusa* as only those cuttings treated with Vitamin B_6, in addition to auxin, rooted. However, *Tripterygium* sp. failed to respond to Vitamin B_6 treatment. *Cinchona*, classified by Audus (1959) as a non-rooter, did not respond to Vitamin treatment, but with auxin gave excellent rooting. *Olea* species, which are non-rooters without auxin, produced fair rooting with auxin treatment, but a decrease in rooting with Vitamin B_1 (Hartmann, 1946). *Lonicera hildebrandiana* is notoriously difficult to root by any treatment.

Vitamin B is necessary for growth of detached roots grown in *in vitro* culture. It is synthesized in the leaves and is transported to the roots where it is necessary for root development and could be termed a hormone (Phillips, 1971). In most cases sufficient supplies are produced by the plant and although some plants have responded to Vitamin B treatment, it is not a normal treatment given to aid cuttings to root.

Vitamin C, as ascorbic acid, is used mainly in tissue culture but some attempts have been made to utilize the plants' responses to applied Vitamin C. Thimann and Behnke-Roger (1950) give details of their usage in propagation. *Ligustrum ovalifolium* var. *aureo-marginata* and *Salix* sp. showed increased rooting with Vitamin C added to the auxin treatment but no rooting at all when used on *Cinchona ledgeriana*. Hubert, Rappaport and Beke (1939) claim that Vitamin C increased the rooting rate of *Eurya japonica* cuttings, while

other reports claim no advantage is gained by using it as a normal treatment of cuttings.

Conclusion

Successful rooting was achieved with the addition of Vitamins B or C to the auxin treatment. Absence of effect does not mean that it is not involved but that it is not present. Its effect may be limited by the presence of endogenous inhibitors or the presence or absence of some necessary factor other than Vitamins. The value of Vitamins B and C in the propagation of Eucalyptus gunnii was therefore tested.

(g) Sugars

Sucrose in culture media exerts a strong influence with auxin in cell differentiation. In high concentrations (4%), differentiation of the callus produces phloem cells whereas at low sucrose levels xylem tissue is formed (Black and Edelman, 1971). It has proved to be successful in increasing the rooting rate in:-

Abies koreana
Allamanda cathartica
Elaeagnus pungens 'Reflexa' (100%)
Gardenia florida (17%)
Lonicera japonica
Picea abies
Pinus caribaea
" palustris
" strobus
Robinia pseudoacacia 'Rectissima' (Thimann and Behnke-Rogers, 1950)

Overbeek et al (1946) found that sucrose greatly increased the rooting of the red-flowered form of Hibiscus rosa-sinensis provided it was also treated with auxin. However, cuttings of Pinus strobus rooted

equally well with a treatment of 1.5% sucrose without IBA and gave 50% rooting. IBA alone at 2000 ppm also gave 50% rooting (Deuber, 1942). Dora et al (1941) found the same species would not root at all with auxin, but with 2.5% sucrose treatment 20% rooting was achieved.

Thomas and Riker (1950) found that auxin with added sugar reduced the rooting rate in Pinus sabiniana.

However, conflicting reports about the value of sugars added to cuttings again may make this a question of specificity. Komissarov (1969), reviewing treatments, states that "in most instances sucrose, fructose and glucose in a pure state have little influence on the rooting of cuttings". It would appear that sufficient carbohydrates were present or were being synthesized by the cutting so that artificial application of sugars was not benefiting the rooting percentage.

Fazio (1964) found that sugar in the form of honey diluted in water caused decay when used on Eucalyptus cuttings. This treatment was then discontinued.

Tissue cultures invariably contain sucrose in concentrations from 0.5 to 8% as a means of sustaining food reserves. However, sucrose in various concentrations failed to stimulate any response from roots of Eucalyptus camaldulensis (Bachelard and Stowe, 1963).

Aneja and Atal (1969) found that Murashige's inorganic salts medium with sugar and other additives, was necessary for callus initiation in tissue of Eucalyptus citriodora.

Conclusion

Sugars are a source of reduced carbon and energy. Lack of rooting in cuttings may be due to their absence in the plant. However, since sugars have a beneficial effect on the rooting of many plants, their effect on Eucalyptus gunnii is worth testing.

(2) Exogenous Growth Retardants

While growth stimulating substances are known to improve rooting in a wide range of plants, recent research has found that by combining a rooting hormone with a shoot growth retardant a further stimulation of rooting occurs.

Growth retardants such as AM01618, Phosfon, Cycocel and B9 are used to restrict shoot growth. Cycocel and B9 have a very wide plant response range. Phosfon does retard growth but side effects, such as vein clearing, make this substance of little use. AM01618 is effective on only a limited range of plants, and again is of little value.

In propagation, when B9 was applied alone at concentrations of 1000, 2500 and 5000 p.p.m. to Geranium, Dahlia and Chrysanthemum cuttings, they produced significantly greater weight and number of adventitious roots than untreated cuttings. Similar treatments with Cycocel caused a marked depression of adventitious root production. As the rate of application increased, so adventitious root production diminished. This suggested to the author that Cycocel was an anti-auxin (Read and Hoysler, 1969).

However, Reid and Crozier (1970) found that Cycocel induced an increase in gibberellin levels in pea seedlings when applied at concentrations of 1 mg/litre. This rate produced a 150-fold increase in endogenous gibberellin levels, while 1000 mg/litre did not appear to provide any significant difference in gibberellin levels from untreated controls.

Doede (1969) found that cuttings of Taxus cuspidata, taken during the four month trial period December to March, were stimulated to root with B9 and Cycocel but only when used in combination with IBA. Cuttings of Juniperus chinensis 'Pfitzeriana', however, only responded to B9 in combination with IBA and then only in December.

B9 improved rooting of Geranium and Dahlia more rapidly than IBA when such treatments were compared with untreated controls. Cycocel was found to retard rooting, and B9, when applied with Cycocel, appeared to reduce the root retarding effect of Cycocel. The suggestion put forward by the author, Reid (1968) was that the root stimulation by B9 was enhancing or replacing the auxin behaviour.

Conclusion

B9 is known to be an anti-gibberellin with some auxin-like effects which promotes rooting. Gibberellin itself antagonises rooting. It could therefore be possible that the restriction of shoot growth diverts some or all of the nutrients from shoot growth to the developing and active root initials. This was therefore tested using B9, Cycocel, Phosphon and GA at appropriate concentrations.

vii Summary of Investigations Undertaken

The foregoing discussion led me to conclude that the following ideas and factors were worthy of, and amenable to, testing. The investigations fall into three parts:

To find (a) the cultural and (b) the environmental conditions most conducive to rooting. These are not well known for Eucalyptus and have therefore first to be investigated with the main aim of preserving cuttings in a viable state for as long as possible, to allow the maximal time for any slow rooting to occur, and

(c) the physiological factors involved in rooting.

Since rooting was so rare, health and survival of cuttings were recorded.

1. Investigations on the Environmental Conditions for Rooting of Cuttings

The following conclusions reached in the introduction were tested by measurements of relative water content, survival and health of cuttings, and degree of pathogenic infection:

(a) That Water is the most important single factor. It must be present in sufficient amounts to keep the cuttings turgid. A high humidity around the cutting and a moist rooting medium are therefore necessary for rooting. However, water content must not be so high as to promote pathogenic infections or anaerobiosis. Different levels and methods of substrate watering and humidifying were therefore also investigated.

(b) Light

Intensity For sunloving plants such as Eucalyptus, better rooting of cuttings should be achieved with the higher light intensity. This, however, is related to (3) temperature. The effects of full daylight and darkness on survival and rooting of cuttings were therefore tested.

Photoperiods Since vegetative growth in Eucalyptus occurs in long photoperiods, rooting of Eucalyptus cuttings may also be achieved. Therefore the effects of three different daylengths were tested.

Wavelength Apart from normal daylight, red or far red light may, through phytochrome-mediated effects, aid rooting. However, effects on Eucalyptus are not known. I have therefore compared the effects of red light and daylight.

(c) Temperature Two main air temperature regimes are popularly employed - 18-21°C and 24-25°C. With plants which prove difficult to root from cuttings, substrate heating is often an advantage. Substrate should then be 2-3°C warmer than the air. The effects of both air temperature regimes with warmed substrate were investigated.

(d) Season In Eucalyptus, juvenile and lignotuber cuttings have commonly been taken during the growing season but are particularly successful in the springtime when the plant grows more actively. Endogenous nutrient and auxin supplies would then be available and, providing the developing root primordia receive sufficient supplies, rooting should take place. Cutting viability and rooting were therefore tested on cuttings taken in spring, late summer and winter. Midsummer soft cuttings have very low viability, do not even transport successfully and were discarded.

2. Cultural Conditions Requiring Investigation

(a) Rooting Medium No overall best rooting medium is known. Media which provide favourable rooting conditions are therefore required. They may vary with the local situation. Sand with peat is a generally accepted commercial rooting medium although perlite with peat gives better control over variability in the medium. Sands can vary widely in pH and in particle size and hence in aeration of the medium. Both mixtures were tested on Eucalyptus.

(b) Fungicidal Treatment Sanitation is regarded as essential throughout the complete rooting process since fungal attack accompanies the death of cuttings. Fungicidal treatment of the cuttings as well as repeated drenches of the rooting medium are common means of further controlling pathogens. The presence, abundance and identity of fungi were therefore investigated in fresh cuttings and in those taken from the culture bench, both alive and dead. The effects of various fungicidal treatments were similarly investigated.

3. Physiological Factors affecting rooting of Eucalyptus Cuttings

The literature reviewed above indicated that the following hypotheses were particularly worth testing. These investigations form the scientific core of my thesis. The basic hypothesis is that growth substances, especially endogenous inhibitors, are the prime factors controlling rooting in Eucalyptus. By contrast, success in rooting of cuttings of easily rooted species varies primarily with cultural conditions.

My investigations tested the following hypotheses:

(a) Exogenous application of Growth Promoting Substances

1. That exogenous applications of auxins are necessary for the successful rooting of cuttings of Eucalyptus gunnii.

2. That exogenous gibberellic acid prevents root initiation (which may be due to the competition for endogenous nutrients when shoot growth is favoured).

3. That high exogenous cytokinin levels favour callus formation while low levels are necessary for root initiation.

4. That casein hydrolysate, a source of organic nitrogen, has a root-promoting effect, and that if so, nutrient limitation may inhibit rooting.

5. That Vitamin B, having proved effective in some of the more difficult to root cuttings, is beneficial for the rooting of Eucalyptus cuttings, in which, therefore, endogenous coenzyme levels may become low.

6. That since Vitamin C increases the rooting rate of some plants, it is worth testing for its effect on Eucalyptus. Success may indicate either an endogenous deficiency e.g. following excision of cuttings, or that rooting inhibitors form by oxidation of endogenous phenolic or other reductants at lowered levels of ascorbic acid.

7. That sugars which promote rooting in some species may also do so in Eucalyptus if endogenous carbohydrate levels are limiting. Comparison of results with added sugars and added casein (5) tests whether the C:N ratio affects rooting.

8. That exogenous chlorogenic acid, an endogenous root-promoting compound which controls internal auxin levels, is an aid, in high concentrations, to rooting of Eucalyptus cuttings.

(b) Exogenous application of Growth Retardants

1. That root growth is adversely affected by shoot growth due to the competition for endogenous nutrients and growth-promoting substances. With the application of exogenous shoot growth retardants, this balance is more favourable to rooting and is therefore worth testing.

(c) Endogenous Growth Substances

1. That Eucalyptus oil is partially responsible for the inhibition of root initiation and root elongation, and further, that the more volatile aromatic oils cause this inhibition.

2. That the presence of endogenous inhibitory growth substances cause the lack of rooting.

3. That abscisic acid is the main natural inhibitor preventing root initiation.

4. That endogenous growth-promoting substances are lacking and thus responsible for the lack of rooting.

5. That endogenous inhibitors other than abscisic acid or oils cause the lack of rooting in Eucalyptus gunnii.

(d) Access of Endogenous Inhibitors to Sites of Root Initiation

1. That easily rooted species containing endogenous rooting inhibitors effectively compartmentalize rooting inhibitors away from potential sites of root initiation, while species difficult or impossible to root allow access of the inhibitors to sites of root initiation.

CHAPTER II

MATERIALS AND METHODS

Materials

From a single plantation in Kent, terminal juvenile stem cuttings from the current year's new growth were obtained from 8 year old trees of Eucalyptus gunnii 'Brightlingsea Strain'. The trees were regularly cut back to induce re-growth of new shoots. This constant cutting back retains the juvenility of the whole plant. Since large numbers of cuttings were used in the experiments, it was impossible to cut them from one tree so cuttings were gathered from trees throughout the whole plantation.

The plants had all been grown from seed taken simultaneously from one tree in Brightlingsea, Essex, and received similar cultural treatment throughout (Waldron, 1968). Any differences occurring in the cuttings would therefore be almost entirely genetic and not principally due to culture, soil conditions, or age of tree.

The plantation is sheltered on all sides by woodland or rows of large forest trees, and the whole area of about 2 acres is grassed down.

The cuttings were carefully placed in a polythene-lined box to keep them fresh during transit (2 days by train, in darkness), and on arrival they were immediately placed in subdued lighting with their bases in water for several hours to revive them fully. The cuttings used in all the experiments were of a standard size containing three pairs of leaves each and were cut just below the node. Various treatments were used and these are detailed under separate headings.

ii Growing Conditions

1. Glasshouse

The experiments were carried out in a small glasshouse 55' x 12'. A partition in the centre of the house enabled different temperature minima to be used: these were $10^{o}C \pm 2^{o}$ and $21^{o}C \pm 2^{o}$. Temperature control was by a rod type thermostat. Higher temperatures, due to solar heat, were controlled by hand operated ventilation.

2. Benches

The rooting benches were of two types. On one side of the house was a standard aluminium propagating bench 4'1" wide and 3'3" high. It was 6" deep and extended the whole length of each section of the glasshouse. The depth of the rooting medium was $2\frac{1}{2}$" in this particular bench system.

The other bench was of deep concrete trough construction and was so designed that various depths of compost could be used. This bench was 3'3" wide and 2'10" high and also ran the length of each section.

3. Soil Warming

Both types of bench had BICC earth screened P2310 1000w 240v electric soil warming cables c onnected to the mains voltage and had a loading of $162w/m^2$. The bottom of the bench was covered with $\frac{1}{4}$" grit to allow proper drainage, and the wires, which were laid out 3" apart across the bench, were placed on a finer grit of $\frac{1}{8}$" particle size. This same grit covered the cable with a 2" layer and above this the rooting medium was placed to a depth of 2-3". A 24" Satchwell rod type thermostat was connected to the heating cables and was positioned $1\frac{1}{2}$" above the cables. This thermostat controlled the temperature at the base of the cuttings to a predetermined $24^{o}C$.

4. Mist

Intermittent mist units were positioned 3 feet apart and were situated in the centre of the bench thus providing a mist spray over the complete area. The mist equipment used was the MacPenny type with "artificial leaf" control. The mist spray was produced by a water pressure of 55-60 lbs. per square inch striking the anvil-type atomizing nozzles giving uniformity of mist over the cutting surface. When the surface electrodes of the "artificial leaf" were dry, an impulse through the control box activated the solenoid valve; this sensor was sited in the cutting bed among the cuttings which were thus kept superficially moist. By altering the position of the sensor in relation to the atomizers the humidity could be varied.

5. Water

The water used in the intermittent mist treatments was normal domestic supply water from a local reservoir. A chemical analysis of this water showed the following chemicals to be present:-

pH	7.0
Ca^{++}	40 ppm
Mg^{++}	12.5 ppm
SO_4^{--}	130 "
Cl^-	20 "
K^+	0.4 "
Na^+	3.0 "

6. Rooting Medium

In the early stages of experimentation, the medium used for rooting was a standard mixture of peat and sand in the ratio 1:3 and was thoroughly moistened before the cuttings were inserted. Later, the mixture was modified to a perlite and peat mixture of equal quantities.

The cuttings were simply pushed into the medium to a depth of ½". This was sufficient to keep them erect.

iii Design and Replication

For each experimental treatment, the numbers and spatial disposition of the cuttings were planned to maximize the statistically reliable output of information on the effects of each treatment and to minimize residual variation arising from other causes. Thus the cutting beds were of large area, of uniform composition, moisture, aeration and temperature; while lighting, humidity control and temperature for the aerial parts of the cuttings were almost completely uniform.

Forty-five to ninety replicate cuttings were used for each treatment, bearing in mind the low proportions expected to root, compromised against cost and amount of work involved. These samples of replicates were each subdivided into three subsamples which were planted out among and between the subsamples for other treatments, to minimize and to permit allowances to be made for, systematic variations in the cultural micro-environment. In early experiments (M2 and M3 series) a Latin Square layout was adopted which assumed some uniformity of the micro-environment. For M2 and M3, three subsamples of fifteen or thirty replicate cuttings per treatment were so laid out. In all later experiments, nine smaller subsamples of five or ten replicate cuttings were planted out in nine randomly selected sites (actually rows) scattered over the cutting bed; these nine sites for each treatment were selected using a table of random numbers. Thus the cuttings were laid out in 3 parallel bands along the bench, each such set of three rows containing 3 subgroups each of five or ten cuttings in two or three parallel rows.

iv Tests of Treatments on Rooting and Survival of Eucalyptus Cuttings

In the tests, 90 replicate cuttings were initially planted for each treatment and control set of replicates. Cuttings were taken at random from the consignment (i.e. population) for all treatments. To allow proper statistical analysis of results of treatments, cuttings were placed randomly in the bands as described above under Methods (p. 72).

The mortality rate in the first month varied, but was generally high, numbers remaining alive varying from 68 to 26 to 5 etc. I have presumed that the causes of the initial mortality rate will include not only any treatment effects, but also weakness in those cuttings which upon receipt were initially or previously damaged, infected, physiologically weak, etc. To avoid confusing these two classes of cause of death, I have ignored the first month's data on the assumption that the early death arising randomly (χ^2; $p = < 0.80, > 0.50$ for 11 d.f.) from causes other than the treatments given to the samples would have completed most or all of their effects. This should minimize such non-treatment effects. By analysing the data only for subsequent months (from 2nd to 7th), I have tried to maximize any non-random, systematic treatment effects. Hence as a basis, the number of cuttings alive at the end of month 1 (the "starting number" or "starting frequency") was used as the reference level for all statistical analyses.

Rooting was randomly distributed among treatments. Since rooting in all cases was very poor (0.40%), survival was taken as a measure of treatment effect. This is based on the established correlation between treatments promoting survival and promoting rooting (χ^2; $p = < 0.10, > 0.05$).

The question of how long to continue observations and analysis of survival and rooting of cuttings led to a compromise based on the following considerations. Observations were continued until all cuttings set in each experiment had finally rooted or died. Statistical analysis of the results was, however, restricted to data for that period after the first month, sufficiently long for different treatment effects to appear distinctively but short enough to minimize long term random causes of death not specifically due to the different treatments. The best compromise period for the data for statistical analysis was from the 2nd to the 7th month after setting cuttings. Very few cuttings survived longer than 7 months in any treatment. This period includes both the following two seasons after setting. Surviving cuttings included those few which had rooted.

V Methods of Statistical Analysis

Different methods were selected as appropriate for the different kinds of experiments. The large quantity of varied data required the use of the shortest justifiable statistical tests, especially those which analysed simultaneously the effects of all the treatments under test and made due allowance for the limited number of degrees of freedom.

1. Binomial and Poisson Distribution Tests

These were rejected primarily because they apply only where there is no mutual influence of response of one individual upon the response of another. There was some risk of microbial infections spreading from one cutting to neighbours, both aerially and within the medium, and there was the risk of mutual shading at low solar angles. Different treatments would have required separate analyses and, where month 1 starting numbers were large, could have involved extensive binomial calculations. Poisson tests would otherwise have been appropriate for analysis of significance of the rare occurrence of rooting. The steep fall, with time, in numbers of cuttings surviving precluded use of the normal approximation in place of a binomial method.

2. χ^2 tests

This was used extensively in my work as the quickest appropriate method for identifying significant correlations between categories of treatments and frequencies of response. Time was saved by the analysis of many treatments simultaneously; potentially significant treatment effects could then be more thoroughly tested by analysis of co-variance as described below. The usual null hypothesis tested was that there were no significant differences between frequencies of response to any category of treatment including the "control"; data frequencies were expected to occur in proportion

to month 1 starting numbers of cuttings tested. The mean of all treatments including the "control treatment" was used as the reference frequency. Expected frequencies always exceeded 5 and referred to actual numbers, not proportions.

Contingency tables of frequencies were set up for χ^2 analysis with r-rows for treatments, c-columns for monthly survival frequencies. The most valid test for χ^2 was based, as discussed above, on the "starting" frequencies of cuttings surviving after the first month and the analysis was conducted on the numbers dying each month since only these frequencies consistently exceeded 5. To ensure that expected frequencies exceeded 5, the data for months 4 to 7 were summed and the totals were used in the tests. Cuttings remaining alive in many instances did not exceed 5 and so a χ^2 test for survival frequency would then be invalid.

3. Analysis of Co-variance

There was the possibility of not only mutual interference between cuttings as mentioned above, but also of non-uniformity in any of the cultural and environmental conditions assumed to be uniform within and between experiments. These latter could weaken the validity of conclusions based on χ^2 tests alone. Hence, the test of analysis of co-variance was used extensively to allow for any such non-uniformity while still providing a means of analysing quickly the data from both within and between experiments for many treatments simultaneously. Anderson and Bancroft (1952) aptly give the justification for using the test: "Its main value is to reduce error variance by eliminating plot to plot variation attributable to fluctuations in the fixed variate and to eliminate

any bias in treated comparisons caused by uneven distribution of the fixed variate to the various treatments". "Fixed variate" refers to all those environmental and other factors (e.g. rooting medium, mist spray uniformity) assumed to be uniform between subsamples and treatments.

The analysis of co-variance is based on the number of cuttings alive at monthly intervals, based on the "starting" number at month 1. Again data for months 4-7 are added together to give adequate frequencies for analysis where these had fallen to very low numbers.

4. Typical Calculations

The analysis (by both χ^2 and co-variance methods) of the frequency data obtained in the typical experiments of Tables 17 and 18 is set out in full in Appendix I. All other data were analysed similarly.

CHAPTER III

RESULTS I: ENVIRONMENTAL AND CULTURAL CONDITIONS AND EXOGENOUS GROWTH SUBSTANCES

RESULTS

i. Investigations on the Environmental Conditions

Environmental conditions are important in the rooting of cuttings, and singly or in combination can be the means of success or failure. It is therefore important that the optimum environmental conditions are provided before testing various other hypotheses.

Under this heading we have the effective rate of water application and humidity levels, light values, temperature regimes, and seasonal influences.

1. Water

Introduction

I have argued above that a cutting's water balance may be a most important factor and must be just sufficiently high to keep the cuttings turgid until they root. If root growth depends on turgor, then cuttings with low relative water content (RWC) and low turgor may be unable to grow roots, or to grow them fast enough. Hence the higher the humidity around the cutting's leaves, the better the chance of rooting due to the prevention of low turgor by excessive water loss, providing the rooting medium also is sufficiently moist. High atmospheric humidity can be achieved by mist propagation ((a) below). An alternative method for decreasing the water loss rate is to coat the cutting with a thin plastic film of an antidesiccant such as 'S600' (c). Mist propagation directly ensures that the rooting medium is kept moist, as well as the air humid.

(a) Mist Propagation

This is used extensively in commerce because it can be automatically controlled. Mist application is governed by the drying

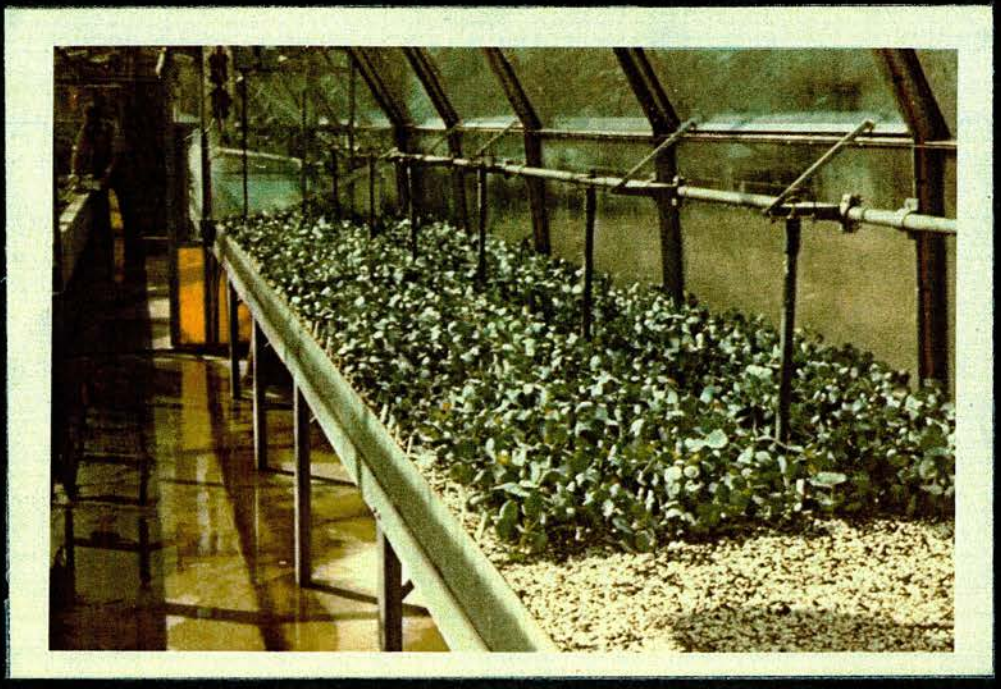
out of the electrodes. In sunny weather this drying out is increased but it is also dependent on the air humidity. The misting can be varied by altering the sizes of water droplets emitted by the atomizers, and by varying the relative spacing of atomizers and sensor unit. The volume of water given off by the nozzles is controlled by the position of these electrodes, and by the threaded rod and screw, from a completely off position to a very large droplet size. The larger the droplet, the sooner the cuttings and sensor are covered with a liquid film, and the shorter the misting periods. Ideally, an even spray of fine particle size is required over the whole rooting bench. See Plates 3 and 4. The finer the droplets, the longer the misting period before leaves and sensor are wetted, and the relatively higher the average atmospheric humidity.

Two misting regimes were tried, and their adequacy determined by measuring changes with time in the relative water content (RWC) of the cuttings to find the correct setting of the water supply control.

Measurement of Relative Water Contents of Misted Cuttings

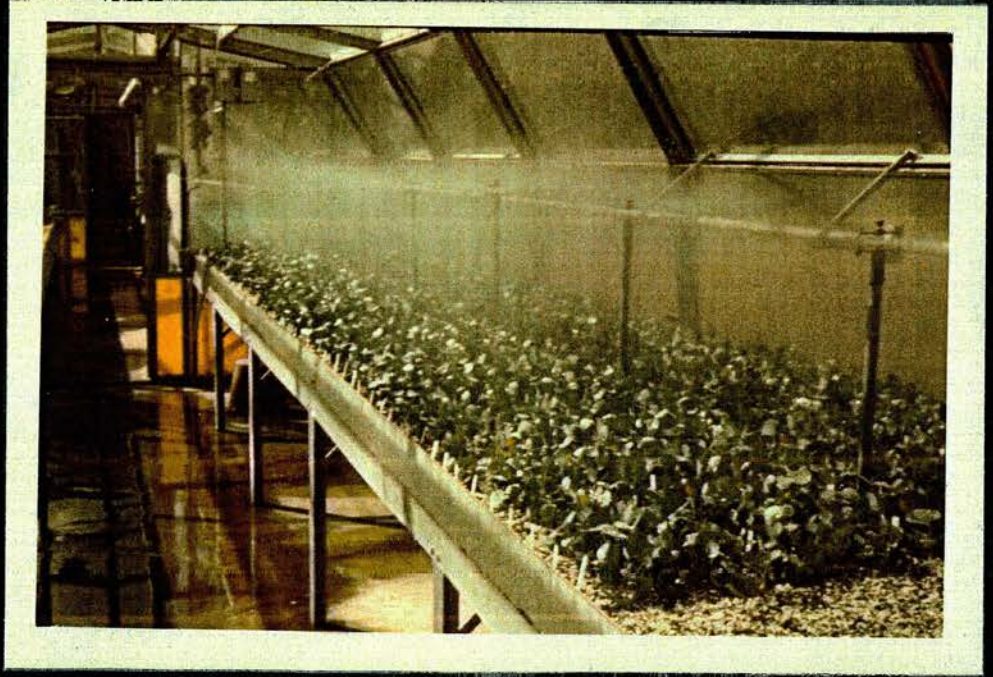
Mist propagation was used throughout the experiment. After having been in the rooting bench for two weeks, eight cuttings were taken at random from the rooting bench. Those which had rotted at the base were retrimmed. All were surface dried and weighed. The cuttings were then placed with their bases in water in a closed glass jar and at 24 hour intervals surface dried and re-weighed. After 3 days the cuttings were desiccated in an oven and again weighed to determine the weight of water. Constancy of dry matter content was assumed during the three days allowed for water saturation.

Plate 3



Cuttings *in situ* in the propagation bench.

Plate 4



Cuttings receiving overhead misting.

Results

The results are presented in Table 1 and Fig. 1

Table 1 Relative Water Contents of Cuttings

Cutting No.	FW1	FW2	FW3	TW	ODW	RWC1	RWC2	RWC3
1	3.19	3.40	3.41	3.51	1.37	87.04	94.86	95.42
2	2.91	3.30	3.42	3.50	1.41	71.78	90.44	96.18
3	1.41	1.61	1.70	1.78	0.59	68.92	85.72	93.28
4	1.79	1.91	1.98	2.19	0.81	70.01	79.71	84.78
5	1.44	1.60	1.64	1.76	0.65	71.17	85.59	89.19
6	0.78	0.81	0.85	0.99	0.31	69.11	73.53	79.41
7	0.69	0.91	0.92	0.99	0.42	47.37	85.96	87.72
8	1.73	1.85	1.81	1.87	0.73	91.85	98.24	94.73
					$\bar{x}$	72.15	86.75	90.08

FW - Field Weights at days 1, 2, 3.
TW - Turgid Weight
ODW - Oven Dry Weight
RWC - Relative Water Content

$$RWC = \frac{FW - ODW}{TW - ODW} \times 100$$

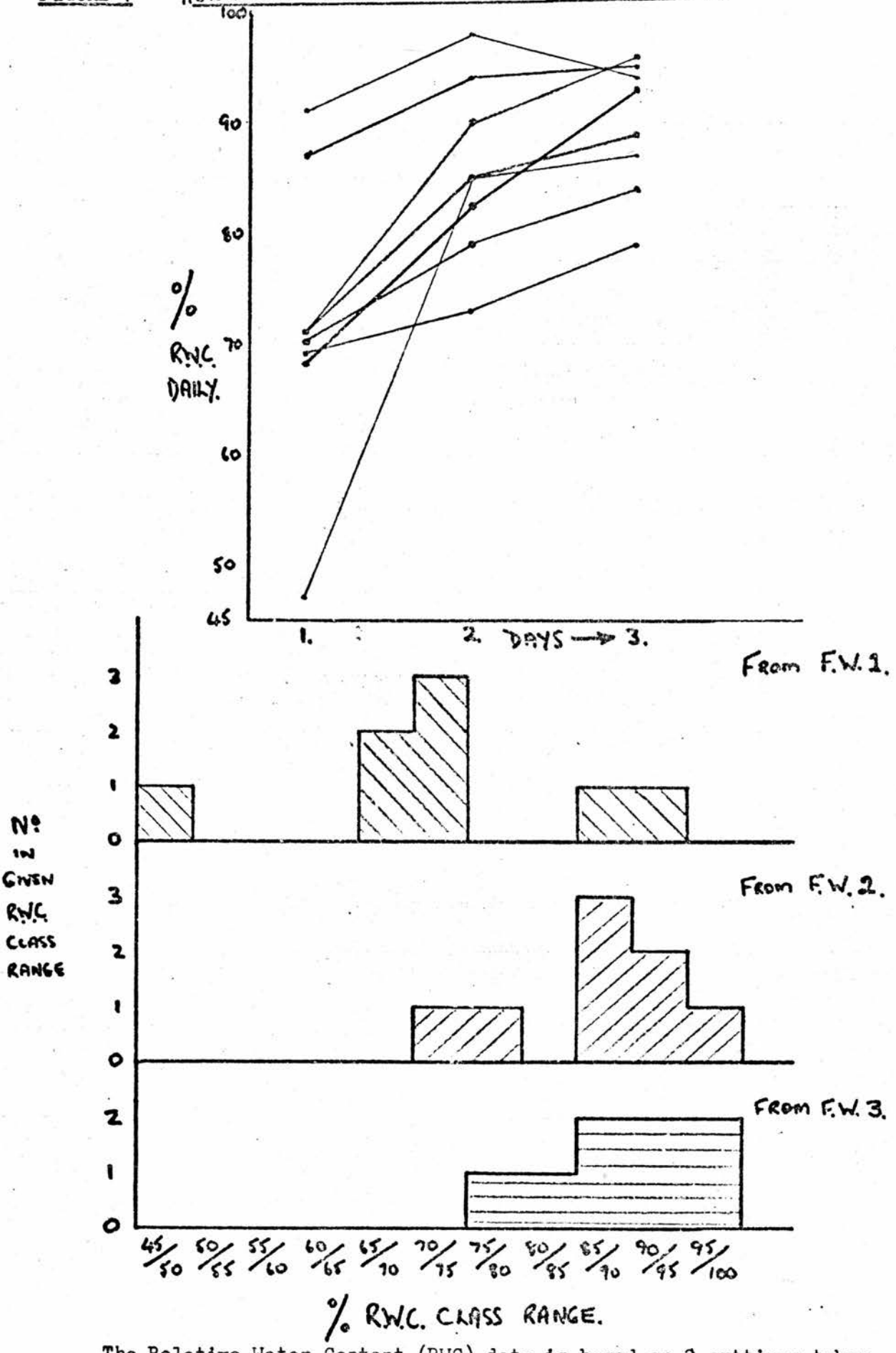
This data is based on the first misting regime to determine if there was sufficient coverage and adequate misting to prevent desiccation of the cutting.

In some cases this measured water balance has been shown to be unreliable owing to slow growth continuing, Barrs & Weatherley* (1962). There were no obvious signs of shoot extension growth in these Eucalyptus cuttings.

These figures clearly show that the cuttings were partially desiccated prior to being placed in water and that the water supply was not sufficient to prevent or replenish water losses from the

*Barrs, H. D. and Weatherley, P. E. 1962. Aust. J. Biol. Soc. 15: 413-428 A Re-examination of the relative Turgidity Technique for Estimating Water Deficiency in Leaves.

FIGURE 1 RELATIVE WATER CONTENTS OF CUTTINGS.



The Relative Water Content (RWC) data is based on 8 cuttings taken from the initial misting regime.

cuttings. In all subsequent treatments the droplet size and spacing of atomizers and sensor were both increased, to raise the humidity of the air and the availability of liquid water to the cuttings. Table 3* and Fig 2 show that this increased misting significantly raised the cuttings' RWC.

Table 3 Relative Water Content of Cuttings Under Improved Misting

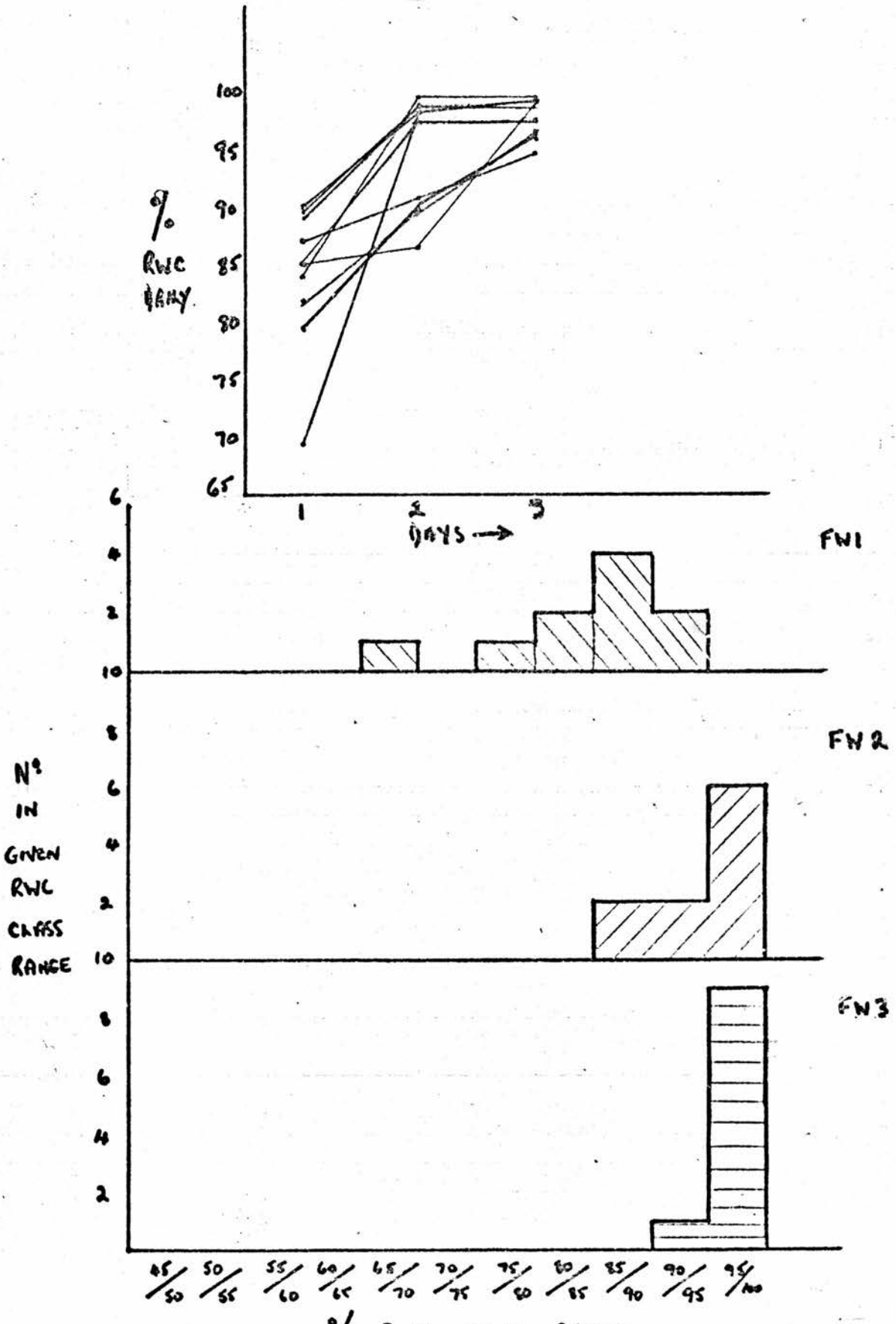
Cutting No.	FW1	FW2	FW3	TW	ODW	RWC1	RWC2	RWC3
1	1.88	1.96	1.97	1.98	0.73	69.60	98.4	99.2
2	1.42	1.50	1.50	1.51	0.55	90.62	98.96	98.96
3	0.61	0.64	0.64	0.65	0.24	90.24	97.56	97.56
4	1.22	1.30	1.30	1.31	0.47	89.28	98.8	98.8
5	0.87	0.89	0.91	0.94	0.39	87.27	90.90	94.54
6	1.14	1.15	1.25	1.26	0.44	85.36	86.58	98.78
7	1.66	1.84	1.84	1.85	0.66	84.03	99.15	99.15
8	1.43	1.50	1.57	1.60	0.61	82.82	89.89	96.96
9	1.18	1.27	1.32	1.35	0.51	79.76	90.47	96.42
10	0.68	0.74	0.74	0.75	0.28	85.10	97.87	97.87
					$\bar{x}$	84.40	94.85	97.82

Ten cuttings were removed from the modified misting regime and the data presented as in Table 1 to determine if the mist coverage was adequate.

The second misting regime resulted in a marked improvement in the survival of cuttings as shown in Table 5.*

*The data in Tables 2 and 4 has been omitted as it is better presented in the form of Figures 1 and 2 respectively.

FIGURE 2 RELATIVE WATER CONTENTS OF CUTTINGS UNDER IMPROVED MISTING



The Relative Water Content (RWC) data is based on 10 cuttings taken from the improved misting regime.

Table 5 Survival of Cuttings under Two Misting Regimes

	Expt. Ref.	No. of Cuttings Set Initially	Cuttings remaining alive at monthly intervals					
			1	2	3	4	5	6
Initial Mist Regime	M2	90	10	-	-	-	-	-
Subsequent Wetter Regime	M10	90	22	5	1	1	1R	Expt. Stopped

Discussion

Results with increased misting show a higher relative water content and a marked improvement in the survival of the cuttings. Cuttings which were given increased irrigation looked fresher longer than the cuttings under the initial misting regime. Gradually, however, under both regimes, all but one of the total number of cuttings died after shedding their leaves. These leaves were then black in colour and soft and mushy to the touch. Kemp (1948) explains that if the protective fatty substances, formed at the wound made by taking the cutting, are leached out and little or no healing takes place, the cuttings then blacken and die from the base upwards. This same rotting was also seen at the wound left by the removal of the lower leaves at the time of making the cutting. Such fatty substances include cutin and perhaps suberin; they hinder microbial attack by preventing ingress of water soluble degradative exo-enzymes from the bacteria and fungi.

Conclusion

Increased misting certainly improves both the relative turgor and the survival of the cuttings and delays the shedding of leaves

by 1-2 weeks. In both misting regimes the leaves ultimately turned black in colour and soft to the touch. It could thus be argued that the cutting/water balance was very important but that the benefits of high RWC were soon masked by rotting.

(b) Leaf Damage due to Excess Water

The tests on the correct rate of misting leave a number of questions unanswered. Could it be that the "hydrophobic"[1] nature of the plants causes this breakdown of the leaf tissues?

Komissarov (1969) states "that leaves of some of the essential oil plants, particularly Eucalyptus, Camphor and Rosmarinus become diseased as a result of protracted systematic watering". Kemp (pers. comm., 1971) is also of this opinion, particularly in the case of Eucalyptus species. Ilex opaca is another plant which is troubled by this phenomenon but only at certain times of the year. It was, however, propagated successfully from cuttings taken in the winter months presumably when they were sufficiently lignified. These were placed under mist sprays (Kemp, 1955).

An experiment was therefore set up to separate the previously interdependent effects of air humidity (and hence water loss rate) and liquid (mist) water supply.

Method

An experiment was set up to compare the increased misting regime (above) with the high humidity inside a clear polythene tent placed over a wet rooting medium, but without overhead sprinkling

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[1] The misused term "hydrophobic" in general horticultural terminology relates to the adverse effects of liquid water on leaves. It causes their ultimate death.

on the cuttings. Some cuttings were dipped into auxin before being placed in the rooting medium; others were not. These treatments are compared separately. Replication and layout were as described in Chapter II. High humidity within the tent was maintained by regular sprinkling of the rooting medium and cuttings 3 times daily by watering can. No intermittent mist sprays were used under the polythene tent but a control plot was used using normal mist propagation techniques without the polythene tent. Misted leaves had a continuously wetted surface almost throughout. Leaves within the tent were covered with a continuous water film only for a short period after drenching. Thereafter, both the leaf surfaces and the inside of the polythene were covered with a fine discontinuous 'mistiness'. Humidity within the tent averaged 97% RH.

Results

The high humidity also adversely affected Eucalyptus cuttings even though water to the leaf surface was restricted to a minimum. The turgidity of the cuttings was not tested but it was assumed to be similar in both treatments, from the appearance of the leaves.

Table 6 shows the results of the tests.

Table 6 Viability of Cuttings under Different Regimes for Maintaining Turgor

Treatment	Auxin Treatment	Expt. Ref.	No. of Cuttings Set Initially	No. of Cuttings Remaining Monthly						
				1	2	3	4	5	6	7
Control Misted	No Auxin	M6-1	90	31	24(2)	15	5	3	3	3
" "	With Auxin (powder)	M6-2	90	26	19(1)	14	1	1	1	1
Control Misted	No Auxin	M7-1	90	39	33(4)	25	11	6	6	5
" "	With Auxin (liquid)	M7-2	90	41	26	17	2	1	1	1
Polythene Tent	No Auxin	M26-1	45	7	4	-	-	-	-	-
" "	With Auxin (liquid)	M26-2	45	4	2	-	-	-	-	-

All experiments commenced in September. Powder and liquid formulations of auxins used at 0.2% concentrations. Both misting and polythene tent techniques were used. The high humidity produced within the tent precluded the need to use misting. The numbers in brackets refer to cuttings rooted.

Discussion

It can be clearly seen that there is little difference in the life of the cuttings up to the second monthly check. After this, the cuttings under the polythene tent succumbed while those under mist, although reducing in number at each check, did not entirely die out until after the seventh count. Thus, mist-treatment favours longevity. The visual effects were so obvious that a statistical test of significance was hardly needed. Only seven cuttings out of a total of 360 rooted: these were all mist-propagated. It could be argued, however, that this experiment was not a fair comparison since the temperature within the tent was as much as 10°C higher than in the misted area beside it; that overheating was obscuring any advantages of high humidity. This may not be a major criticism, however, since

viability and even successful rooting were greatest in the open glasshouse with misting when day temperatures regularly reached even higher levels of up to 24°C for several weeks in 1970. This is further considered in A.3.

Conclusion

While this indicates that some other factors are playing prominent roles, high humidity and a proper water balance in the cuttings are very important. It indicates that mist propagation is an adequate method to use for subsequent work.

(c) Turgor Control by Antidesiccant Film

So far I have used misting and still, moisture-saturated air to maintain cutting turgor. There is another easy way which has only recently been employed for the propagation of cuttings - to coat the plant with a thin antidesiccant film by dipping. This could, if successful, decrease the need for expensive misting equipment. It could decrease the microbial hazards associated with a continuous liquid water film over the cuttings' surface. Moreover, spores of micro-organisms subsequently falling on the film-covered surfaces would be less likely to infect the cutting than parasites or saprophytes.

Hence, the viability of cuttings coated with antidesiccant was compared, as before, with that of mist-cultured cuttings.

Method

Replication, experimental layout and mist culture techniques were carried out as described above. Both non-auxin and auxin treated (liquid dipped and dried) cuttings were used. The

antidesiccant, S-600 (Synchemicals Ltd., Bermondsey, London) was used. It is an aqueous P.V.C. emulsion which dries over dipped plants to form a continuous film of low permeability to liquid or vapour water. It was diluted 1 in 5 with water. The cuttings were briefly immersed, removed, and any large droplets shaken off. After the film dried, which occurred quickly, the cuttings were planted and watered well into the peat/perlite medium. Subsequently sufficient water was regularly applied to the rooting medium 2-3 times daily. At the same time, the humidity of the glasshouse was raised by damping the floor and other surfaces. The cuttings were not wetted.

Results

The viabilities of the cuttings treated with S-600 and by misting are compared in Table 7.

Table 7 Survival of Cuttings in Two Turgor Controlling Mechanisms

Treatment		Expt. Ref.	No. of Cuttings Set Initially	No. of Cuttings Remaining Monthly						
				1	2	3	4	5	6	7
Mist	Control No Auxin	M7-1	90	39	33(4)	25	11	6	6	5
	Auxin NAA 2g/lt	M7-15	90	32	26	18	-	-	-	-
	Auxin IBA 2g/lt	M7-13	90	31	26	15	7	6	6	6
S-600	Control No Auxin	M24-1	25	25	-	-	-	-	-	-
	Auxin NAA 2g/lt	M24-2	25	25	-	-	-	-	-	-
	Auxin IBA 2g/lt	M24-3	25	25	-	-	-	-	-	-

All experiments commenced in September. Number in brackets refers to cuttings rooted.

After one month, the S-600 cuttings were still green and healthy, but before two months they all started to turn pallid, then yellow and finally to brown at death. Signs of leaf rotting were absent. During the period of the experiment not a single leaf dropped from the 75 cuttings even when these died. Further, S-600 actually prevented the formation of an abscission layer. By comparison, under mist-propagation the leaves started to blacken and a few days later to drop at 4-5 weeks, and the majority had dropped, or were only lightly attached to the cutting, by 8-10 weeks: an abscission zone ultimately formed in all these leaves.

It could be argued that dehydration rather than attack by micro-organisms or their exo-enzymes caused the ultimate death of the S-600 cuttings. By contrast, mist-grown cuttings blacken, first at each open or cut surface, then all over each leaf which then abscinds. Finally blackening progressively covers the stem until the whole cutting dies. This may indicate that surface moisture plus exposed inner tissues render a cutting liable to microbial invasion which is then the final cause of death.

Conclusion

It is obvious that optimal conditions for maintaining the viability of the cuttings have not yet been found. It may be that the antidesiccant is also effective as a waxy sealant hindering entry of microbial exo-enzymes and microbial invasion. It may now be suggested that newly received cuttings should be surface-

sterilised, sealed with S-600, and cultivated under a light mist for high humidity and adequate water supply, with regular fungicidal drenches. Time has not, alas, allowed this to be tested.

2. Fungal Attack

If this be true, it follows that mist-cultured cuttings should be tested for the presence internally of parasitic and saprophytic fungi and bacteria. The extent of any such infection should therefore be compared between dead cuttings taken from mist-culture; living, mist-cultured, fungicidally treated cuttings; and fresh cuttings directly they are received from the Nursery. One might predict some decrease in extent of infection in this series, if infection were a major contributory cause of death.

Method

In order to establish the cause of death, 23 dead and dying cuttings were removed from the rooting bench where they had been receiving intermittent mist treatment for six weeks. The cuttings were removed to a mycology culturing room which was rendered free from fungal spores by continual spraying with 0.05% Thymol. There was also an extractor fan present to remove quickly any spores which were taken in on the person. The air filter was of the type which screened out any fungal spores in the air intake. The cuttings were dipped in 95% alcohol and flamed to ensure that the surface was free from infection. Any microorganism present would therefore be within the tissue of the cutting. Juvenile Eucalyptus cuttings were used in the tests. Dead and dying Myrtus cuttings were also tested.

Isolates were taken at various places, mainly at infected areas, basal, leaf scar, vascular tissue. Small pieces of tissue were removed with a scalpel, flamed and then placed on Malt Agar* plates. These were then left to incubate in the dark at 26°C. Some fungi appeared very quickly (5-7 days) while others took some weeks to develop.

Results

A variety of fungal and bacterial organisms as well as yeasts were isolated from 19 of the 23 dead and dying cuttings. The details of these are given in Table 8.

Discussion

We do know that some fungal genera such as Rhizoctonia and Botrytis cause considerable damage to cuttings and plants. These two species were isolated in only 7 of the 23 positive plates, while yeasts and bacteria accounted for the majority of the remainder. Trichoderma is a green mould, as is Penicillium, and both are more than likely to be a secondary and saprophytic infection. These are prominent in moist conditions. The value of yeasts as the causative organism is debatable. (See Plate 5)

Conclusion

On the whole, there is little evidence to support the theory that fungal pathogens are the prime cause of mortality.

(a) Infection in the Living Cutting

As a check for primary infection, living cutting material was removed from the bench and treated in similar manner to the dead and dying cuttings.

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* "Malt Agar" contains 2% malt extract and 2% agar. This non-selective medium is routinely used for isolation and culture of a wide range of fungi.

Table 8 Isolation and Identification of Pathogens from Dead or Dying Cuttings

Cutting No.	Type of Cutting	Isolated From	Description		Plate
1	Eucalyptus	Basal rot	(1) Brown mycelium (2) White mycelium with sclerotia (3) Blue green mycelium	Rhizoctonia Botrytis Penicillium	5
3	"	Basal rot		Feathery Yeast	
11	"	Bud Scar, black	Yeast	Yeast	
12	"	Vascular tissue	Yeast	Yeast	
15	"	Vascular tissue	White mycelium becoming green	Trichoderma	
20	Myrtus	Base	White mycelium green centre	Trichoderma	
21	"	Nodal tissue	White mycelium green centre	Trichoderma	
22	"	Vascular tissue	White mycelium green centre	Trichoderma	
23	"	Base	(1) Feathery Yeast (2) Yeast	Feathery Yeast Yeast	
24	"	Base	Feathery Yeast	Feathery Yeast	
24a	Eucalyptus	Vascular tissue	Brown mycelium	Rhizoctonia	
25	"	Vascular tissue	White mycelium green centre	Trichoderma	
26	"	Vascular tissue	Brown mycelium	Rhizoctonia	
27	"	Vascular tissue	Brown mycelium	Rhizoctonia	
28	"	Leaf callus, rotted	Feathery Yeast	Feathery Yeast	
29	"	Vascular tissue	Feathery Yeast	Feathery Yeast	
30	"	Vascular tissue	White mycelium with sclerotia	Botrytis	
31	"	Vascular tissue	Brown mycelium	Rhizoctonia	
42	"	Vascular tissue	White circular	Yeast	
43	"	Basal rot	Feathery Yeast	Feathery Yeast	

This information is based on 32 tissue samples surface sterilized and cultured on Malt Agar plates.

Results

These are given on Table 9.

Discussion

From the 11 living juvenile cuttings removed from the rooting medium, 6 cuttings produced microbial isolates: two different fungi, together with a watery bacterium and a red yeast. The yeast we can discount because it is a common saprophyte and has been isolated from the phyllosphere by Edinburgh University Department of Tree Biology. It is common on many species. This yeast appears to cause no adverse effect on the plant.

The ash-grey sporing fungus and the isolate showing concentric ringing we have been unable to identify, (see Plate 6). Isolate No. 19 (Plate 7), which produced a translucent light brown colour, was found in three of the living cuttings removed from the rooting medium. Since replating produced no growth, and nothing microbial was visible under the microscope, this proved to be a solute from the cutting.

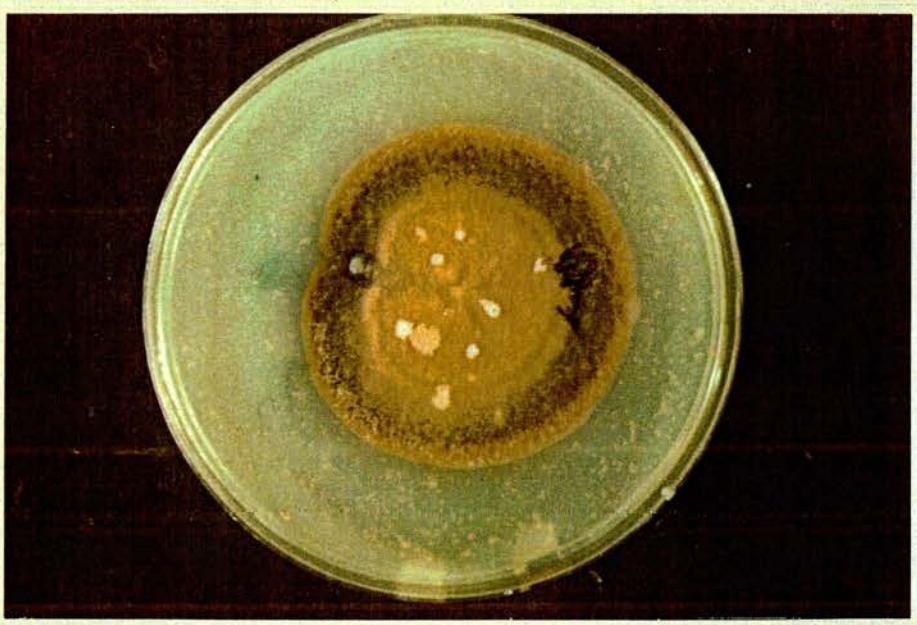
Conclusion

Fungal as well as other organisms are present in live tissue. We are not aware of the effect these have on the cutting nor do we know for certain if they are the cause of death. Treatment with fungicides at the time of taking the cutting may help to reduce infection.

Plate 5

Some of the identified fungi isolated from Eucalyptus.

Plate 6



The Unidentified fungus isolated.

Table 9 Identification of Isolates from Living Eucalyptus Cuttings Removed from the Medium

Cutting No.	Isolated From	Description	Identification	Plate
4	Vascular tissue	(1) Watery bacterial (2) Ash grey sporing fungal	Bacterial (watery) Unidentified	
5	Callus	Dark brown concentric fungal	Unidentified	6
16	Petiole	Red Yeast	Red Yeast	
19	Vascular tissue	Translucent light brown	Solute from cutting	7
45	Nodal tissue	Light brown translucent	(19)	
46	Vascular tissue	Light brown translucent	(19)	

Eight samples were initially cultured on Malt Agar plates.

(ℓ) The Effect of Fungicidal Treatment on Living Cuttings

Introduction

Regular fungicidal treatments have been used successfully in commerce to control and/or reduce infection in cuttings grown under mist propagation techniques.

Fungicides are included in many of the powder preparations containing growth hormones and by dipping the cuttings into the powder, subsequent infection is reduced. Fungicidal drenches applied regularly over the cuttings can reduce infection and hence the losses due to fungal attack.

Fungicidal treatments may then hold the balance between success and failure.

It was therefore necessary to determine if a fungicidal application with the auxin treatment, together with an overhead weekly drench, would prevent infection and thereby increase rooting.

Method

The cuttings were treated with various preparations which would either render them sterile or, hopefully, prevent infection.

Some cuttings were immersed in chloros for 10 seconds to kill off any pathogenic organisms which might be present externally. Half of the cuttings were then treated with auxin powder. The remainder were dipped in talc. A further batch of cuttings received a combined single auxin and fungicidal treatment, while others received a combined two auxin and fungicidal treatment.

In case bacterial infection was the cause of mortality, still more cuttings were dipped in Cicatrin - an antibactericidal powder. Half of these also received auxin, while the control received neither auxin nor bactericidal treatment.

The fungicide Captan was also used as a weekly overhead drench over the cuttings in the M6 series.

Results

The results and treatments are shown in Table 10.

Discussion

The initial results show that none of the antipathogenic treatments is better than the control samples which received no treatment at all, whatever the time of year. The χ^2 test which is valid for the fungicidal treatments in the M6 series gives a significant difference between control and the various fungicidal treatments $p = <0.01, >0.001$ for 12 d.f. This indicates that there are unknown overriding factors concerned in the survival of cuttings when treated with fungicides and auxins.

When the χ^2 test was conducted on the Cicatrin treatments with auxin and non-treatment controls, again in the M6 series, a non-significant result was given. $p = <0.95, >0.90$ for 6 d.f.

Auxin with Captan produced the best results in the fungicidal treated cuttings in the M3/5 series but was poorly placed in the later series. Here Thiram with two auxins, together with the weekly overhead fungicidal drench, produced the best results in the antipathogenic treatments.

Cicatrin alone consistently provided good results but the survival rate was not generally as good as the non-treatment controls. However, both Cicatrin, and Cicatrin and auxin together, produced the best rooting compared with all other treatments - five in all.

Table 10 Survival of Cuttings under Differing Fungicidal and Bactricidal Treatments

Treatment	Expt. Ref.	No. of Cuttings Set Initially	No. of Cuttings Remaining Monthly							
			1	2	3	4	5	6	7	Total
Control No auxin	M3/5-1	90	33	26	17	15	11	5	1	75
No fungicide	M 6-1	90	31	24(2)	17	5	3	3	3	55
Control IAA	M3/5-2	90	22	16(3)	13	11	8	3	3	54
No fungicide	M 6-2	90	26	19(1)	14	1	1	1	1	37
Chloros	M3/5-19	90	20	13	9	5	3	1	-	31
	M 6-19	90	26	17	9	1	-	-	-	27
Chloros + IAA	M3/5-20	90	25	19	15	10	5	-	-	29
	M 6-20	90	27	22	4	-	-	-	-	26
NAA + IAA +	M3/5-21	90	14	8(1)	5	4	4	2	1	24
Thiram	M 6-21	90	30	25(1)	14	4	1	1	1	46
NAA + Captan	M3/5-22	90	26	18	11	10	6	3	1	49
	M 6-22	90	36	17	10	-	-	-	-	27
Cicatrin	M3/5-17	90	20	16(3)	16	10	7	5	3	57
	M 6-17	90	26	22	13	1	1	1	1	39
IAA + Cicatrin	M3/5-18	90	25	19	11(1)	5	5	4	1	45
	M 6-18	90	22	17(1)	11	2	1	1	1	33

M3/5 series was started in April while the M6 series began in September

The numbers in brackets refer to cuttings rooted.

Cuttings treated with the weekly drench were initially better than undrenched cuttings, but this effect was reversed after two months.

Conclusion

Fungicidal pre-treatments, as well as drenches, would therefore appear to be the best treatment. Pre-treatment by fungicides is not always possible especially where other treatments are being used. However, since the overhead drench alone produced the best results, this has been continued for the remainder of the tests.

(c) The Effect of Systemic Fungicidal Treatment on Cuttings

Introduction

The above information shows that overhead drenches reduce fungal infection, using the fungicide Captan in liquid suspension.

A newer, more efficient, systemic fungicide, Benomyl, has recently been marketed and is used to control a wide spectrum of plant pathogenic fungi.

Object

It was therefore necessary to determine the effect of fungicidal treatment on living cuttings.

Method

On arrival from the source, thirty cuttings were immersed for a few seconds in Benomyl and then their bases were placed in a solution of Benomyl and auxin for 16 hours. Subsequently they were pushed into the rooting medium. At weekly intervals these cuttings and their rooting medium were drenched with the same fungicide. No replicated plots were used. The cuttings were simply lined into the rooting medium. After 6 weeks, 5 cuttings (2 living and 3 dead) were

selected and taken to the mycological laboratory where they were treated as described above.

Results

Results are given in Table 11 where it can be seen that from all the dead samples, yeasts and one bacterium were isolated, while from the living samples only the solute, corresponding to No. 19, was evident. No cellular organisms were detected by microscopic examination. It was not possible to detect the solute in the other plates since the fungal and bacterial isolates were produced much quicker than the solute which was very slow to become evident.

Discussion

It could be argued that the Benomyl treatment reduced the fungal infection of the cuttings tested. Numbers were, however, too small to be of any significant value.

Fungal infection, however, was still present but it is interesting to note that living tissues did not produce any fungal isolate.

Conclusion

It therefore appears that fungicidal treatment is beneficial in reducing infection in cuttings. However, it is not clear whether the cuttings are contaminated before delivery from the Nursery or whether they become infected during the operation of preparing the cuttings, treating them, or while occupying space in the rooting medium.

Table 11 Fungal Isolates from Benomyl Treated Juvenile Eucalyptus Cuttings

Cutting No.	Isolated From	Description	Identification
47	Cutting dead, base.	Yeast	Yeast
48	Cutting dead, vascular tissue.	Yeast	Yeast
49	Cutting dead, node.	(1) Water bacteria (2) Feathery Yeast	(1) Water bacteria=4 (2) Feathery Yeast=28
50	Living, vascular tissue.	Light brown translucent	Solute=19
51	Living, vascular tissue.	Light brown translucent	Solute=19

These isolates were produced from 5 tissue samples cultured on Malt Agar plates.

(d) Fungal Tests on Freshly Picked Eucalyptus Shoots

Introduction

Fungal and bacterial organisms have been isolated from living cuttings removed from the rooting medium after fungicidal treatment, and in living cuttings removed from the rooting bench without being treated with fungicides. It is a possibility that these organisms may be present in the cuttings received direct from Kent. It is known that some of the trees in the plantation are infected with Stereum purpureum.

Object

To determine, by isolation on agar plates, the fungal flora of freshly received, living and healthy Eucalyptus gunnii shoots.

Method

Shoots received direct from the Kent plantation were taken to the mycology laboratory immediately on arrival. Vascular tissue was removed from each of the shoots, dipped in 95% alcohol, flamed and placed on malt agar plates in similar manner to previous cultures.

Results

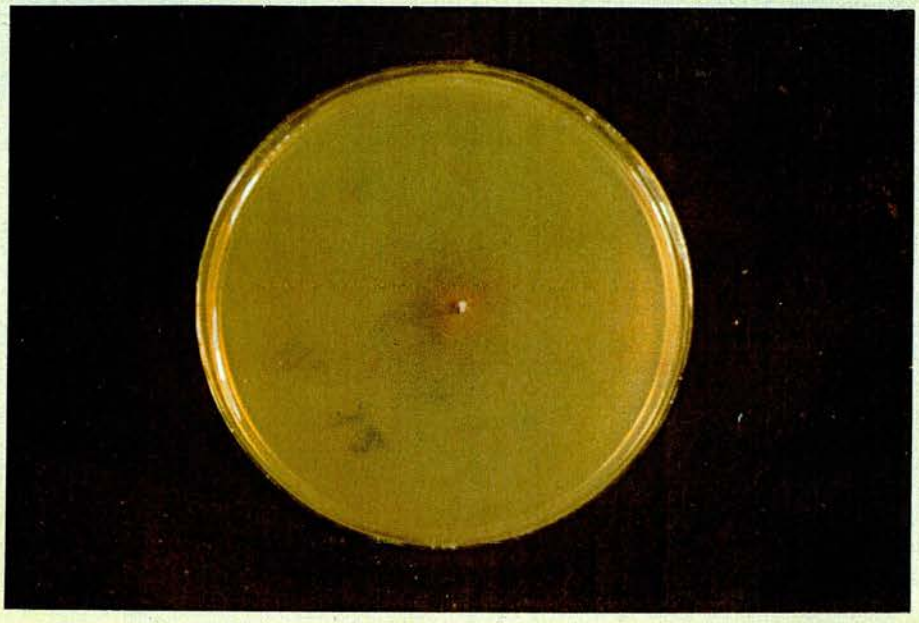
Of the 10 samples of the Kent material, 7 produced isolates, none of which was a known pathogen. It is interesting to note that all of them produced the same light brown translucent coloration identical to No. 19 which was found in living tissues of earlier isolates. See Table 12 for details. There was no fungal mycelium present in any of the isolates.

Table 12 Samples of Material Received Fresh from Kent

Cutting No.	Type of Cutting	Isolated From	Description	Identification	Plate
32	Eucalyptus	Vascular tissue	Light brown translucent	(19) solute	7
35	"	"	" " "	"	
36	"	"	" " "	"	
38	"	"	" " "	"	
39	"	"	" " "	"	
40	"	"	" " "	"	
41	"	"	" " "	"	

Ten tissue samples were initially cultured on Malt Agar plates.

Plate 7



Malt agar plate with tissue from typical, freshly received and benomyl-treated cuttings. Mycelium is absent, a soluble pigment is present.

Plate 8

Cuttings remaining in the propagation bench after two months.

Discussion

There are no indications of the presence of pathogenic organisms in freshly received cuttings. A systemic fungicidal treatment is still thought desirable to prevent the spread of any infection. It is also necessary to control the fungal pathogens and saprophytes of all types from the base of the cutting by dipping the cutting in the fungicidal solution and also drenching the rooting medium. Bacterial and other antiobiotics are also recommended to prevent further mortality.

Conclusion

Only the solute was found in all the cultures. It would therefore appear that fungal infection probably occurs either during treatment or in the rooting medium. Infection has been controlled by fungicidal treatment to the cuttings at time of preparation and also as an overhead drench. Nevertheless, cutting mortality was unaffected by these measures to control both fungal pathogens and saprophytes.

The tissues of the cutting may therefore become physiologically senescent and simply rot. Plate 8 shows a typical sample of cuttings remaining after 2 months in the medium.

3. Light

Introduction

Illumination must be considered under three categories - (a) light intensity, (b) light duration or photoperiod, and (c) wavelength.

(a) Light intensities - Different species require different optimal light intensities. Basically they can be divided into two main groups - the shade tolerant species and the light demanding

species. Eucalyptus gunnii, a Tasmanian alpine species, is classified as a sun species, i.e. light demanding. This latter group must have high light intensities to aid shoot growth.

It is known that high light intensities are required to promote extension growth in adult Eucalyptus shoots. This is assumed to be a necessary precondition for vegetative propagation.

(b) Photoperiod, however, belongs to a different category and as such demands attention. It is known that many species have their own optimum photoperiod for vegetative growth as well as for flowering. It is known that long photoperiods favour incremental growth in Eucalyptus but nothing is known about the daylength which will favour the successful rooting of Eucalyptus cuttings. Some species of plants will, however, root in complete darkness provided ample supplies of food reserves are present in the cuttings; Stoutemyer (1961) mentions Euonymus, Gardenia and Aucuba as examples of this.

A check for any photoperiodic effects on Eucalyptus was therefore necessary.

Method

Cuttings were taken in January and were grown in a recorded air temperature of 22-23°C. Substrate temperatures were maintained at 24°C. Some cuttings were treated with auxin, others were not. Three daylengths were used - (i) total darkness, (ii) 8 hours light, and (iii) 16 hours light.

(i) Cuttings were grown under a black polythene tent in a cool glasshouse. Due to the confined microclimate, temperatures increased and these were recorded at 22-23°C. The cuttings were well ripened shoots and would therefore have good reserves of food materials.

(ii) Since the cuttings were taken in January, the "normal" daylength at that time of year is 8 hours and thus gives a short day treatment.

(iii) Long day treatment was provided by using two 6' long 60 watt "Grolux" lights suspended 3' above the cuttings to supplement the 8 hour daylight. These were connected to a time clock mechanism which provided the necessary long day photoperiod.

All the cuttings were grown under mist propagation.

Results

The results comparing these three photoperiods are given in Table 13.

Table 13 Survival of Cuttings under Various Photoperiods

Photoperiod	Cutting Treatment	Expt. Ref.	No. of Cuttings Set Initially	No. of Cuttings Remaining Monthly						
				1	2	3	4	5	6	7
0 hours tent	No Auxin	M19-8	45	32	-	-	-	-	-	-
0 " "	Auxin	M19-1	45	37	-	-	-	-	-	-
8 " open & mist	Auxin	M21-1	45	1	-	-	-	-	-	-
16 " " " "	No Auxin	M12-3	90	15	6	4	3	3	3	3
16 " " " "	Auxin	M12-4	90	2	2	1	-	-	-	-

Three photoperiods, 0, 8 and 16 hours daylength, were used. Auxins, where applied, were used at 4000 ppm IBA. Cuttings were set in December.

Looking at the no auxin data, almost all of the cuttings in the darkness treatment remained alive after one month but all perished between 4 to 5 weeks. The long photoperiod (16 hours) alone favoured survival in the long term. This photoperiod also produced one rooted cutting. As mentioned later on p.126 auxin significantly decreased survival of cuttings. Moreover, no positive interaction between auxin and photoperiod was found. Only one cutting from the 8 hour photoperiod remained alive after 4 weeks, but died between weeks 6 and 7. In the long photoperiod (16 hours) auxin treated cuttings also died out very quickly; the last survivor between weeks 9 and 10.

Discussion

There may very well have been another factor involved in the darkness treatment - that of high air humidity. If the cuttings were not ultimately dying for the same reason as in other treatments, then it was almost certainly due to the depletion of food reserves in the cuttings. It could also be possible that all the cuttings received a temperature too high for rooting. Comparisons of two different temperature regimes are covered in A.3.

Conclusion

Long day treatment favours the viability of the cuttings. It was also under this photoperiod that the one cutting rooted.

(c) Wavelength is another factor which appears to be specific for rooting in certain species. In general, blue light treatment of cuttings produces an inhibiting effect on rooting but red and far red light produce a variety of plant responses. Red and far red light offer distinct advantages with regard to rooting. While no information is available regarding the response of Eucalyptus to red and far red light, it is concluded above that cuttings of Eucalyptus might respond to these wavelengths.

The effect on Eucalyptus gunnii cuttings of red and far red light was therefore tested.

Method

Some cuttings were treated with auxin and kinetin; others received no such treatment. Methods of application, as well as the statistical layout, are described above.

Cuttings were placed into the rooting medium of peat and perlite in September in a temperature of 10°C. No misting was used. The red and far red light was provided by covering the batch of cuttings with a plastic tent constructed with red perspex. This allowed only the red and far red light to reach the cuttings. For comparison, another polythene tent was constructed with clear film which allowed all visible wavelengths to pass. The tents retained the moisture of the atmosphere thereby retaining the turgidity of the cuttings. Daylength averaged 14 hours initially, a relatively long, and hence favourable, photoperiod.

Results

Results comparing the wavelength are given in Table 14.

Table 14 The Effect of Different Wavelengths on Eucalyptus Cuttings

Treatment	Cutting Treatment	Expt. Ref.	No. of Cuttings Set Initially	No. of Cuttings Remaining Monthly 1	2	3	4	5
Normal	No Auxin	M26-1	45	7	4	-	-	-
Daylight	NAA	M26-2	45	4	2	-	-	-
	IBA	M26-3	45	12	8	-	-	-
	Kinetin	M26-4	45	15	6	-	-	-
Red and	No Auxin	M25-1	45	-				
Far Red	NAA	M25-2	45	-				
Wavelengths	IBA	M25-3	45	-				
	Kinetin	M25-4	45	-				

Cuttings were set in September 1973 and given a normal daylength of 14 hours full spectrum light. Red and Far Red filters were also used. Auxins were applied at 4000 ppm and kinetin at 2 mg/litre.

Discussion

Some cuttings were still alive under normal daylight conditions at the first monthly count, whereas none survived under the red filters. Normal daylight provides the better conditions of the two treatments. As stated above, other factors must be involved because the survival times again were very short.

Conclusion

Full visible spectrum maintains viability better than red and far red wavelengths.

4. Temperature

Introduction

Two main air temperature regimes are widely used in research and in commerce. For the cool temperate plants, an air temperature of 10-12°C is used, while for the warm temperate and tropical species, the higher temperatures 21-27°C are used. Eucalyptus species in general grow in nature under temperatures within the high range, although Eucalyptus gunnii is a Tasmanian alpine species.

Method

Substrate heating is deemed necessary for species which prove difficult to root from cuttings and therefore this has been used throughout this work. Minimum air temperatures of 10°C and 21°C were provided. Both short days (8 hours) and long days (16 hours) were also used. Cuttings used in the tests were pushed into the rooting medium of peat and perlite.

Results

Comparisons are provided in Table 15.

In most cases the survival rate of the cuttings was low. Survival was better at the lower temperature and this was distinctly linked with long day treatment. In comparison, the cool short day treatment provided a very poor result. The survival rate of the higher temperature cuttings was generally poor apart from one sample which was comparable with the mean result of the cooler temperature, longer photoperiod cuttings.

Discussion

As shown in a previous test, the short day treatment was not beneficial whatever the temperature, although the cooler temperature produced the better result.

Table 15 Viability of Cuttings under Different Temperature and Photoperiods

Temperature Treatment	Daylength	Auxin Treatment	Expt. Ref.	No. of Cuttings Set Initially	No. of Cuttings Remaining Monthly						
					1	2	3	4	5	6	7
10°C	S	None	M11-3	90	7	2	2	-	-	-	-
10°C	S	Auxin	M11-4	90	1	1	-	-	-	-	-
10°C	S	Auxin	M11-5	90	5	-	-	-	-	-	-
10°C	L	None	M10-1	90	22	5	2	1	1(1)	1	1
10°C	L	Auxin	M10-2	90	14	3	3	3	3(1)	3	3
10°C	L	Auxin	M10-3	90	18	8	6	3(1)	1	1	1
21°C	L	None	M12-3	90	15	6(1)	4	3	3	3	3
21°C	L	Auxin	M12-4	90	2	2	1	-	-	-	-
21°C	L	Auxin	M12-5	90	5	1	1	-	-	-	-
21°C	S	Auxin	M21-1	45	1	-	-	-	-	-	-

All the cuttings were prepared and set in December/January 1970-71

Daylengths of 8 and 16 hours were used. Auxins were applied at two concentrations, 4000 and 8000 ppm IBA. Numbers in brackets refer to cuttings rooted.

In the long day treatments, the cooler temperatures gave better survival. It is so clearly evident that no statistical analysis is necessary.

Conclusion

The cooler temperatures were beneficial and produced the best results from the viability and rooting aspects. There was certainly an interaction of temperature with photoperiod. A cool temperature and long photoperiod were therefore necessary for success.

5. Seasonal Effect

Introduction

There is considerable variety in seasonal responses of different species to rooting.

Deciduous plants root best during the actively growing season while evergreen plants generally root best in the winter months although this is far from the truth in some species.

Information regarding the optimal time for Eucalyptus propagation clearly indicates the actively growing season but, as the general conditions (weather, season and plant) vary considerably, there is no known best time for the conditions prevailing during this work.

Object

It was therefore necessary to determine the best time of year to root Eucalyptus cuttings in the conditions relating to this work.

Method

It was seen at an early stage that cuttings taken in midsummer were not suitable. It was found that young shoots and leaves were tender, subject to damage, and wilted very quickly and therefore did

not survive the journey from the cutting source in Kent. These cuttings were therefore excluded from further tests.

Thereafter, cuttings were taken at three times of the year - spring (April), late summer (September), and winter (December).

Some cuttings received auxin treatment, others did not. These were then pushed into the rooting medium of perlite and peat. The same temperature regime (10°C) was used throughout and the cuttings received the normal daylength for that particular time of year.

Results

Table 16 shows that winter-taken cuttings produced a low survival record, whereas spring and autumn cuttings at least remained alive longer. Rooting was very limited in these seasons and no rooting occurred in the winter-taken cuttings.

Daylength also was another factor which was involved, but see the results of A.2. Results for one season in different years were statistically undistinguishable.

Conclusion

This test indicated that the cuttings must be actually growing if rooting was to occur. Hence spring and autumn were both suitable. Summer cuttings were "soft", poorly lignified and had much more primary tissues, and therefore easily lost turgor, visibly drooped, and rotted readily.

Table 16 Seasonal Response to Rooting

Season	Expt. Ref.	Treatment	No. of Cuttings Set Initially	No. of Cuttings Remaining Monthly						
				1	2	3	4	5	6	7
Spring 22.4.70	M3/5-1	Control No Auxin	90	33	26	17	15	11	5	1
	5-2	Auxin 0.2% IAA	90	22	16(3)	13	11	8	3	3
	5-3	Auxin 2.0% IAA	90	8	5(1)	3	2	2	2	1
Summer 15.6.70	M2 -1	Control No Auxin	90	0	-	-	-	-	-	-
	-2	Auxin 0.2% IAA	90	0	-	-	-	-	-	-
	-3	Auxin 2.0% IAA	90	0	-	-	-	-	-	-
Autumn 12.9.70	M6 -1	Control No Auxin	90	31	26(2)	15	5	3	3	3
	-2	Auxin 0.2% IAA	90	26	17(1)	14	1	1	1	1
	-3	Auxin 2.0% IAA	90	25	16(2)	8	2	2	2	2
Winter 14.12.70	M11 -3	Control No Auxin	90	7	2	2	-	-	-	-
	-4	Auxin 4000 IBA	90	1	1	-	-	-	-	-
	-5	Auxin 8000 IBA	90	5	-	-	-	-	-	-

Cuttings were set at four times during the year. Numbers in brackets refer to cuttings rooted.

ii. Investigations into Cultural Factors in Relation to Propagation of Eucalyptus from Cuttings

1. Rooting Medium

Natural rooting media (peat, sand etc.) vary considerably from one locality to another. The peat/sand medium offers a reasonably reliable mixture but some sands can be substandard. Peat and perlite both conform to standard grades and, although fairly recently used in propagation, offer a costlier but less variable medium.

The rooting capacity is the criterion for success and it was therefore necessary to determine if the locally obtained sand, together with the granulated peat, was superior or not to the peat/perlite mixture. The use of peat and sand, and peat and perlite was therefore compared as rooting media.

Method

The peat used in both mixtures was the medium grade Irish Moss peat (Bord na Mona, Dublin) with a pH of about 5. This was mixed with sand in the ratio of one in four. The sand was a fine aggregate obtained as a washed Grade (2) sand from a local sand pit (Scottish Aggregates, Wormit, Fife). Perlite was obtained from Johns-Manville Ltd., London, and mixed with equal parts of the medium grade peat.

The mixtures (kept separate) were used to fill up the rooting bench and covered the substrate heating cables by a depth of 4". The media were then firmed and well watered to settle the particles. Cuttings were simply pushed into each media.

Results

There was little difference in the actual numbers of cuttings remaining alive after one month. In both cases cutting mortality was high. Those which rooted in sand had fleshy roots which were easily damaged, while those in perlite were fleshy to start with but then developed a fibrous root system.

Discussion

Moss and algal growth was common and occurred freely in the sand and peat mixture, while this did not prove to be the case with the perlite/peat mixture. There was less compaction of the perlite mixture which thus aided oxygen exchange. Sand proved to be very variable and contained a variety of particle sizes and therefore could not be considered suitable. While a better grade, high quality, quartz sand could have been obtained at very high cost from a distant source, it was decided to use the perlite and peat mixture for all other tests on rooting. Repeated fungicidal drenching of this rooting medium has been discussed above.

Conclusion

While no difference could be found between the two mixtures from the rooting viewpoint, there were considerable advantages culturally in the long term in using the peat/perlite medium.

2. Fungicidal Treatment

This aspect was discussed at the end of "water regimes" since it was relevant at that part (see pp. 89-102).

iii. PHYSIOLOGICAL FACTORS AFFECTING ROOTING OF EUCALYPTUS CUTTINGS

1. Exogenous application of Growth Promoting Substances

Auxins are known to aid rooting in most cuttings and, as discussed in the Introduction (pp. 49-53), endogenous auxin/inhibitor levels are of the utmost importance. By increasing the endogenous auxin levels in the cutting, inhibition has been partially overcome in some of the species and rooting occurred. The exogenous application of auxin to Eucalyptus gunnii, which is an extremely difficult plant to root, may therefore assist rooting. The naturally occurring auxin, indole-3-acetic acid, was used in some series of experiments, while in others the more stable IBA and NAA were also tested. Further tests were conducted on auxin solvents, and on the methods of application. These tests are described below.

Methods

Both powder and liquid formulations of the auxins were used. Powder formulations used talc as the carrier. Concentrations of 0.2% and 2.0% w/w IAA were used. Liquid formulations of IAA, IBA and NAA using 45% ethyl alcohol as the solvent were prepared to concentrations of 20, 2000 and 4000 ppm by adding water.

The bases of the cuttings were simply pushed into the powder preparation and the surplus removed by tapping the cuttings on the side of the container, while the bases of other cuttings were dipped into the liquid formulation for a period of 10 seconds or in the low concentration as an overnight soak. Control cuttings were treated in like manner but with the carriers alone. Thereafter all cuttings were transferred to the rooting medium. Each treatment contained

90 cuttings and these were re-treated at monthly intervals with freshly prepared formulations. This was also a convenient time for the check on rooting.

(a) The effect of indole-3-acetic acid on rooting and survival of Eucalyptus gunnii cuttings

Indole-3-acetic acid was applied in both powder and liquid formulations. Powder formulations were used at concentrations of 0.2% and 2.0% w/w IAA. Liquid formulations were used at 2000 and 4000 ppm as a quick dip treatment, at monthly intervals.

Results

Table 17 gives the details of the experimental layout and the results obtained.

Table 17 The effect of auxin treatments on Eucalyptus gunnii cuttings

Treatment	Expt. Ref.	Alive/ Dead	No. of Cuttings Remaining Monthly							
			1	2	3	4	5	6	7	Total 2-7
Control No Auxin P	M6-1	Alive	31	24(2)	17	5	3	3	3	55
		Dead		7	7	12	2	0	0	28
Control No Auxin L	M7-1	Alive	39	33(4)	25	11	6	6	5	86
		Dead		6	8	14	5	0	1	34
Auxin 0.2% P	M6-2	Alive	26	19(1)	14	1	1	1	1	37
		Dead		7	5	13	0	0	0	25
Auxin 2000 ppm L	M7-2	Alive	41	26	17	2	1	1	1	48
		Dead		15	9	15	1	0	0	40
Auxin 2.0% P	M6-3	Alive	25	16(2)	10	2	2	2	2	34
		Dead		9	6	8	0	0	0	23
Auxin 4000 ppm L	M7-3	Alive	35	20	13	1	1	0	0	35
		Dead		15	7	12	0	1	0	35
P = Powder L = Liquid	Total	Alive	197							295
	Total	Dead		59	42	84				185

All treatments commenced in September 1970. Liquid and powder preparations were used and are indicated. The numbers in brackets refer to rooted cuttings.

The Effect of Various Concentrations of Indole-3-acetic acid on the Survival of Eucalyptus gunnii cuttings

Fig. 3

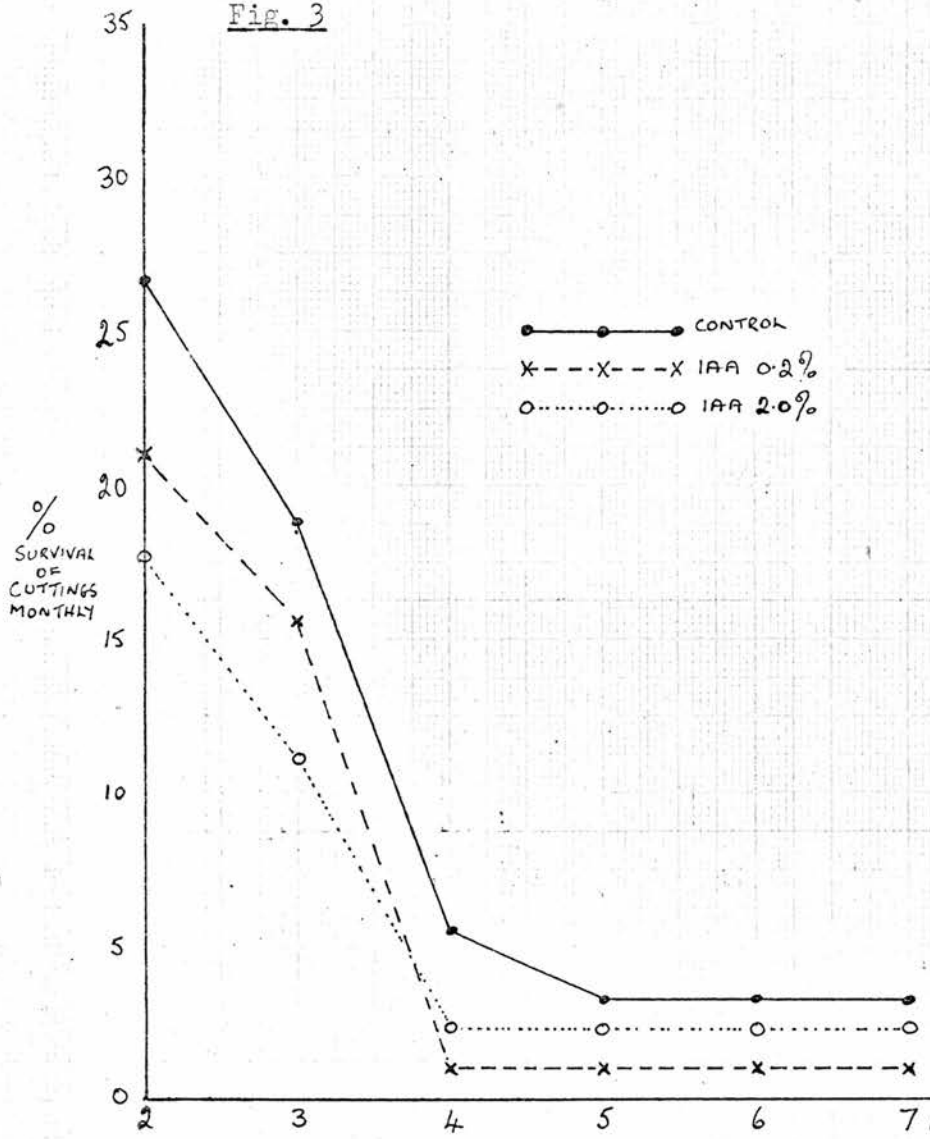
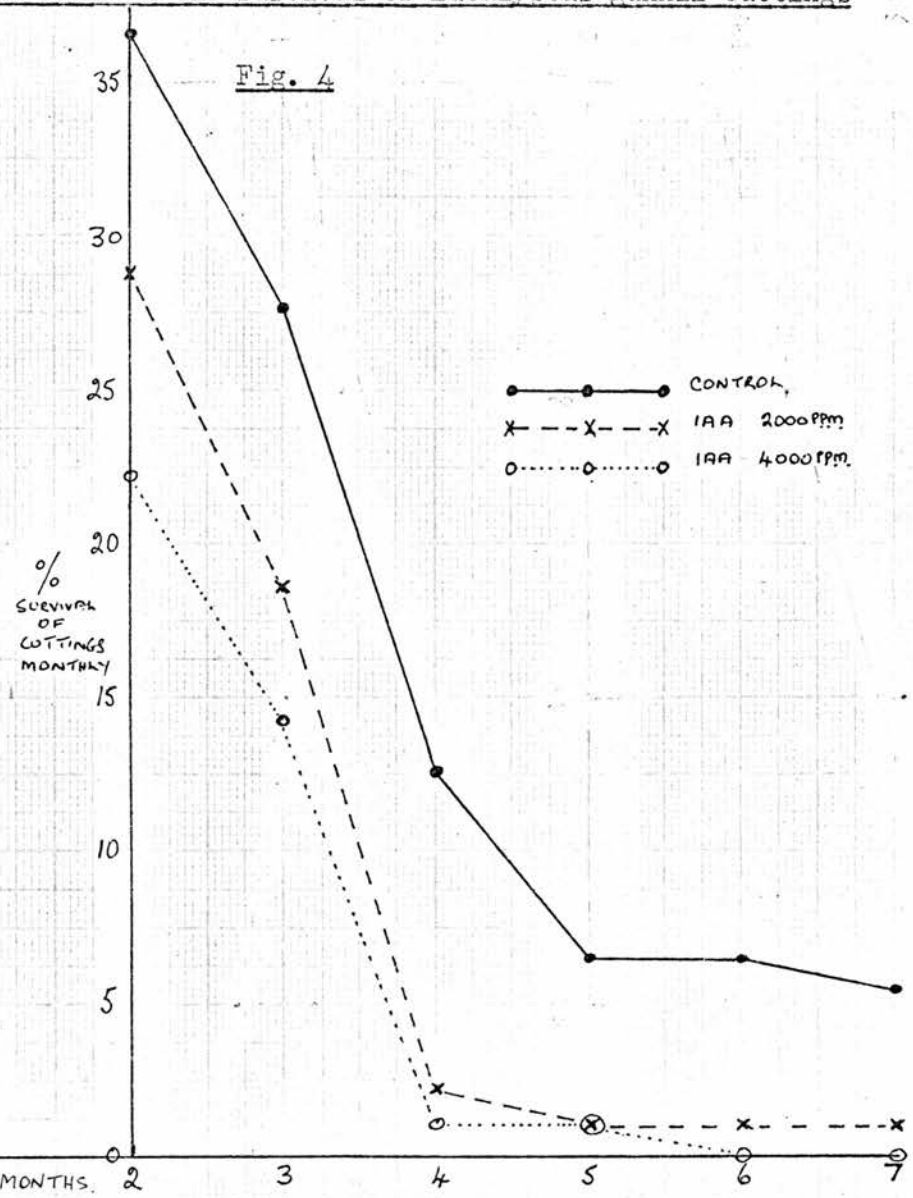


Fig. 4



Ninety cuttings were set in each treatment and the survivors counted at monthly intervals. Both powder and liquid formulations were used.

Interpretation

The data has been fully analysed by two different methods which are typical of the methods used throughout the tests in Chapter 2 - Methods of Statistical Analysis (pp74-77). In the χ^2 test where the most valid and independent analysis was conducted on the monthly death rate of cuttings, the analysis was designed to show up any significant differences in the pattern of treatment effects. In this case with 10 d.f. $p = < 0.80, > 0.50$ - a non-significant result. A further method of comparing treatment effects is shown in Figs. 3 and 4. In the comparable treatments in other series of experiments, it can be clearly seen that a similar pattern of death occurs irrespective of starting numbers. The Analysis of Co-Variance is based on the numbers of cuttings alive at the start, and comparisons are related to the summation of the survivals from months 2 to 7. Here we find that p is almost down to 0.05 for $n_1 = 5$ and $n_2 = 11$. The random component accounts for the greater part of the variance and therefore the treatment effects are at the limits of acceptable significance.

Conclusion

From both methods of analysis of the tests no significant difference was found between cuttings with the auxin IAA treatment and on non-auxin treatment controls.

Effect of Different Auxins on Eucalyptus Cuttings

It is known that the auxin, IAA, is readily oxidised in vivo, and thus its effect on the plant is transitory. It could be possible that IAA is not the most satisfactory auxin to use on

cuttings. IBA and NAA are substances which are not naturally found in the plant but are synthetic in origin. They are known to be more stable to endogenous oxidation and their effect on the cuttings should therefore be more permanent. It is therefore possible that both IBA and NAA, used singly and together, have a more lasting positive effect on rooting, and were therefore tested.

Method

Applications and treatments are described in Chapter 2. IAA, IBA and NAA all at 2000 ppm and dissolved in 45% ethyl alcohol, were used as a quick dip single treatment of cuttings. Various mixtures of these auxins were also used together with a non-auxin treatment which acted as a control.

Results

Results are given in Table 18 and Figs. 5 and 6.

Interpretation

Analysis of the data gives significant results, although the results are not wholly valid since two of the 21 figures are below 5. The χ^2 test for 12 d.f. giving $p = < 0.01, > 0.001$ shows that there are significant treatment effects. The $\chi^2 = 29.27$ is mostly weighted by three figures and it is interesting to note that in one case IBA is involved and in the other two cases NAA used singly and also in combination with IBA are the auxins used. These auxins are persistent in the plant and/or cutting therefore their effect is more prolonged. However, as shown in the graph of cutting survival, the non-auxin control produced the higher survival

Table 18 Effects of various auxins on Eucalyptus cuttings

Treatment	Expt. Ref.	Alive/ Dead	No. of Cuttings Remaining Monthly							
			1	2	3	4	5	6	7	Total 2-7
Control No Auxin	M7-1	Alive	39	33(4)	25	11	6	6	5	86
		Dead		6	8	14	5	0	1	34
IAA	M7-14	Alive	32	21(1)	18	3	2	1	1	46
		Dead		11	3	15	1	1	0	31
IBA	M7-13	Alive	31	26(6)	15	7	6	6	6	66
		Dead		5	11	8	1	0	0	25
NAA	M7-15	Alive	32	26	18	0	0	0	0	44
		Dead		6	8	18	0	0	0	32
IBA/IAA	M7-16	Alive	26	18	11	1	0	0	0	30
		Dead		8	7	10	1	0	0	26
IBA/NAA	M7-17	Alive	24	10(2)	5	2	2	2	2	23
		Dead		14	5	3	0	0	0	22
IAA/NAA	M7-18	Alive	28	22	16	0	0	0	0	38
		Dead		6	6	16	0	0	0	28
	Total	Alive	212							333
	Total	Dead		56	48		94			198

All treatments started in September 1970. All auxin treatments were used at 2000 ppm as a quick dip single treatment. The numbers in brackets refer to rooted cuttings.

The Survival Record of Eucalyptus gunnii Cuttings after Treatment with Several Auxins Alone and in Various Combinations

Fig. 5

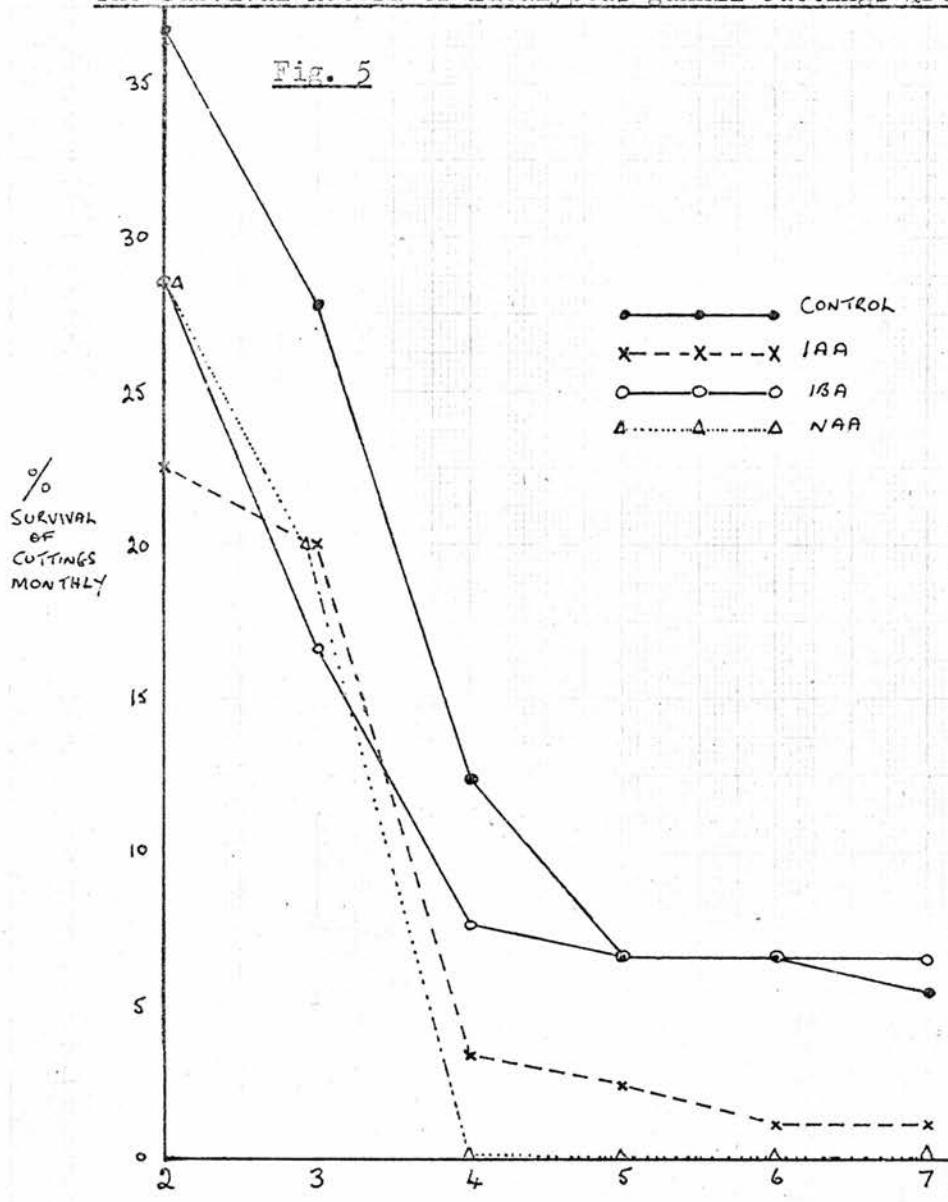
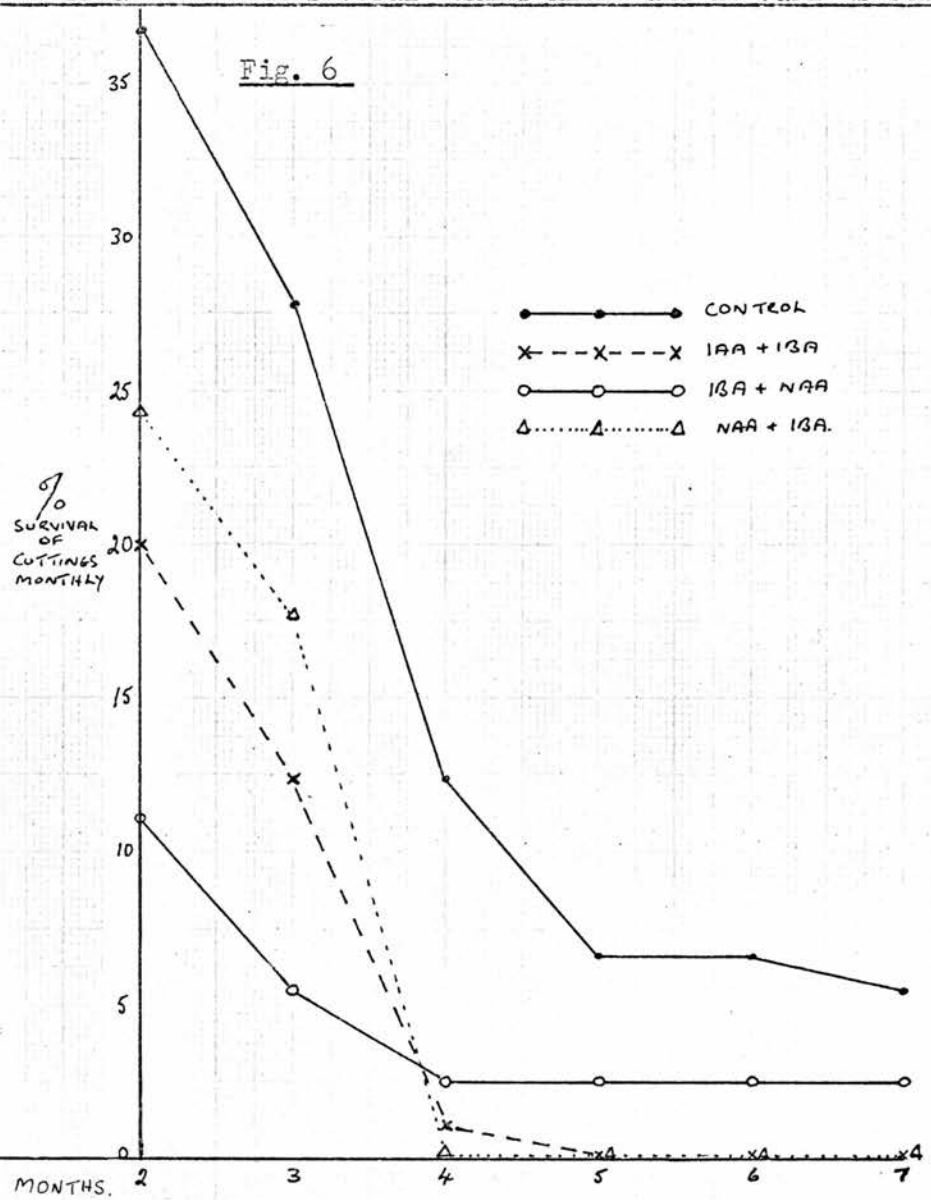


Fig. 6



Ninety cuttings were set in each treatment. Auxins were used at 2000 ppm in every case. The survival rate was recorded monthly.

rate while the more persistent auxins provided the lowest rate. All treatments produced a very low survival record after month four.

In the analysis of co-variance the situation arises where the error component is equal to the treatment component and therefore there is no significant difference from this analysis for survival differences.

Conclusion

The more persistent auxins used singly and together produced a significant deleterious effect compared with the non-auxin treatment. It is therefore clear that auxins, using powder or alcohol/water as carriers, as a basal dip are not beneficial for survival. This adverse effect could be caused by the auxins or by the solvent or talc.

The effect of two solvents on the action of auxin on Eucalyptus Cuttings

The efficiency of auxins on rooting and/or survival may be due to the solvent used. Auxins are generally dissolved in 45% ethyl alcohol and diluted to the required concentration with distilled water. Recently DMSO (dimethylsulphoxide) has proved to cause a beneficial response when used by itself with a variety of cuttings.

The effects of DMSO with and without auxin were therefore tested.

Method

Ethyl alcohol was used as a control solvent for the auxin IBA, which was used at concentrations of 0, 20, 4000, and 8000 ppm IBA. DMSO was used at two concentrations, 100, and 1000 ppm in water, containing the same range of IBA concentrations. Cuttings were

Table 19 The effect of auxin solvents on the rooting and survival of cuttings

Treatment	Expt. Ref.	Alive/ Dead	No. of Cuttings Remaining Monthly							
			1	2	3	4	5	6	7	Total 2-7
Alcohol Solvent										
Control	M12-3	Alive	15	6(1)	4	3	3	3	3	22
No Auxin		Dead		9	2	1	0	0	0	12
Auxin	M12-2	Alive	13	7(1)	6	4(1)	3	3	3	26
20 ppm		Dead		6	1	2	1	0	0	10
Auxin	M12-4	Alive	2	2	1	0	0	0	0	3
4000 ppm		Dead		0	1	1	0	0	0	2
Auxin	M12-5	Alive	5	1	1	0	0	0	0	2
8000 ppm		Dead		4	0	1	0	0	0	5
DMSO 100 ppm Solvent										
Control	M12-6	Alive	4	1	1	1	1	1	1	6
No Auxin		Dead		3	0	0	0	0	0	3
Auxin	M12-7	Alive	8	3	3	2	2(1)	2	2	14
20 ppm		Dead		5	0	1	0	0	0	6
Auxin	M12-9	Alive	1	0	0	0	0	0	0	0
4000 ppm		Dead		1	0	0	0	0	0	1
Auxin	M12-10	Alive	11	6	6	3(1)	2	2	2	21
8000 ppm		Dead		5	0	3	1	0	0	9
DMSO 1000 ppm Solvent										
Control	M12-13	Alive	2	1	1	1	1	1	1	6
No Auxin		Dead		1	0	0	0	0	0	1
Auxin	M12-12	Alive	13	6	4	1	1	1	1	14
20 ppm		Dead		7	2	3	0	0	0	12
Auxin	M12-14	Alive	1	0	0	0	0	0	0	0
4000 ppm		Dead		1	0	0	0	0	0	1
Auxin	M12-15	Alive	3	0	0	0	0	0	0	0
8000 ppm		Dead		3	0	0	0	0	0	3
	Total	Alive	78							114
	Total	Dead		45	6	11	2	0	0	64

Ethyl alcohol acted as a control treatment with and without the auxin concentrations. DMSO at 100 and 1000 ppm were used as solvents for similar auxin concentrations. Auxin at 20 ppm was applied as an overnight soak, while the higher concentrations i.e. 4000 and 8000 ppm were applied as a quick dip single treatment. The numbers in brackets indicate cuttings rooted. 90 cuttings were set in each treatment.

treated as described in Chapter 2 (pp.69-74). The 20 ppm auxin treatment was applied as an overnight soak, whereas the higher concentrations were applied as quick dip treatments.

Results

The results of the tests are shown in Table 19.

Interpretation

Comparison of the treatments using the χ^2 test is not possible due to the low frequency of the data. Even when the auxin treatments for each solvent were added together, the χ^2 test was not valid. The analysis of co-variance produced a non-significant result i.e. $n_1 = 5$ and $n_2 = 2$ giving $p = 0.20$ when each of the solvent treatments were grouped together. The auxin treatment with the consistently higher data was 20 ppm IBA as an overnight soak, irrespective of solvent used. The analysis of co-variance in this test for $n_1 = 5$ and $n_2 = 11$ gave $p = 0.20$, a non-significant result.

Conclusion

There is no difference in the solvents' effect and the combined effect of solvent and auxin. However, the effect of high auxin concentrations was again generally deleterious on cutting survival. DMSO similarly produced an unfavourable effect on cutting survival.

The effect of auxin application as a top dip compared with the accepted basal dip method

The high concentrations of auxin are normally applied as a basal dip treatment and their effects have proved to be harmful to survival and rooting. Another method of application so far untested is to apply the auxin to the apical part of the plant. The endogenous

auxins are synthesized mainly in the apical regions of the shoot and their effect on cuttings is felt mainly at the base of the cutting. Hence the basal application treatment is applied where the effect is known. There is no simple method of finding the rate of absorption and concentration of applied auxin, and the lack of auxin uptake from the base of the cutting may be the cause of this poor rooting and survival effect. However, auxins move polarly downwards in the shoot. Uptake of nutrients and other chemicals through the leaves is known, so therefore the exogenous application of auxin to the shoot apex should lead to absorption by the plant. The auxins should then be transported in similar manner to the endogenous auxins and ultimately produce the required effect at the base of the cutting. This apical treatment of auxin was therefore tested.

Method

Two batches of cuttings were prepared. The first batch was treated with the auxin IBA at 4000 and 8000 ppm concentrations using both 45% ethyl alcohol and 100 and 1000 ppm DMSO as the solvents. These were treated in the normal fashion by the basal dip method. The second batch was given the similar auxin and solvent treatment but this batch of cuttings received an apical dip. The time of dipping in all cases was 10 seconds.

Results

Table 20 gives the results of the treatments.

Table 20 The effects of the basal and apical dip treatments

Treatment		Alive/ Dead	No. of Cuttings Remaining Monthly							
			1	2	3	4	5	6	7	Total 2-7
(a) Basal Dip										
Ethyl Alcohol 45% Solvent										
Control	M12-3	Alive	15	6(1)	4	3	3	3	3	22
No Auxin		Dead		9	2	1	0	0	0	12
Auxin	M12-2	Alive	2	2	1	0	0	0	0	3
4000 ppm		Dead		0	1	1	0	0	0	2
Auxin	M12-5	Alive	5	1	1	0	0	0	0	2
8000 ppm		Dead		4	0	1	0	0	0	5
DMSO 100 ppm Solvent										
Control	M12-6	Alive	4	1	1	1	1	1	1	6
No Auxin		Dead		3	0	0	0	0	0	3
Auxin	M12-9	Alive	1	0	0	0	0	0	0	0
4000 ppm		Dead		1	0	0	0	0	0	1
Auxin	M12-10	Alive	11	6	6	3(1)	2	2	2	21
8000 ppm		Dead		5	0	3	1	0	0	9
DMSO 1000 ppm Solvent										
Control	M12-13	Alive	2	1	1	1	1	1	1	6
No Auxin		Dead		1	0	0	0	0	0	1
Auxin	M12-14	Alive	1	0	0	0	0	0	0	0
4000 ppm		Dead		1	0	0	0	0	0	1
Auxin	M12-15	Alive	3	0	0	0	0	0	0	0
8000 ppm		Dead		3	0	0	0	0	0	3
	Total	Alive	44							60
	Total	Dead		27	3	6				
(b) Apical Dip										
Ethyl Alcohol 45% Solvent										
Control	M13-1	Alive	7	4	2	1	0	0	0	7
No Auxin		Dead		3	2	1	1	0	0	7
Auxin	M13-2	Alive	2	1(1)	1	1	1	1	1	6
4000 ppm		Dead		1	0	0	0	0	0	1
Auxin	M13-3	Alive	1	0	0	0	0	0	0	0
8000 ppm		Dead		1	0	0	0	0	0	1
DMSO 100 ppm Solvent										
Control	M13-4	Alive	12	4	2	2	2	2	2	14
No Auxin		Dead		8	2	0	0	0	0	10
Auxin	M13-5	Alive	9	5	5	5	1	1	1	18
4000 ppm		Dead		4	0	0	4	0	0	8
Auxin	M13-6	Alive	1	1	1	1	1(1)	1	1	6
8000 ppm		Dead		0	0	0	0	0	0	0
DMSO 1000 ppm Solvent										
Control	M13-7	Alive	6	1	0	0	0	0	0	1
No Auxin		Dead		5	1	0	0	0	0	6
Auxin	M13-8	Alive	4	1	1	1	0	0	0	3
4000 ppm		Dead		3	0	0	1	0	0	4
Auxin	M13-9	Alive	4	2	1	0	0	0	0	2
8000 ppm		Dead		2	1	1	0	0	0	4
	Total	Alive	46							58
	Total	Dead		27	6	8				

All cuttings received a quick dip single treatment and were set in January 1971. They were grown under a 16 hour photoperiod and checked at monthly intervals. Numbers in brackets refer to rooted cuttings. 90 cuttings were set in each treatment.

Discussion

The χ^2 test was not valid in the analysis of this data since expected values for almost 2/3 of the data are below 5. Since 90 cuttings were initially set and the present analysis is based on the number of cuttings remaining at the end of one month, there is obviously a non-treatment factor affecting the cuttings so individual analysis is impossible. However, by lumping the data into two groups, that for basal dip and also for apical dip, the data can be analysed by χ^2 and analysis of co-variance. The χ^2 test gives a non-significant result at 2 d.f. $p = < 0.99, > 0.98$. Analysis of co-variance likewise gives a similar result. With $n_1 = 11$ and $n_2 = 5$, $p = > 0.20$. Certainly it could be argued that the results are due to non-treatment random effects since the actual number of cuttings remaining alive were extremely low. Seasonal effect is thought likely to be the cause of this (see pp. 110-112).

Conclusion

No definite pattern of effect is shown and it can only be assumed that there is no difference due to the site of application.

Summary of Auxin Conclusions

All the auxins tested produced effects which were deleterious to survival and rooting. This was particularly evident with the higher concentrations. However, even non-auxin treatment controls produced poor results.

It must therefore be noted that for some physiological reason, auxins alone have a damaging effect on cuttings of Eucalyptus gunnii. The physiological effect of endogenous inhibitors is suggested as one of the reasons for the mortality of cuttings. This is tested in the chromatographic analysis below (pp. 162-185).

We have already discussed the possibility of fungal infection and found that disease can be prevented with systematic fungicidal treatment.

Auxin treatment on Eucalyptus gunnii did not produce the anticipated rooting and survival which occur normally in most genera. Further tests using other growth substances alone and in combination with auxin have proved beneficial in many genera. Such substances include Gibberellic Acid, Kinetin, Chlorogenic Acid, vitamins and sugars.

(b) The effects of Gibberellic Acid on the rooting and survival of cuttings

As stated earlier in this work (p. 54) there are two known possible actions by GA in propagation. Firstly that it has an inhibiting effect on rooting and survival due to the increase in shoot growth at the expense of root activity, and secondly, that GA inhibits the activity of auxin oxidase thus stimulating the effect of auxins. Recent research certainly shows that auxin levels are significantly

increased after treatment with GA. Research has also found that GA inhibits rooting in plants which otherwise would root readily. Similarly GA treatment in combination with auxin has proved to be of little benefit in rooting. However, keeping in mind that GA stimulates the effects of auxin, and that different plants' responses to one chemical or compound are not always similar, then the effect of GA alone and in combination with various auxins, is worth testing.

Method

Liquid formulations of auxins, IAA, IBA and NAA were used at 2000 ppm in 45% ethyl alcohol. GA was used at 1 mg/litre. Cuttings were simply dipped into the liquid for 10 seconds and then immediately inserted into the rooting medium in accordance with the statistical layout described in Chapter 2. There was one non-treatment control. Other treatments included the three auxins used separately and in combination with GA and a GA treatment alone. Ninety cuttings were set in each case.

Results

Table 21 gives details of the tests together with the data for each month.

Interpretation

Both the χ^2 test and the analysis of co-variance produded non-significant results. From a cursory glance at the data it is clear that the GA and combined GA/auxin give poor survival rates. Auxins on their own provide slightly better survival rates, but the non-treatment control provided the best survival record.

Table 21 The effect of GA and auxins on rooting and survival

Treatment	Expt. Ref.	Alive/ Dead	No. of Cuttings Remaining Monthly							
			1	2	3	4	5	6	7	Total 2-7
Control No Auxin	M7-1	Alive	39	33(4)	25	11	6	6	5	86
		Dead		6	8	14	5	0	1	34
IAA	M7-14	Alive	32	21	18	3	2	1	1	46
		Dead		11	3	15	1	1	0	31
IBA	M7-13	Alive	31	26	15	7	6	6	6	66
		Dead		5	11	8	1	0	0	25
NAA	M7-15	Alive	32	26	18	0	0	0	0	44
		Dead		6	8	18	0	0	0	32
GA	M8-1	Alive	20	14(1)	7	4	3	3	2	33
		Dead		6	7	3	1	0	1	18
IAA/GA	M8-5	Alive	21	11(2)	6	2	2	2	2	25
		Dead		10	5	4	0	0	0	19
IBA/GA	M8-4	Alive	22	14	4	0	0	0	0	18
		Dead		8	10	4	0	0	0	22
NAA/GA	M8-6	Alive	17	11	5	0	0	0	0	16
		Dead		6	6	5	0	0	0	17
	Total	Alive	214							334
	Total	Dead		58	58	82				198

Auxins were applied at 2000 ppm and GA at 1 mg/litre as a single quick dip treatment. The numbers in brackets indicate cuttings rooted. 90 cuttings were set in each treatment.

Conclusion

Gibberellic acid alone or in combination with auxins has no significant effect on the survival and rooting of cuttings.

(c) The effect of Kinetin on Eucalyptus gunnii cuttings

Since most of the experimentation on the effect of kinetin has been conducted in *in vitro* culture, the effect on cuttings is uncertain. However, there are clear indications that a high kinetin/low auxin ratio is necessary for callus formation, and a low kinetin/high auxin ratio for root initiation.

With other genera which have proved difficult to root, callus formation seems to be a prerequisite before rooting can take place. It is known from anatomical studies that root initiation can take place in the cambium cells of the callus and therefore the application of kinetin to cuttings is worthy of further study outwith the confines of *in vitro* culture.

Method

The kinetin (6-furfuryl-amino purine) was used at 2% w/w as a powder and at 2 mg/l in liquid formulation. The powder formulation used talc as the carrier and distilled water was used as the liquid solvent. Kinetin was used on its own as a first test but with a non-kinetin treatment to act as a control sample. Samples of cuttings were tested at different times of the year and are compared below.

Results

These are shown in Table 22.

Table 22 The effect of Kinetin on rooting and survival

Treatment	Expt. Ref.	Alive/ Dead	No. of Cuttings Remaining Monthly							
			1	2	3	4	5	6	7	Total 2-7
Control No Kinetin P	M3/5-1	Alive	33	26	17	15	11	5	1	75
		Dead		7	9	2	4	6	4	32
Kinetin 2% w/w P	M3/5-4	Alive	19	11	8	8	8	5	0	40
		Dead		8	3	0	0	3	5	19
Control No Kinetin P	M6-1	Alive	31	24(2)	17	5	3	2	2	55
		Dead		7	7	12	2	0	0	28
Kinetin 2% w/w P	M6-4	Alive	36	28	13	0	0	0	0	41
		Dead		8	15	13	0	0	0	36
Control No Kinetin L	M7-1	Alive	39	33(5)	25	11	6	6	5	86
		Dead		6	8	14	5	0	1	34
Kinetin 2 mg/l L	M7-4	Alive	39	29(1)	16	4	4	3	3	59
				10	13	12	0	1	0	36
Kinetin 2 mg/l L	M7-19	Alive	36	32	18	1	0	0	0	51
		Dead		4	14	17	1	0	0	36
Control No Kinetin L	M10-1	Alive	22	5	1	1	1	1	1	10
		Dead		17	4	0	0	0	0	21
Kinetin 2 mg/l L	M10-4	Alive	13	1	1	1	0	0	0	3
		Dead		12	0	0	0	0	0	12
P = Powder	Total	Alive	258							520
L = Liquid	Total	Dead		79	83		100			262

Cuttings were taken in April (M3/5), September (M6 and M7) and in December (M10). These all received a single initial treatment. Cuttings involved with the liquid formulations received a quick dip treatment. All numbers in brackets refer to cuttings rooted.
90 cuttings were set in each treatment.

Interpretation

The only valid set of data for the χ^2 test is from the M6 series. The result in this instance produced a non-significant effect: $p = < 0.50, > 0.20$. Similarly by adding the M6 and M7 series a non-significant result was found ($p = < 0.10, > 0.05$). When the data was tested by the analysis of co-variance again a non-significant result was found ($p = < 0.10, > 0.05$), for $n_1 = 8$ and $n_2 = 17$.

During the course of the eight month study, only 1 cutting rooted in the kinetin treated samples, whereas 7 rooted in the control samples. Survival rates were also generally better in control samples.

Conclusion

Kinetin on its own appears to have little or no effect on cuttings of Eucalyptus gunnii applied as a routine propagation treatment.

(d) The effect of Kinetin and Auxin on Eucalyptus Cuttings

As stated above, callus and root initiation is controlled by the ratio of endogenous levels of kinetin and auxin. Both have been applied in in vitro culture on Eucalyptus camaldulensis. Kinetin, present in coconut milk, was found to be essential for the growth of roots, while auxin on its own produced a poor effect. Together, and including other micronutrients, their effect was not as good as kinetin alone, (Bachelard and Stowe, 1963). By applying both auxin and kinetin singly as a means of control, a test was conducted on their effects in combination on E. gunnii.

Method

Kinetin was applied throughout at 2 mg/l furfuryl amino purine in water in the tests, except for the non-treatment control sample. The auxin IAA was used as a liquid formulation at two concentrations 0.2% and 2.0% w/w IAA.

Results

Table 23 shows both the treatments and the results.

Table 23 The effect of differing auxin concentrations and kinetin on cuttings

Treatment	Expt. Ref.	Alive/ Dead	No. of Cuttings Remaining Monthly							
			1	2	3	4	5	6	7	Total 2-7
Control No Auxin	M7-1	Alive	39	33(4)	25	11	6	6	5	86
		Dead		6	8	14	5	0	1	34
IAA 0.2%	M7-2	Alive	41	26	17	2	1	1	1	48
		Dead		15	9	15	1	0	0	40
IAA 2.0%	M7-3	Alive	35	20	13	1	1	0	0	35
		Dead		15	7	12	0	1	0	35
Kinetin 2 mg/l	M7-4	Alive	39	29(1)	16	4	4	3	3	59
		Dead		10	13	12	0	1	0	36
Kinetin + IAA 0.2%	M7-5	Alive	37	25(1)	17	3	1	1	1	48
		Dead		12	8	14	2	0	0	36
Kinetin + IAA 2.0%	M7-6	Alive	35	19(1)	13	5	4	4	3	48
		Dead		16	6	8	1	0	1	32
	Total	Alive	226							324
	Total	Dead		74	51		88			213

Kinetin was applied as a standard 2 mg/l, alone and in combination with auxin which was used at 0.2% and 2.0%. Cuttings received a single quick dip treatment and were all inserted into the rooting medium in September 1970. The numbers in brackets refer to cuttings rooted.

Interpretation

The data is suitable for both the χ^2 test and the analysis of co-variance. Both give a non-significant result: $\chi^2 = 10.85$ with 10 d.f. $p = < 0.50, > 0.20$, and the analysis of co-variance for $n_1 = 5$, $n_2 = 11$, giving $p = < 0.20, > 0.05$.

It is, however, interesting to note that the kinetin treatments both with and without auxin produced 1 rooted cutting in each, while for the non-treatment control sample of a similar number of cuttings produced 4 rooted cuttings. These numbers in themselves are not validly significant nor can they in themselves indicate a trend.

If other series of similar tests are compared (the M3/5 series) we find with powder application a non-significant result in the analysis of co-variance - for $n_1 = 5$, $n_2 = 11$, $p = > 0.20$. The χ^2 is not valid since several numbers are lower than 5. However, in this case rooting (3 and 1) occurred only in the two single auxin treatments which further confuses the issue. If we look at the M6 series, a similar series of tests with powder formulators, we find again non-significant results - $\chi^2 = 17.98$ for 10 d.f., $p = < 0.10, > 0.05$, and with the analysis of co-variance for $n_1 = 5$, $n_2 = 11$, giving $p = > 0.20$. In this instance rooting occurred in the non-kinetin treated cuttings, albeit in low numbers i.e. Control = 2; IAA at 0.2% = 1; IAA at 2.0% = 2.

Conclusion

Thus we find that there is no significant difference between the treatments. Rooting occurred in all tests when taking the three series into account but at totally unacceptable levels.

(e) The effect of GA on the auxin/kinetin ratio

Since auxin, kinetin and gibberellic acid have all been tested singly and in pairs, and their effect with cuttings which root readily is generally unfavourable, there is still a possibility that when all are used in association a favourable result will be achieved.

Method

All growth substances were applied in liquid formulations. Kinetin was prepared to 2 mg/litre concentrations, while GA was applied at 1 mg/litre. The auxin NAA was applied at 2000 ppm in 45% ethyl alcohol. Ninety cuttings were used in each treatment.

Results

The layout of the experiments and the results are shown in Table 24.

Table 24 The effect of various growth substances on survival and rooting

Treatment	Expt. Ref.	Alive/ Dead	No. of Cuttings Remaining Monthly							
			1	2	3	4	5	6	7	Total 2-7
Non-treatment Control	M7-1	Alive	39	33(4)	25	11	6	6	5	86
		Dead		6	8	14	5	0	1	34
Auxin NAA	M7-15	Alive	32	26	18	0	0	0	0	44
		Dead		6	8	18	0	0	0	32
Kinetin	M7-19	Alive	36	32	18	1	0	0	0	51
		Dead		4	14	17	1	0	0	36
GA	M7-21	Alive	35	28(2)	23	7	4	3	2	68
		Dead		7	5	16	3	1	1	33
GA	M8-1	Alive	20	14(1)	7	4	3	3	2	33
		Dead		6	7	3	1	0	1	18
GA+Kinetin	M8-2	Alive	17	13	8	0	0	0	0	21
		Dead		4	5	8	0	0	0	17
GA+NAA	M8-6	Alive	17	11	5	0	0	0	0	16
		Dead		6	6	5	0	0	0	17
GA+NAA+Kinetin	M8-3	Alive	14	12	6	0	0	0	0	18
		Dead		2	6	6	0	0	0	14
	Total	Alive	210							338
	Total	Dead		41	59		101			201

All treatments were started in September 1970. The auxin NAA was applied at 2000 ppm; GA at 1 mg/litre, and kinetin at 2 mg/litre. Ninety cuttings were set in each treatment and the numbers in brackets refer to those cuttings which rooted. 90 cuttings set in each treatment

Interpretation

Early impressions of the data fail to imply any beneficial effect. In fact, the combined treatments of all the growth substances indicate a deleterious effect. In the statistical analysis of the data no χ^2 test is valid, but in the analysis of co-variance a non-significant result was obtained: $p = > 0.20$ for $n_1 = 5$, $n_2 = 17$.

Conclusion

GA used singly, decreased survival compared with controls and in combination with auxin or kinetin, or both, decreased survival even further. Thus no indication of any of the major growth substances which will aid rooting or the survival of cuttings, is evident.

(f) The effect of organic nitrogen treatment on cuttings

I have argued above that the cuttings require a plentiful supply of nutrients in order to sustain growth for rooting. Certainly the cuttings chosen were in active growth; had at least two pairs of leaves and a sturdy stem. Food materials should therefore have been adequate.

Organic nitrogen as a nutrient source in the form of casein hydrolysate is used in *in vitro* culture to provide the developing cells with nutrients and therefore the hypothesis, that nutrient limitation may be the cause of the lack of rooting, was tested.

Method

In the first instance auxin and casein hydrolysate, the organic nitrogen source, were tested to compare their effects. Auxin was

used at two concentrations 0.2% and 2.0% w/w IAA; casein hydrolysate was used at the concentration 3 gm per litre; and kinetin at 5 mg/litre was added to determine the further effect of another growth substance.

Results

The full table of results is given below. This also shows the experimental references and the treatments.

Table 25 The effect of organic nitrogen on the influence of auxin and kinetin in rooting

Treatment	Expt. Ref.	Alive/ Dead	No. of Cuttings Remaining Monthly							
			1	2	3	4	5	6	7	Total 2-7
Non-treatment Control	M7-1	Alive	39	33(4)	25	11	6	6	5	86
		Dead		6	8	14	5	0	1	34
IAA 0.2%	M7-2	Alive	41	26	17	2	1	1	1	48
		Dead		15	9	15	1	0	0	40
IAA 2.0%	M7-3	Alive	35	20	13	1	1	0	0	35
		Dead		15	7	12	0	1	0	35
Kinetin	M7-4	Alive	39	29(1)	16	4	4	3	3	59
		Dead		10	13	12	0	1	0	36
IAA 0.2% + Kinetin	M7-5	Alive	37	25(1)	17	3	1	1	1	48
		Dead		12	8	14	2	0	0	36
IAA 2.0% + Kinetin	M7-6	Alive	35	19(1)	13	5	4	4	3	48
		Dead		16	6	8	1	0	1	32
Casein	M7-7	Alive	32	24(1)	18	6	4	3	2	57
		Dead		8	6	12	2	1	1	30
IAA 0.2% + Casein	M7-8	Alive	48	35	20	3	0	0	0	58
		Dead		13	15	17	3	0	0	48
IAA 2.0% + Casein	M7-9	Alive	34	27(1)	17	2	1	1	1	49
		Dead		7	10	15	1	0	0	33
Casein + Kinetin	M7-10	Alive	30	18	10	0	0	0	0	28
		Dead		12	8	10	0	0	0	30
IAA 0.2% + Casein + Kinetin	M7-11	Alive	39	33	24	1	0	0	0	58
		Dead		6	9	23	1	0	0	39
IAA 2.0% + Casein + Kinetin	M7-12	Alive	38	30(1)	22	3	2	2	1	60
		Dead		8	8	19	1	0	1	37

Kinetin at 5 mg/litre and casein hydrolysate at 3 gm/litre were used throughout the tests. Auxin (IAA) was used at two concentrations 0.2% and 2.0%. Ninety cuttings were initially set. Numbers in brackets refer to cuttings rooted. 10 cuttings set in each treatment

Interpretation

Comparison of the auxin treatments, M7-1, 2 and 3, with added casein hydrolysate, M7-7, 8 and 9, produced a non-significant result in the χ^2 test (χ^2 = 10.23 for 10 d.f., p = $< 0.50, > 0.20$). Likewise in the analysis of co-variance a similar result was obtained for $n_1 = 5$, $n_2 = 11$, giving p = 0.05.

By adding kinetin in the form of 6-furfuryl-amino-purine to the auxin and casein treatments (M7-10, 11 and 12) and comparing with the auxin supply (M7-1, 2 and 3) and kinetin alone (M7-4, 5 and 6), the χ^2 test still gives a non-significant result: (χ^2 = 24.41 with 16 d.f., p = $< 0.10, > 0.05$), and in the analysis of co-variance for $n_1 = 8$, $n_2 = 17$, giving p = $< 0.20, > 0.05$). Rooting occurred throughout the treatments - the majority (4) being in the non-treatment control sample. Therefore no indication can be found at all for the most suitable treatment to adopt.

Conclusion

There is thus no clear indication on which growth substance or group of substances, or indeed organic nutrients, will provide the answer to the lack of rooting and poor survival record.

(g) The synergistic effect of vitamins on rooting

Vitamins have been known to assist rooting in many species which prove difficult to root. The synergistic properties of vitamins and auxins is illustrated above (pp. 58-62).

Vitamins B and C both seem to have a species selectivity effect. They assist some species to root but do not help others. The action of Vitamin B is to promote endogenous coenzyme activity in the cutting.

Vitamin B was firstly tested to see if Eucalyptus gunnii was a species responsive to vitamin application.

Method

Auxin and vitamin B were applied singly and in combination. There was also a non-treatment control sample. Auxin was applied at 0.2% w/w IAA and vitamin B at 1% w/w at three seasons of the year. The vitamin B was prepared by grinding tablets containing thiamine hydrochloride. Both powder and liquid formulations were used.

Results

The results are shown in three separate tables, 26, 27 and 28. Each indicates a different season, and valid statistical analysis was conducted on each separately to prevent confounding the results.

Table 26 The effect of vitamin B and auxins on cuttings taken in the spring

Treatment	Expt. Ref.	Alive/ Dead	No. of Cuttings Remaining Monthly							
			1	2	3	4	5	6	7	Total 2-7
Non-treatment Control	M3/5-1	Alive	33	26	17	15	11	5	1	75
		Dead		7	9	2	4	6	4	32
IAA 0.2% w/w	M3/5-2	Alive	22	16	13	11	8	3	3	54
		Dead		6	3	2	3	5	0	19
Vitamin B 1%	M3/5-13	Alive	28	16	11	9	5	2	1	44
				12	5	2	4	3	1	27
IAA+Vitamin B	M3/5-14	Alive	27	18	15	11	9	6	4	63
		Dead		9	3	4	2	3	2	23
	Total	Alive	110							236
	Total	Dead		34	20	47				101

Cuttings were treated in April 1970 using the auxin IAA at 0.2% w/w and vitamin B_1 at 1% w/w as a powder formulation.

Table 27 The effect of vitamin B and auxins on cuttings taken in the summer

Treatment	Expt. Ref.	Alive/ Dead	No. of Cuttings Remaining Monthly							
			1	2	3	4	5	6	7	Total 2-7
Non-treatment Control	M6-1	Alive	31	24(2)	17	5	3	3	3	55
		Dead		7	7	12	2	0	0	28
IAA 0.2% w/w	M6-2	Alive	26	19(1)	14	1	1	1	1	37
		Dead		7	5	13	0	0	0	25
Vitamin B 1%	M6-13	Alive	22	15	13	6	2	1	1	38
		Dead		7	2	7	4	1	0	21
IAA+Vitamin B	M6-14	Alive	27	20(2)	9	2	2	2	2	37
		Dead		7	11	7	0	0	0	25
	Total	Alive	106							167
	Total	Dead		28	25	46				99

Cuttings were treated in September 1970 using powder formulations of IAA at 0.2% w/w and vitamin B_1 at 1% w/w. Numbers in brackets refer to cuttings rooted.

Table 28 The effect of vitamin B and auxins on cuttings taken in the winter

Treatment	Expt. Ref.	Alive/ Dead	No. of Cuttings Remaining Monthly							
			1	2	3	4	5	6	7	Total 2-7
Non-treatment Control	M10-1	Alive	22	5	1	1	1	1	1	10
		Dead		17	4	0	0	0	0	21
IAA 0.2% w/w	M10-2	Alive	14	3	3	3	3	3	3	18
		Dead		11	0	0	0	0	0	11
Vitamin B 1%	M10-15	Alive	28	5	2	2	1	1	1	12
		Dead		23	3	0	1	0	0	27
IAA+Vitamin B	M10-16	Alive	21	5	1	1	0	0	0	7
		Dead		16	4	0	1	0	0	21
	Total	Alive	85							47
	Total	Dead		67	11	2				80

Cuttings were treated in December 1970 with liquid formulations of IAA at 0.2% and vitamin B_1 at 1%. Ninety cuttings were set in each treatment and these were checked at monthly intervals.

Interpretation

The analysis of co-variance in the M3/5 series of experiments gives a non-significant result - for $n_1 = 7$, $n_2 = 3$ giving $p = > 0.20$.

Similarly in the M6 series, which also used powder formulations, a non-significant result is given - for $n_1 = 3$, $n_2 = 7$ giving $p = > 0.20$. Thus there is no significant difference in the treatment effects with powder applications.

The same experiment was conducted using liquid preparations (M10 series) and in the winter time, but again a similar non-significant result was obtained - for $n_1 = 7$, $n_2 = 3$ giving $p = < 0.20, > 0.05$.

Conclusion

Vitamin B has no significant effect on Eucalyptus gunnii cuttings irrespective of method of application or seasonal effect.

The effect of vitamin C on Eucalyptus cuttings

Vitamin C has also a species selectivity record. It has certainly proved valuable in increasing the rooting rate of some genera.

Method

Vitamin C was applied at 1% w/w as ascorbic acid in both powder and liquid formulations. The auxin IAA at 0.2% w/w was used singly and in combination with vitamin C. There was also a non-treatment control sample. Cuttings were treated at three seasons of the year.

Results

Tables 29, 30 and 31 give the treatments and their results.

Table 29 The effects of vitamin C and auxins on cuttings taken in the spring

Treatment	Expt. Ref.	Alive/ Dead	No. of Cuttings Remaining Monthly							
			1	2	3	4	5	6	7	Total 2-7
Non-treatment Control	M3/5-1	Alive	33	26	17	15	11	5	1	75
		Dead		7	9	2	4	6	4	32
IAA 0.2% w/w	M3/5-2	Alive	22	16	13	11	8	3	3	54
		Dead		6	3	2	3	5	0	19
Vitamin C 1%	M3/5-15	Alive	28	22	18	15	10	4	2	71
		Dead		6	4	3	5	6	2	26
IAA+Vitamin C	M3/5-16	Alive	27	19	15	10	4	2	1	51
		Dead		8	4	5	6	2	1	26
	Total	Alive	110							251
	Total	Dead		27	20		56			103

Cuttings were treated in April 1970 using the auxin IAA at 0.2% w/w and vitamin C at 1% w/w as a powder formulation.

Table 30 The effects of vitamin C and auxins on cuttings taken in the summer

Treatment	Expt. Ref.	Alive/ Dead	No. of Cuttings Remaining Monthly							
			1	2	3	4	5	6	7	Total 2-7
Non-treatment Control	M6-1	Alive	31	24(2)	17	5	3	3	3	55
		Dead		7	7	12	2	0	0	28
IAA 0.2% w/w	M6-2	Alive	26	19(1)	14	1	1	1	1	37
		Dead		7	5	13	0	0	0	25
Vitamin C 1%	M6-15	Alive	28	19	13	1	0	0	0	33
		Dead		9	6	12	1	0	0	28
IAA+Vitamin C	M6-16	Alive	30	20(2)	12	3	3	3	3	44
		Dead		10	8	9	0	0	0	27
	Total	Alive	115							169
	Total	Dead		33	26		49			108

Cuttings were treated in September 1970 using powder formulations of IAA at 0.2% w/w and vitamin C at 1% w/w. Numbers in brackets refer

Table 31 The effects of vitamin C and auxins on cuttings taken in the winter

Treatment	Expt. Ref.	Alive/ Dead	No. of Cuttings Remaining Monthly							
			1	2	3	4	5	6	7	Total 2-7
Non-treatment Control	M10-1	Alive	22	5	1	1	1	1	1	10
		Dead		17	4	0	0	0	0	21
IAA 0.2% w/w	M10-2	Alive	14	3	3	3	3	3	3	18
		Dead		11	0	0	0	0	0	11
Vitamin C 1%	M10-17	Alive	18	2	0	0	0	0	0	2
		Dead		16	2	0	0	0	0	18
IAA+Vitamin C	M10-18	Alive	21	7	2	0	0	0	0	9
		Dead		14	5	2	0	0	0	21
	Total	Alive	75							39
	Total	Dead		58	11		2			71

Cuttings were treated in December 1970 with liquid formulations of IAA at 0.2% w/w and vitamin C at 1% w/w. Ninety cuttings were set in each treatment and these were checked at monthly intervals.

Interpretation

Not only can the effects of vitamin C alone and in combination with auxin be compared but the seasonal exposure of cuttings to these treatments can be determined.

The χ^2 test is only valid in the M6 series and there is no significant difference - $\chi^2 = 2.57$ and for 6 d.f. $p = < 0.90, > 0.80$. Similarly, when the data from the M6 series was tested by the analysis of co-variance, there was no significant difference, with $p = > 0.20$.

In the M3/5 series, by the analysis of co-variance the result showed a similar trend, $p = < 0.20, > 0.05$. The M10 series

produced such poor results that a visual scan of the data clearly shows again that there is no significant difference in the treatments. By the analysis of co-variance test, $p = < 0.20, > 0.05$.

Conclusion

Clearly the best times for setting cuttings are in the actively growing seasons. No preference can be seen for either April or September on the numbers of cuttings alive at the start of the analysis, although more cuttings remained alive at the end of the test with the springtime setting. However, on the analysis of treatment effects, no significant difference was found between Vitamin C and control treatments.

(R) The effect of sugars on the rooting of Eucalyptus cuttings.

There have been mixed results to the application of sugars to cuttings. Some degree of specificity is suspected. Fazio (1964) indicated that sugars caused decay when applied to Eucalyptus rostrata cuttings, and Bachelard and Stowe (1963) failed to see any response from Eucalyptus camaldulensis roots grown in vitro.

However, Thimann and Behnke-Rogers (1950) list several genera which responded quite remarkably to added sucrose treatment. Its effect on Eucalyptus gunnii was therefore tested.

Method

Cuttings were obtained in March 1972 and treated with four preparations which included sucrose. Sucrose at 2% plus a phosphate buffer was used alone for one treatment. To this was added NAA and IBA as a combined auxin treatment at 2000 ppm. Kinetin was applied at 2 mg/litre together with sucrose, and finally all the substances were used in a fully combined treatment.

Results

The results are shown in Table 32.

Table 32 The effect of sugar, alone and combined with auxin and kinetin, on Eucalyptus gunnii cuttings.

Treatment	Expt. Ref.	Alive/ Dead	No. of Cuttings Remaining Monthly							
			1	2	3	4	5	6	7	Total 2-7
Sucrose	M23-2	Alive	27	9	6(3)	3	3	3	3	27
		Dead		18	3	3	0	0	0	24
Sucrose+IAA+IBA	M23-1	Alive	23	13	8(5)	5	5	5	5	41
		Dead		10	5	3	0	0	0	18
Sucrose+Kinetin	M23-3	Alive	31	8	4(3)	3	3	3	3	24
		Dead		23	4	1	0	0	0	28
Sucrose+Kinetin+ IAA+IBA	M23-4	Alive	30	12	6(3)	3	3	3	3	30
		Dead		18	6	3	0	0	0	27
	Total	Alive	111							122
	Total	Dead		69	18		10			97

Sucrose was applied as a 2% solution containing a phosphate buffer. The auxins, IAA and IBA, were used as a combined auxin treatment at 2000 ppm with sucrose. Similarly, kinetin at 2 mg/litre was added to sucrose for a further test. Finally, all substances were fully combined. Cuttings were treated in March 1972. The numbers in brackets refer to cuttings rooted.

Interpretation

While 14 cuttings rooted (an overall percentage of 7.78%), the highest yet recorded in all the tests, the analysis of the data still gives a non-significant result. In the χ^2 test $p = < 0.50, > 0.20$, while the analysis of co-variance gives a similar value i.e. $p = < 0.50, > 0.20$. From the point of view of rooting, these results

are encouraging even though survival values are disappointing. There could be justification for further experiments with the further usage of sucrose as a combination with other growth substances.

Conclusion

The results from application of sugars and phosphate with and without auxins are non-significant but give higher rooting than any other treatment. Sucrose and a phosphate buffer were used in all the tests. Further tests separating the sucrose and phosphate treatments and with and without auxins may show more clearly that sucrose or phosphate, or both, are beneficial for survival and rooting.

(c) The effect of chlorogenic acid in propagation

Chlorogenic acid has been known to stimulate root formation in young seedlings (see p. 56). This work has been carried out mainly in in vitro culture.

Chlorogenic acid is known to be present in higher concentrations in juvenile or young tissue than in adult or more mature growths. Juvenile shoots of many plants root much more easily than their adult growths. Chlorogenic acid then may be one of the major factors influencing the rooting of these juvenile forms. This hypothesis was therefore tested.

Method

Juvenile Eucalyptus shoots were received in early May. Their bases were placed in water containing the systemic fungicide Benomyl, for 24 hours before making the cuttings. The various treatments were applied to the cuttings by a single "quick dip" method in a liquid preparation.

Chlorogenic acid was applied at the concentration 10^{-3}M alone and in combibation with auxin.

The auxin, IBA, was used at a concentration of 4000 ppm, while *Ethrel was used at two concentrations i.e. 0.01% and 0.40%. There was a mixture of all three substances and in this case Ethrel was used at the 0.01% concentration. A non-treated sample acted as a control.

Results

Mortality was very high from the start with the exception of the non-treated control samples. The treatments and results are given in Table 33.

Table 33 The effect of chlorogenic acid, alone and combined with auxin and ethrel on the survival and rooting of Eucalyptus gunnii cuttings

Treatment	Expt. Ref.	No. of Cuttings Remaining Alive Monthly			
		1	2	3	Total 1-3
Non-treatment Control	M29-1	11	10	8(1)	29
IBA 4000 ppm	M29-2	7	3	0	10
Chlorogenic Acid 10^{-3}M	M29-3	7	6	3	16
IBA+Chlorogenic Acid	M29-4	5	2	0	7
Ethrel 0.01%	M29-5	4	1	1	6
IBA+Ethrel 0.01%	M29-6	6	2	0	8
Ethrel 0.40%	M29-7	3	0	0	3
IBA+Ethrel 0.40%	M29-8	6	1	0	7
IBA+Chlorogenic Acid+ Ethrel 0.40%	M29-9	3	0	0	3

Thirty cuttings were set in each treatment in May 1974. They received normal daylight conditions and a cool temperature of 15°C. They were pushed into a peat/perlite mixture. Numbers of cuttings rooted are shown in brackets.

*Ethrel is the trade name for 2-chloro-ethyl-phosphonic acid.

Interpretation

The non-treated control sample produced the best record of survival and the only rooted cutting at the end of three months. This same sample accounted for 66% of the total survivors of all the treatments.

The chlorogenic acid applied alone produced a reasonably good survival record but all other treatments were totally unsatisfactory.

Auxin treatments again produced a deleterious effect on rooting and survival, and Ethrel, at both concentrations, gave an even worse survival record.

Statistical analysis is unnecessary with this data.

Conclusion

Chlorogenic acid alone and in various combinations has not produced stimulation in rooting. Hence the hypothesis that rooting should be favoured by maintaining endogenous auxin levels by applying exogenous inhibitors of auxin oxidase is unsatisfactory in the case of Eucalyptus gunnii.

2. Exogenous application of Growth Retardants

The effect of shoot growth retardants on the rooting of Eucalyptus gunnii cuttings

One of the basic requirements of the rooting medium, and indeed the whole propagation environment, is to maintain a warmer temperature in this medium than in the air above it. The object of this is to encourage cellular activity (leading hopefully to rooting) at the base of the cutting while discouraging shoot growth. Shoot growth may use food materials faster than the leaves can photosynthesise them. In fact, shoot growth retardants which slow down the rate of

shoot growth have been known, in combination with auxins, to stimulate rooting as predicted. Hence a series of experiments was conducted to see if growth retardants would have any root inducing effects on Eucalyptus gunnii cuttings. The basic mechanisms by which these substances interfere with shoot growth are still unsettled. They affect endogenous IAA and GA activity.

Method

Phosphon, Cycocel and B9 were used singly and in combination with the auxin, IBA, dissolved in 45% ethyl alcohol and made up to 2000 ppm with distilled water. Phosphon was used at 25 ml per litre, Cycocel at 5 ml per litre and B9 at 2.5 ml per litre, all diluted by or dissolved in distilled water. Cuttings were set in September (M8 series) and in December (M9 series).

Results

Treatments, experimental references and results are given in Table 34.

Interpretation

The analysis of co-variance test on the M8, M9 and combined M8 and 9 series of experiments all produced no significantly differing results. These were $p = < 0,20, > 0.05$; $p = > 0.20$; and $p = > 0.20$ respectively.

It therefore indicates that whatever the time of year, auxins and shoot growth retardants have not proved to be beneficial in rooting or survival. While there was no increase in top growth, due possibly to the growth retardants, there was likewise no beneficial effect on the rooting activity of the cutting.

Table 34 The effect of growth retardants on rooting and survival of Eucalyptus gunnii cuttings

(a)

Treatment	Expt. Ref.	Alive/ Dead	No. of Cuttings Remaining Monthly							
			1	2	3	4	5	6	7	Total 2-7
IBA+Phosphon	M8-7	Alive	10	4	2	0	0	0	0	6
		Dead		6	2	2	0	0	0	10
IBA+Cycocel	M8-8	Alive	27	20	13	1	1	0	0	35
		Dead		7	7	12	0	1	0	27
IBA+B9	M8-9	Alive	30	20	14	3	2	1	1	41
		Dead		10	6	11	1	1	0	29
Phosphon	M8-10	Alive	11	6	3	1	1	1	1	13
		Dead		5	3	2	0	0	0	10
Cycocel	M8-11	Alive	21	17	9	3	1	0	0	30
		Dead		4	8	6	2	1	0	21
B9	M8-12	Alive	23	18	11(1)	2	1	1	1	34
		Dead		5	7	9	1	0	0	22
	Total	Alive	122							159
	Total	Dead		37	33		49			119

Cuttings were treated in September 1970.

(b)

Treatment	Expt. Ref.	Alive/ Dead	No. of Cuttings Remaining Monthly							
			1	2	3	4	5	6	7	Total 2-7
IBA+Phosphon	M9-7	Alive	18	3	0	0	0	0	0	3
		Dead		15	3	0	0	0	0	18
IBA+Cycocel	M9-8	Alive	16	3	0	0	0	0	0	3
		Dead		13	3	0	0	0	0	16
IBA+B9	M9-9	Alive	15	3	1	0	0	0	0	4
		Dead		12	2	1	0	0	0	15
Phosphon	M9-10	Alive	21	6	2	1	0	0	0	9
		Dead		15	4	1	1	0	0	21
Cycocel	M9-11	Alive	22	8	4	2	2	0	0	16
		Dead		14	4	2	0	2	0	22
B9	M9-12	Alive	24	5	1	1	1	0	0	8
		Dead		19	4	0	0	1	0	24
	Total	Alive	116							43
	Total	Dead		88	20		8			116

Cuttings were treated in December 1970. IBA was used at 2000 ppm: phosphon at 25 ml/litre; Cycocel at 5 ml/litre, and B9 at 2.5 ml/litre. All were used as a quick dip single treatment. The number in brackets indicates the only cutting which rooted. 90 cuttings were set in each treatment.

Conclusion

No shoot growth was noted in controls or retardant treated cuttings. Only one rooted cutting out of a total of 1080 cuttings set in control and retardant treatments does not reflect a trend and can therefore be accounted for as a random chance rooting. The hypothesis of diversion of essential nutrients from root to shoot is not an adequate explanation of failure of Eucalyptus gunnii cuttings to root.

There may well be endogenous growth substances present in the cutting which so clearly and disastrously affect the rooting and survival of Eucalyptus gunnii.

An endogenous growth inhibitor has been suspected and therefore having attempted to determine means of encouraging rooting and survival, and failed to find any group of growth substances able to provide this, the next obvious step is to try to determine whether such an endogenous inhibitor is present, and, if so, what this, or these, inhibitor(s) might be.

CHAPTER IV

RESULTS II: ENDOGENOUS PLANT GROWTH SUBSTANCES

iv. Endogenous Growth Substances affecting Rooting of Eucalyptus Cuttings

1. Investigations into the inhibitory effect of Eucalyptus oil on the germination of Mustard and Cress

The results of the tests conducted on cuttings of *Eucalyptus gunnii* so far indicate that they contain endogenous inhibitors of rooting. Popular lore associates inhibition of vegetative regeneration and of seedling germination in *Eucalyptus* stands and around the bases of *Eucalyptus* trees, with their content of *Eucalyptus* oils which is claimed as being responsible for such inhibition of growth.

To test this hypothesis, various concentrations of *Eucalyptus* and Medical Paraffin oils were used.

Materials and Methods

Eucalyptus and Paraffin oils were used at various concentrations. Water and ethyl alcohol were used as controls. Mustard and Cress seeds were used in the germination test since they both readily germinate and grow quickly. *Eucalyptus* and Paraffin oils were diluted in 45% ethyl alcohol to concentrations of 0.2, 1.0, and 10%. Petri dishes containing cotton wadding under filter paper each received 5 ml. of each treatment. Fifty seeds of Mustard and Cress were counted into the petri dishes and the lids were set in place.

Two dishes of each treatment were used in daylight conditions and a further two dishes were placed in the dark. Both received the same temperature i.e. 21°C. Data was gathered at precisely 24 hour periods and the number of germinated seedlings recorded.

Results

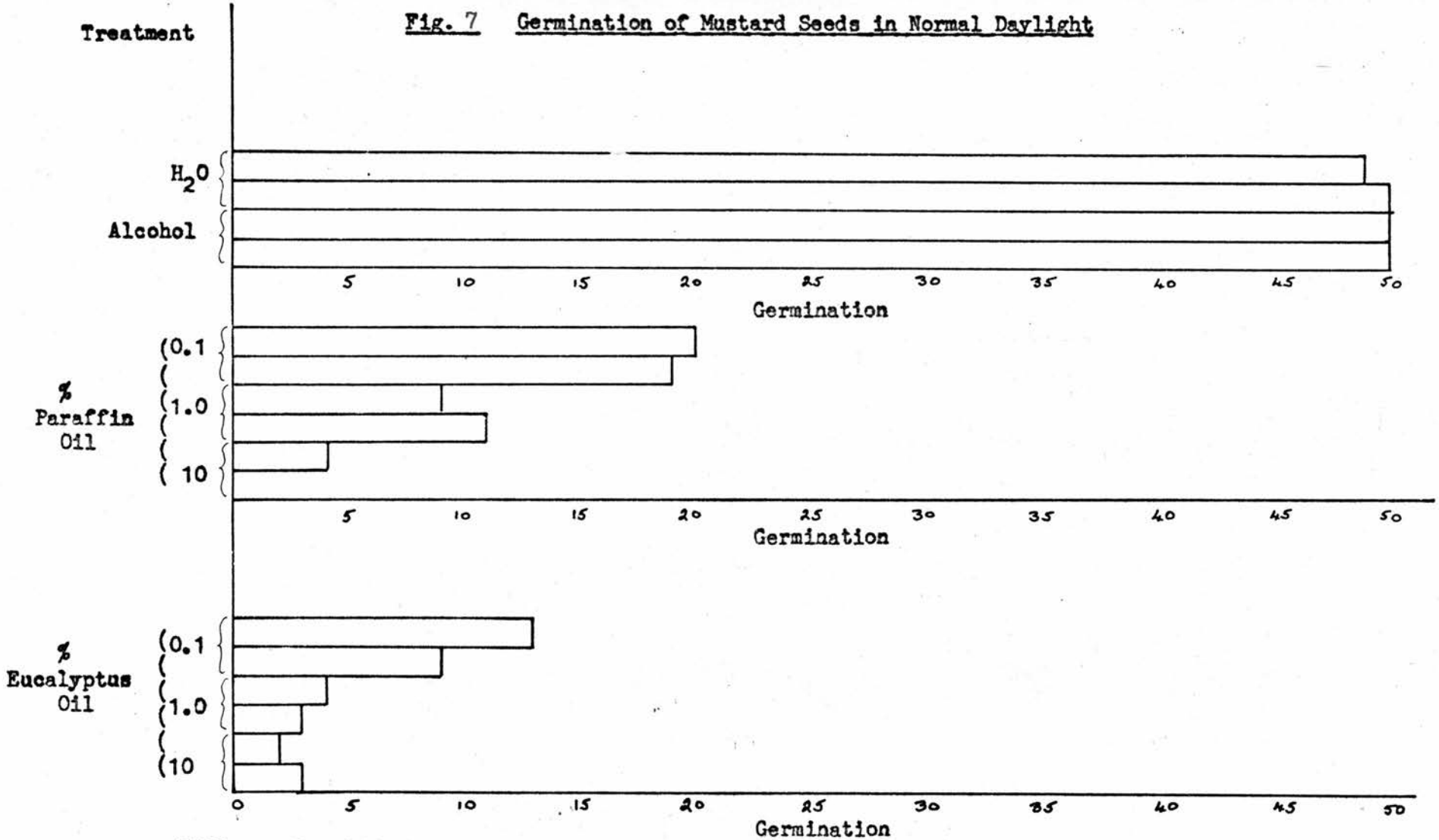
These are given in Tables 35 and 36, and for easy visual analysis in Figs. 7 to 10.

Table 35 The Effect of Various Concentrations of Eucalyptus and Paraffin Oil on Seed Germination in daylight

Treatment		Expt. Ref.	Seed Mustard Cress	No. of Seeds Germinated Each Day									
				1	2	3	4	5	6	7	8	9	Total
H_2O		14-1	M	43	6	0	0	0	0	0	0	0	49
			C	44	3	2	0	0	0	0	0	0	49
		14-2	M	39	10	1	0	0	0	0	0	0	50
			C	49	0	1	0	0	0	0	0	0	50
Alcohol		14A-1	M	43	7	0	0	0	0	0	0	0	50
			C	46	3	1	0	0	0	0	0	0	50
		14A-2	M	35	14	0	0	0	0	1	0	0	50
			C	41	4	3	0	0	0	0	0	0	48
	(0.1%	14A-9	M	0	7	6	5	2	0	0	0	0	20
			C	0	6	18	9	7	1	0	0	0	41
		10	M	0	6	9	0	1	3	0	0	0	19
Paraffin			C	0	7	31	0	1	0	0	0	0	39
Oil in	(1.0%	11	M	0	0	1	4	0	4	0	0	0	9
Alcohol			C	0	0	0	0	0	0	0	0	0	0
		12	M	0	4	6	0	0	1	0	0	0	11
			C	0	4	16	8	5	2	0	0	0	35
	(10%	13	M	1	2	0	1	0	0	0	0	0	4
			C	1	0	0	0	0	1	0	0	0	2
		14	M	0	0	0	0	0	0	0	0	0	0
			C	0	0	0	0	0	1	0	0	0	1
	(0.1%	14A-5	M	0	0	4	4	5	0	0	0	0	13
			C	0	0	0	3	5	1	3	0	1	13
		6	M	2	0	4	0	0	1	1	0	1	9
			C	0	0	2	5	6	1	0	0	0	14
Eucalyptus													
Oil	(1.0%	7	M	1	0	0	1	2	0	0	0	0	4
in			C	0	0	0	0	1	0	0	0	0	1
Alcohol		8	M	0	0	0	0	2	1	0	0	0	3
			C	0	0	1	1	1	0	0	0	0	3
	(10%	3	M	0	0	1	0	1	0	0	0	0	2
			C	0	0	0	0	0	0	0	1	0	1
		4	M	0	0	1	0	0	0	0	2	0	3
			C	0	0	1	0	3	1	0	0	0	5

Fifty seeds were set in each treatment

Fig. 7 Germination of Mustard Seeds in Normal Daylight



Fifty seeds of Mustard were placed on wet filter paper. Medical Paraffin and Eucalyptus oils at three concentrations were used together with water and 45% ethyl alcohol as control treatments. Germination was recorded during a period of 9 days.

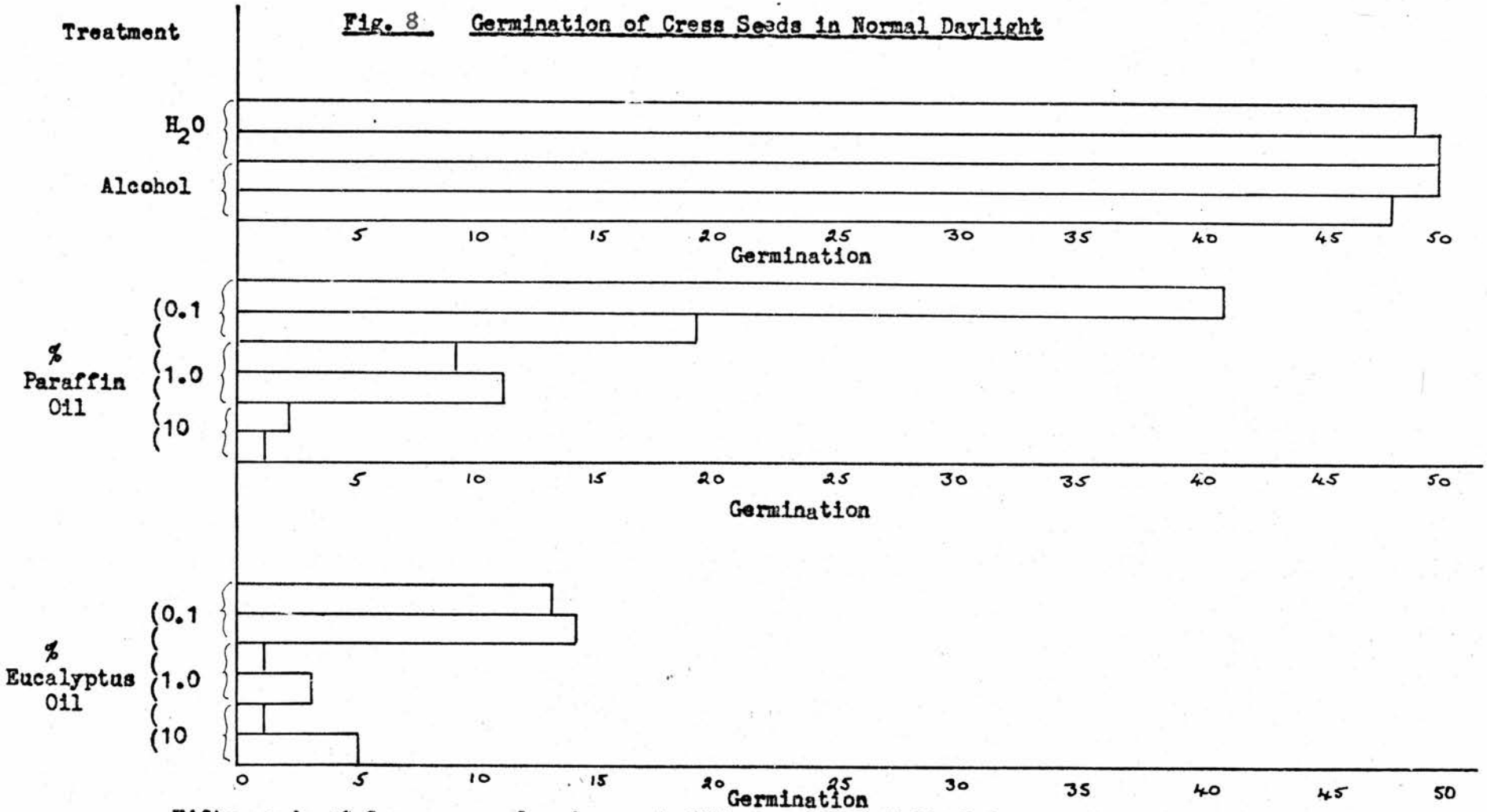


Fig. 8 Germination of Cress Seeds in Normal Daylight

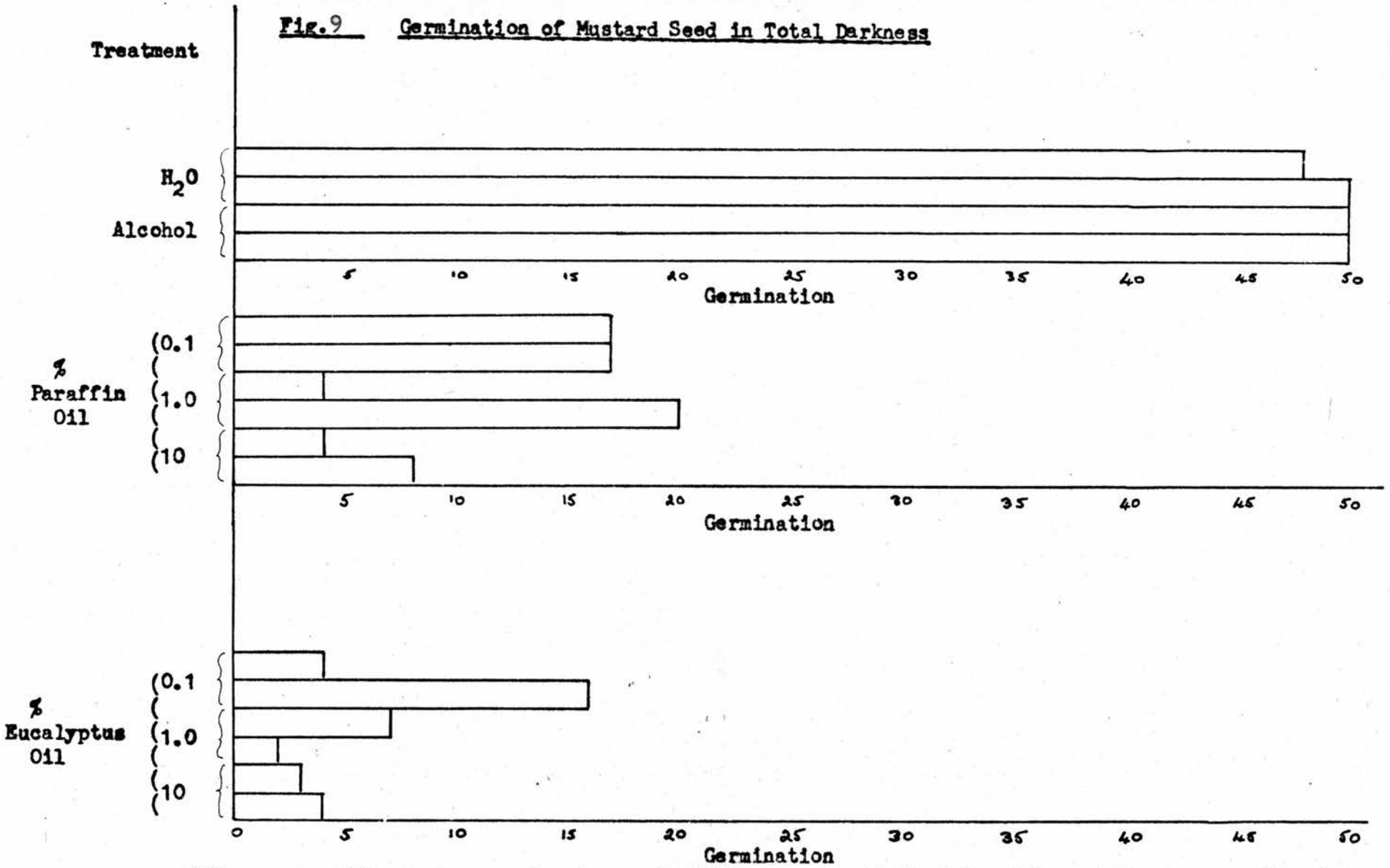
Fifty seeds of Cress were placed on wet filter paper. Medical Paraffin and Eucalyptus oils at three concentrations were used together with water and 45% ethyl alcohol as control treatments. Germination was recorded during a period of 9 days.

Table 36 The Effect of Various Concentrations of Eucalyptus and Paraffin Oil on Seed Germination in Complete Darkness

Treatment		Expt. Ref.	Seed Mustard Cress	No. of Seeds Germinated Each Day 1	2	3	4	5	6	7	8	9	Total
H_2O		14-7	M	41	7	0	0	0	0	0	0	0	48
			C	46	3	0	0	0	0	0	0	0	49
		14-8	M	35	13	2	0	0	0	0	0	0	50
			C	37	8	2	0	0	0	0	0	0	47
Alcohol		14A-15	M	46	3	1	0	0	0	0	0	0	50
			C	42	4	4	0	0	0	0	0	0	50
		16	M	39	9	2	0	0	0	0	0	0	50
			C	45	0	2	0	0	1	0	0	0	48
	(0.1%	14A-23	M	1	4	7	0	5	0	0	0	0	17
			C	3	0	9	7	2	0	0	0	4	25
		24	M	0	3	9	4	0	1	0	0	0	17
			C	0	3	8	1	3	0	1	0	0	16
Paraffin Oil	(1.0%	25	M	0	0	0	2	0	0	2	0	0	4
			C	0	0	0	0	1	0	2	2	0	5
in Alcohol		26	M	1	10	5	3	0	1	0	0	0	20
			C	0	6	12	8	0	1	14	0	0	41
	(10%	27	M	0	0	2	2	0	0	0	0	0	4
			C	0	0	0	0	1	0	1	3	0	5
		28	M	0	0	0	0	3	0	1	4	0	8
			C	0	0	0	0	0	0	1	3	1	5
	(0.1%	14A-19	M	1	1	0	1	0	0	1	0	0	4
			C	0	0	0	1	0	1	1	0	2	5
		20	M	0	2	7	3	1	2	1	0	0	16
			C	0	0	5	3	3	6	0	0	3	20
	(1.0%	17	M	0	0	1	2	4	0	0	0	0	7
			C	0	0	0	0	2	0	0	1	3	6
Eucalyptus Oil		18	M	1	0	1	0	0	0	0	0	0	2
			C	0	0	0	0	1	0	4	0	3	8
in Alcohol	(10%	21	M	0	0	0	0	1	1	0	0	1	3
			C	0	0	0	0	0	0	2	0	2	4
		22	M	0	0	0	0	0	2	0	0	2	4
			C	0	1	0	0	0	0	1	0	0	2

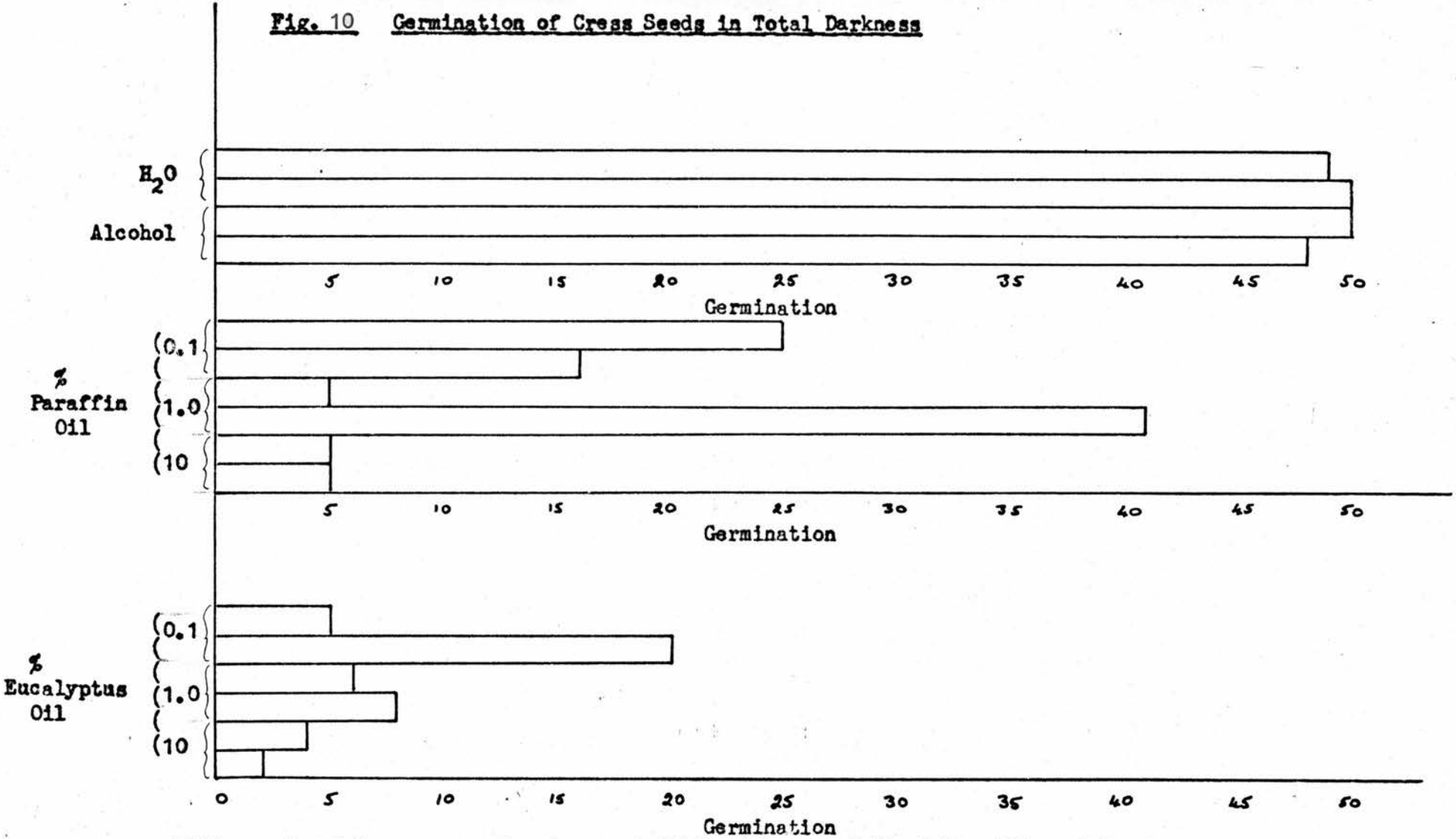
Fifty seeds set in each treatment.

Fig. 9 Germination of Mustard Seed in Total Darkness



Fifty seeds of Mustard were placed on wet filter paper. Medical Paraffin and Eucalyptus oils at three concentrations were used together with water and 45% ethyl alcohol as control treatments. Germination was recorded during a period of 9 days.

Fig. 10 Germination of Cress Seeds in Total Darkness



Fifty seeds of Cress were placed on wet filter paper. Medical Paraffin and Eucalyptus oils at three concentrations were used together with water and 45% ethyl alcohol as control treatments. Germination was recorded during a period of 9 days.

Interpretation

The non-oil treatments, water and alcohol, both produced almost maximum germination and a steady growth rate thereafter, which indicated that the seed was viable. However, Paraffin oil and Eucalyptus oil treatments produced a falling germination rate as the concentration increased and at the same ratio irrespective of seed used or whether they were in daylight or complete darkness.

By comparison, using Paraffin oil as a control, the numbers germinating were not significantly different from those treated with corresponding concentrations of Eucalyptus oil. It indicates that Eucalyptus oil in increasing concentrations reduces the germination rate.

Conclusion

While reduced germination rates were found to be directly caused by increasing concentrations of Eucalyptus oil, the results, compared with similar concentrations of Paraffin oil, did not show any significant difference. The effect of Eucalyptus oil and Paraffin oil in reducing germination is probably due to the film of oil decreasing air passage through the micropyle and testa rather than the effect of inhibitors of growth in Eucalyptus oil.

2. Volatile Aromatic Oils in Eucalyptus

A further test was conducted to determine if the volatile or heat-labile aromatic oils in Eucalyptus could in any way be responsible for this poor germination. Volatile oils can be removed simply by heating to 90°C.

Methods

Eucalyptus and Paraffin oils were heated to 60°C and to 90°C for 30 minutes in open vessels. They were then made up, as before, into 0.1% and 10% concentrations with 45% ethyl alcohol. Mustard and Cress seeds (50 in number) were counted into petri dishes as above and counted daily for germination.

Results

These are given in Table 37, and Fig. 11.

Interpretation and Conclusion

Eucalyptus and Paraffin oils (preheated to 60°C and to 90°C) do not significantly alter germination compared with oil free controls. Therefore a volatile or heat labile and partially inhibitory constituent appears to be present in both whole Eucalyptus and whole liquid paraffin oils.

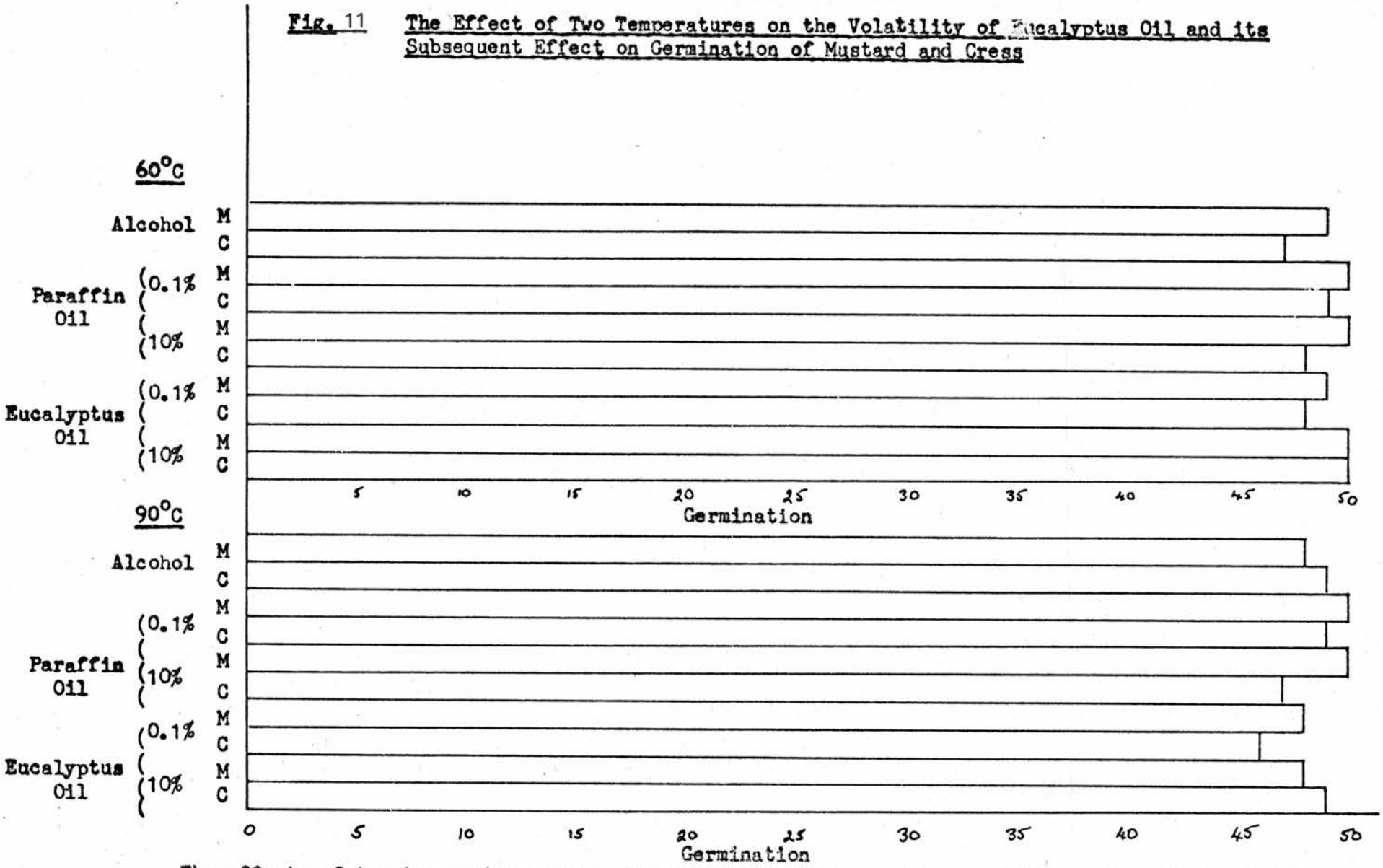
Endogenous inhibitors, present in Eucalyptus, must therefore prevent the rooting. Extraction and paper partition chromatography can isolate these inhibitors for further study.

Table 37 The Effect of Eucalyptus Oil unheated and preheated at two temperatures on seedling germination

Treatment	Expt. Ref.	Seed Mustard Cress	No. of Seeds Germinated Each Day									
			1	2	3	4	5	6	7	8	9	Total
60°C for 30 minutes												
Control	16-1	M	1	43	2	2	1	0	0	0	0	49
45% Alcohol		C	32	5	6	3	1	0	0	0	0	47
0.1% Eucalyptus	2	M	4	42	0	3	0	0	0	0	0	49
Oil in Alcohol		C	33	6	7	2	0	0	0	0	0	48
10% Eucalyptus	3	M	1	12	31	6	0	0	0	0	0	50
Oil in Alcohol		C	7	12	25	5	0	0	1	0	0	50
0.1% Paraffin	4	M	0	41	8	0	0	1	0	0	0	50
Oil in Alcohol		C	24	22	1	0	0	1	0	0	1	49
10% Paraffin	5	M	2	36	10	1	0	0	1	0	0	50
Oil in Alcohol		C	36	9	1	1	0	1	0	0	0	48
90°C for 30 minutes												
Control	16-6	M	1	43	2	1	0	1	0	0	0	48
45% Alcohol		C	22	22	2	1	0	1	0	0	1	49
0.1% Eucalyptus	7	M	1	37	7	0	0	0	0	3	0	48
Oil in Alcohol		C	24	18	3	0	0	0	1	0	0	46
10% Eucalyptus	8	M	0	25	20	3	0	0	0	0	0	48
Oil in Alcohol		C	17	12	14	3	0	1	2	0	0	49
0.1% Paraffin	9	M	5	39	2	1	0	0	0	1	2	50
Oil in Alcohol		C	25	21	2	1	0	0	0	0	0	49
10% Paraffin	10	M	1	45	4	0	0	0	0	0	0	50
Oil in Alcohol		C	23	19	3	1	0	0	1	0	0	47

Fifty seeds were set in each treatment

Fig. 11 The Effect of Two Temperatures on the Volatility of Eucalyptus Oil and its Subsequent Effect on Germination of Mustard and Cress



The effects of two temperature pretreatments of the oils are given. Fifty seeds of Mustard (M) and Cress (C) were placed on wet filter papers each containing the various concentrations of medical Paraffin and Eucalyptus oils. Germination was recorded over a period of 9 days. Total germination is given.

3. Analysis of extracts of leaves of 3 Eucalyptus types, and of Myrtus communis.

The first hypothesis that a deficiency of endogenous growth promoters alone could explain lack of growth and rooting cannot hold, since the exogenous application of such substances alone and in various combinations did not cause significant growth, and, in some cases, produced significantly earlier death. In this section it is established by bioassays of Eucalyptus leaf extracts that endogenous growth promoters are present in cuttings as received (at concentrations typical of the same hormones in other species; auxin less than 10^{-8}M; gibberellins (as GA_3) at about 10^{-6}M; and kinetins at about 10^{-8}M). Hence there is no deficiency of endogenous hormones in the cuttings. There remains then an alternative hypothesis that endogenous plant growth inhibitors (known to be present in many plants) are the main, if not the complete, cause of the lack of rooting in Eucalyptus cuttings.

Since there was no significant response by the Eucalyptus cuttings to any environmental or biochemical factor tested which enabled rooting to take place, endogenous inhibitors were next suspected as being the cause of failure. To test this hypothesis, fractions of various Eucalyptus leaf extracts were assayed for all the growth substances listed above.

This hypothesis was found adequate to explain failure to root in a few other species as discussed in the introduction. Indeed the older the plant part from which cuttings were taken, the greater the measured content of rooting inhibitor present and the less rooting occurred.

Some natural phenols and terpenoids are known endogenous inhibitors of shoot and root. ABA, or endogenous terpenoid substances grouped collectively under the term "abscisin" have also been shown to inhibit root as well as shoot growth, but whether ABA (at natural endogenous concentrations) inhibits rooting of cuttings has not before been tested. I have now tested this and found ABA present in Eucalyptus. In addition, however, the natural terpenoids of Eucalyptus leaf extracts which chromatograph at Rf 0.85-0.95 (below) have also been tested. It will be shown here in this section that finally, as some cuttings did root, there is a possibility that they were genetically distinct from those which failed to root. They may have a low endogenous inhibitor level because of genetic inability to produce or maintain inhibitors as in other cuttings. This was tested. If a low content of rooting inhibitors is found and is heritable, and can be bred into otherwise 'elite' trees, then mass propagation of clonal material by cuttings will become a reality with tremendous economic potential throughout the world. Hence, to test these three ideas, extracts of leaves of unrooted adult Eucalyptus, rooted and unrooted juvenile Eucalyptus, and Myrtus communis were then made and chromatographed to separate, by paper partition chromatography, endogenous growth promoters and inhibitors. These fractions were then bioassayed to determine whether differences in endogenous hormone contents were detectable.

The presence of inhibitors in a homogenate of macerated tissue does not indicate that it is necessarily active in whole tissue in vivo. The relevance of this warning will become evident when I deal with

Myrtus communis later in this work, where it will be shown that although an endogenous growth inhibitor is abundant, it is in fact inactive in vivo in the very same tissue.

Plant Material

Eucalyptus shoots were obtained from the same source as the cuttings used earlier in this work. Adult Eucalyptus shoots were cut from trees 30-40 feet high and 20-25 years old. Eucalyptus shoots showing juvenility in leaf form were obtained from 7-8 year old trees. These are pruned severely annually to retain this juvenile growth stage. On arrival the shoots were placed in water to restore them to full turgidity. Some were kept in complete darkness, while the remainder were placed in a well lit position. The temperature of the room was 15°C ± 2 deg. throughout this stage.

Shoots of Eucalyptus, rooted during the course of this work, and of Myrtus communis, an easily rooted member of the same family, were also used in the tests described below.

Extraction

15 gm. of fresh leaves of each of the four samples were macerated and extracted with 50 ml. of 95% methanol for 24 hours in the dark at -5°C. The macerate was filtered through Whatman No. 3 filter paper into a Buchner flask aided by a water vacuum suction pump. The filtrate was stored in the dark at -5°C. 25 ml. of the filtrate were then reduced to 1 ml. by rotary evaporation in vacuo at a bath temperature not exceeding 50°C.

Chromatography

(a) This reduced extract was streaked on to 23 cm. Whatman No. 3 chromatography paper, 3 cm. from the base of the paper. The chromatograms suspended above the solvent - 80/20 Isopropanol 0.15 N Ammonia* - were equilibrated in the dark for 12 hours in an airtight Shandon glass tank. The tank was lined with filter paper to aid vapour saturation. The chromatograms were developed at room temperature for 5-6 hours by ascending chromatography till the solvent front was about 18 cm. from the base line. They were air dried, then cut into horizontal 1 cm. strips. An untreated 1 cm. strip cut from the area above the solvent front acted as a control sample. Each strip was soaked overnight in 2 ml. of distilled water in a covered petri dish prior to the bioassay. During the bioassay the strip was still present.

(b) In order to locate the Rf of various groups of growth substances on the chromatogram, known concentrations of IAA, kinetin, GA and ABA were similarly treated by ascending chromatography. Their positions on the Rf were then determined by bioassays.

Bioassay Methods

Paton et al (1970) found that the Cress germination bioassay provided a quick yet reliable test for the presence of inhibitors. I have therefore used this as a means of determining if inhibitors

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*The solvent used - 80/20 Isopropanol/0.15 N Ammonia - "gives satisfactory separation of the active substances in the extracts and retained the brown resinous material at the starting line" Phillips and Wareing (1958). J. Expt. Bot. 9: 350-64.

are present in Eucalyptus gunnii. I have also used the Cress Root Growth and Cress Hypocotyl Growth as bioassays which might differ usefully in selectivity to test further the presence of growth inhibitors as well as positive growth hormones.

Details of the bioassay methods are given below.

1. Cress and Rice germination assays

Seeds of Cress 'Fine Green Curled' and Rice (Oryza sativa) were counted onto moist chromatographic paper strips containing the eluate. These were placed in the dark at room temperature i.e. 15°C. Germination was recorded after 24 hours and 5 days respectively.

2. Cress root assay

Cress 'Fine Green Curled' seeds were germinated on damp filter paper, and seedlings selected for uniformity after 24 hours. Twenty-five were placed in the 8 cm. petri dishes containing the eluate and the increase in root length, after 24 hours in the dark at 15°C, was measured.

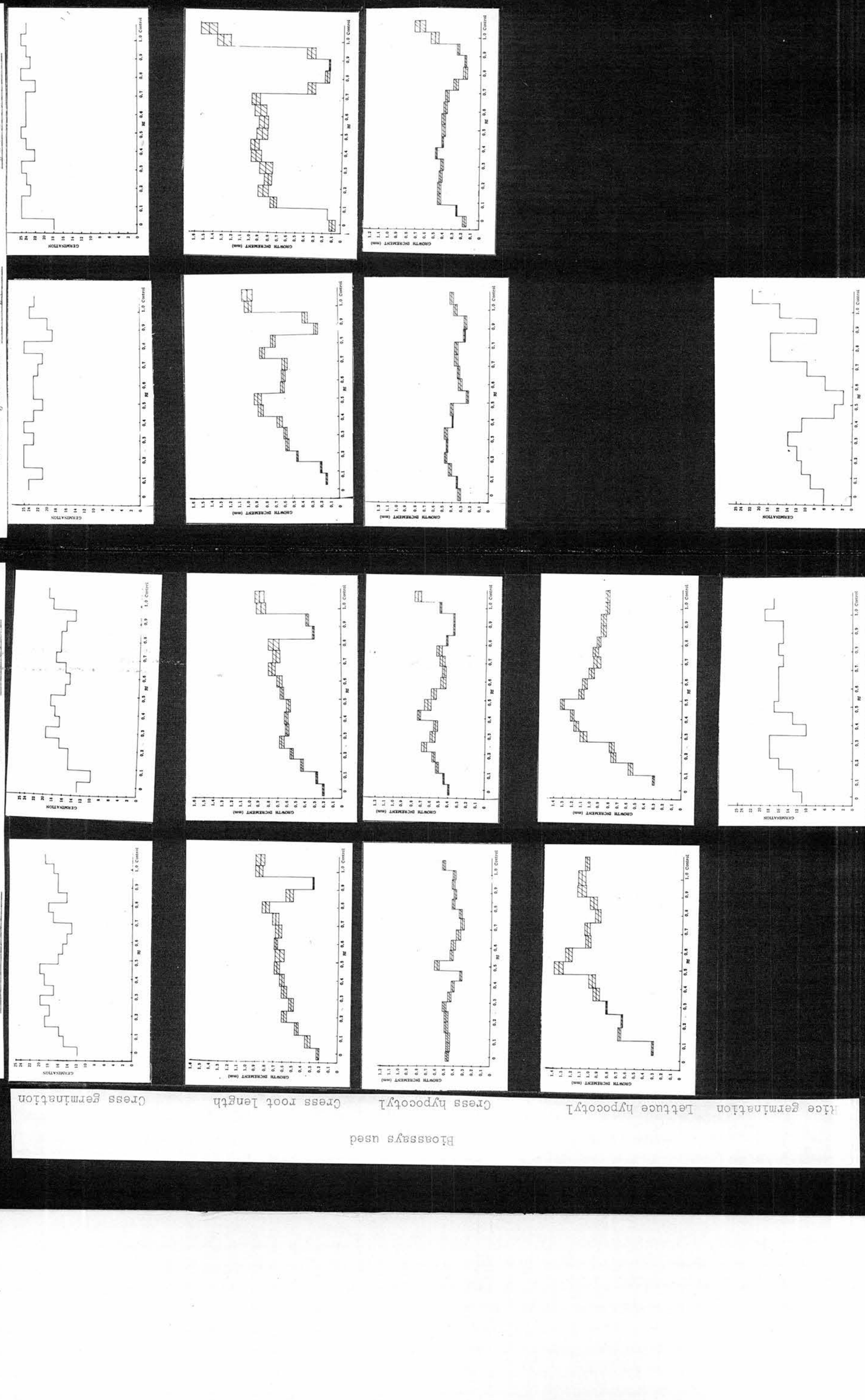
3. Cress and Lettuce hypocotyl assays

Cress seedlings of the above were retained and the resultant shoot growth measured after 24 hours. Seeds of Lettuce 'Grand Rapids' were germinated as above and 20 were selected for uniformity after 2 days. After a further 2 days the hypocotyls were measured.

Results

It will be evident from Fig. 12 that there are differences in the chromatograms of the four separately extracted leaf extracts. In the Cress germination bioassay, inhibition of growth is seen at Rf 00 to 0.05 in all but the rooted juvenile Eucalyptus extract. Inhibition of germination was found at 0.4 to 0.7 more prominently

Fig. 12 Bioassays of growth substances in chromatograms of leaf extracts of adult and juvenile Eucalyptus gunnii and Myrtus communis



in adult and juvenile Eucalyptus and was positioned higher up the Rf scale at 0.7 to 0.75 in rooted Eucalyptus and Myrtus communis leaf extracts. A further inhibition was found in all cases at Rf 0.85 to 0.95 although more prominent in the adult and unrooted juvenile Eucalyptus extracts. Growth peaks were common to all extracts at Rf 0.15 to 0.3 and again the region 0.35 to 0.45. There was a further peak region at Rf 0.7 to 0.85.

In the Cress root bioassay, two prominent positions of the Rf scale denote the presence of inhibitors in all extracts. These are positioned at the beginning (Rf 0.01) and near the end (Rf 0.85 to 0.95). Further inhibition of root growth is shown with all but the juvenile Eucalyptus chromatograms at Rf 0.3 to 0.35. Growth peaks are produced in all extracts at Rf 0.5.

The Cress shoot bioassay produced inhibition of growth at 0.0 to 0.05 and at 0.80 to 0.95 Rf in juvenile and rooted Eucalyptus and in Myrtus extracts. There was a general depression in growth in the adult tissues except for a peak at Rf 0.45 which was also noted in the other chromatograms between 0.35 to 0.45 but with slight deviations in each case.

The Lettuce hypocotyl bioassay indicates strong inhibitors present at 0 to 0.1 Rf and growth promotors at 0.5 to 0.6 Rf. This Rf region has been identified at the position of GA and is present in both adult and juvenile shoots.

In the Rice germination bioassay presence of inhibitors is noted at the 0.4 to 0.6 Rf region and this inhibitor appears to be present at higher concentrations in the rooted cutting extract than in the unrooted extract. An inhibitor is also present in the rooted cutting extract at 0.85 to 0.95 Rf.

Interpretation

Later in the thesis, these growth peaks and inhibitor valleys have been identified as groups of growth substances. Inhibitors at the base line are known to include phenolics and a mixture of insoluble chemicals produced by the extraction and concentration procedures. The 0.85 and 0.95 Rf inhibitors are known to include various terpenoid inhibitors, among them farnesol and geraniol. Paton et al (1970), using the Cress germination bioassay, also found that inhibition of rooting of Eucalyptus grandis occurred with the substances at Rf 0.9. Whether farnesol and geraniol are inhibitors of rooting has not yet been tested.

Conclusion

Differences in the four extracts are evident from the chromatograms. The identification of the various growth substances present in Eucalyptus gunnii is as yet unknown. It is therefore important to identify these.

4. Infrared Spectroscopy of Eucalyptus and Myrtus leaf extracts

Differences have been shown in the bioassay tests on the paper chromatograms of the four plant extracts. The identification of the substances has not been possible.

Two ways to identify growth substances present were attempted:

(a) By Infrared Spectroscopy and

(b) Bioassays which are specific for groups of hormones.

The IR Spectroscopic method can show differences in the chemical composition of various extracts from their resultant IR absorption spectral pattern. These can then be identified.

I am greatly indebted to Professor F. D. Gunstone and his assistants for obtaining the Spectograms from my materials.

Method

Leaf extracts of adult Eucalyptus, rooted and unrooted juvenile Eucalyptus and Myrtus communis were made, concentrated and separated by paper partition chromatography. The points on the Rf scale which consistantly produced inhibition of growth by bioassay methods were subjected to IR Spectroscopy.

Two areas on the Rf scale were tested i.e. Rf 0 to 0.1 and Rf 0.85 to 0.95. The ABA section (Rf 0.5 to 0.7) was not tested since I have shown below that this substance is not responsible for lack of rooting.

In order to provide a higher concentration of inhibitor, a total of 5,* and in some cases 7, comparable strips from the specific Rf area were used together. These were extracted with a minimal volume of carbon disulphide as the solvent and mulled into the measuring cells with a few drops of Nujol.

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*Results for 5 only are presented in this thesis as typical.

For comparison of leaf extract absorption spectra with known growth substances, spectrograms of pure IAA, GA_3, kinetin, and ABA were similarly obtained by the above methods.

Results and Discussion

The pure known growth substances gave distinctive IR spectra with sharply defined absorption bands. If, however, all four separate spectra were superimposed, the resulting pattern would be indistinct owing to mutual cancellation of the absorption and transmission regions of the different hormones. See Figs. 13, 14, 15.

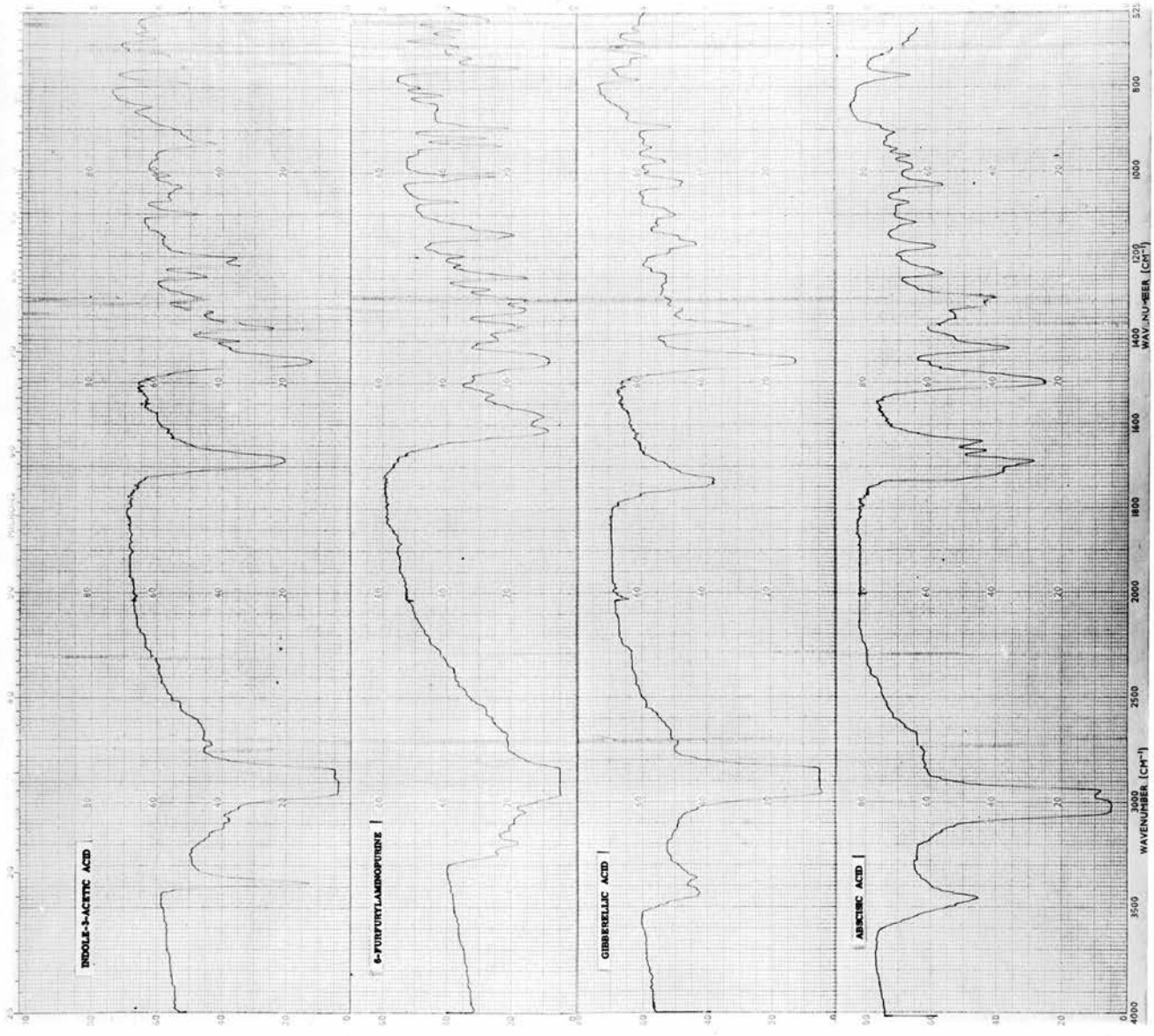
The general absorption and the lack of sharp peaks obtained with the fractions of the whole leaf extracts is therefore to be expected.

The combination of paper chromatography with bioassays relatively specific for different groups of hormones allows detection of the latter at very low concentrations in the presence of other non-hormonal organic substances at each Rf region. All the molecules present at each Rf, however, will contribute to the IR spectrum and mask any distinctive hormonal peaks.

Conclusion

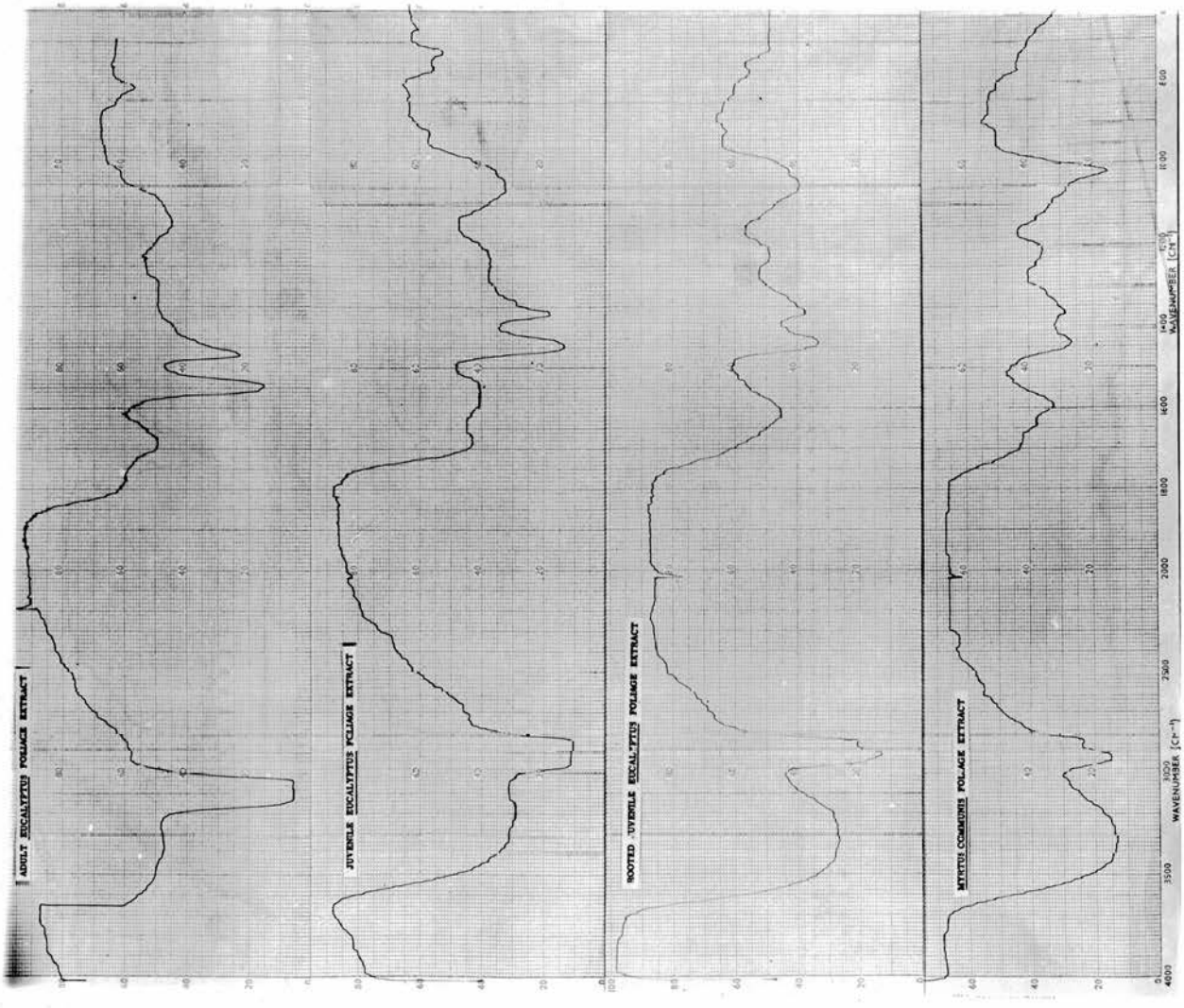
More elaborate and effective means of initially separating the components in the leaf extracts are necessary before using IR Spectroscopy for identification. Gas liquid chromotography is suggested as being a suitable method of separating the endogenous substances. This is being considered for future work.

Fig. 13 Infrared spectrograms of pure growth substances.



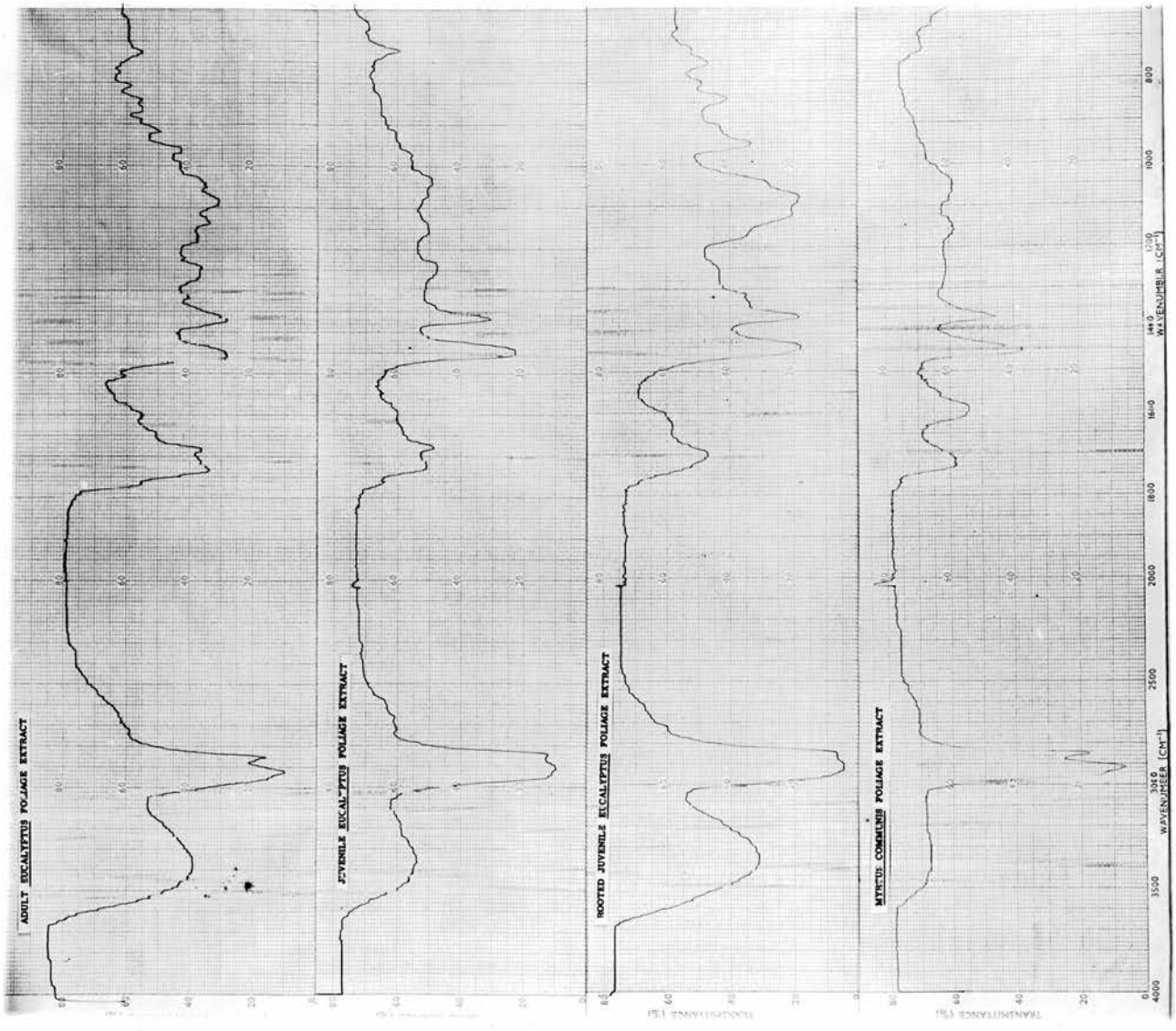
Nujol was used as the mulling agent. The scan speed was fast.
2mg were used in each case.

Fig. 14 Infrared spectrogams of leaf extracts of three Eucalyptus gunnii and of Myrtus communis.



The 0.05 to 0.10 Rf fraction of each of the leaf extracts was produced by paper partition chromatography. Nujol mull was used and scan speed was fast.
Five papers were used to concentrate the fraction in each case.

Fig. 15 Infrared spectrogams of leaf extracts of three Eucalyptus gunnii and of Myrtus communis from the 0.85 to 0.95 Rf fraction.



The Rf fraction was produced by paper partition chromatography.
Nujol mull was used and the scan speed was fast.
Five papers were used to concentrate the fraction in each case.

5. Identification of Growth Substances by using Bioassay Methods

Since all chemicals present will contribute to the IR spectra, identification of the separate substances, all present together, have proved impossible without further prior separation.

Bioassay methods, which are specific for groups of growth hormones, offer a second method of analysis. By comparing the chromatograms of known growth substances against the chromatograms of the four leaf extracts already presented above in Fig. 12, the identity of such substances is possible.

Method

In order to compare the Rf of substances on the leaf extract chromatograms, known concentrations of IAA, kinetin, GA_3 and ABA, alone and in various combinations, were treated as above by ascending chromatography. Their positions on the Rf were then determined by bioassay.

Since ABA is suspected of being one of the major causes of failure of rooting in Eucalyptus, various concentrations of ABA were added to adult and juvenile leaf extracts to determine if ABA was the cause of inhibition of growth.

Bioassay Methods Used

1. Germination assay

Seeds of Cress 'Fine Green Curled'; Lettuce 'Grand Rapids', a non-light demanding variety; and also of Rice (Oryza sativa) were counted onto moist chromatographic paper strips containing the eluate. These were placed in the dark at room temperature i.e. 15°C. Germination was recorded at 24 hours, 48 hours and 5 days respectively.

2. Cress root assay

Cress 'Fine Green Curled' seeds were germinated on damp filter paper, and seedlings selected for uniformity after 24 hours. Twenty-five were placed in the 8 cm. petri dishes containing the eluate and the increase in root length after 24 hours in the dark at 15°C was measured.

3. Cress hypocotyl assay

Cress seedlings of the above were retained and the resultant shoot growth measured after 24 hours.

4. Dwarf Maize coleoptile and primary leaf section: straight growth assay

Maize 'Kelvedon Glory' was soaked for 4 hours then sown in shallow trays in damp sand. These were placed in the dark at 15°C in a humid atmosphere and left to germinate. On reaching 20 mm. the coleoptile was removed and cut into 10 mm. sections, removing the tip each time. This section, however, contained an enclosed section of primary leaf. These were then transferred to covered petri dishes containing the eluate. The coleoptile section was then measured after 24 hours.

5. Pea 1st internode assay

Pea 'Alaska' was similarly treated and a 10 mm. section of the epicotyl used, again removing the growing point. These sections were also measured after 24 hours.

6. Lettuce hypocotyl assay

Thirty seeds of Lettuce 'Grand Rapids' were placed on the moistened chromatographic paper and kept in the dark at 15°C. After

two days the seeds were checked for germination and 10 removed leaving 20 which were uniform in growth. After a further 2 days the hypocotyls were measured.

7. Radish cotyledon bioassay (modified from Letham, 1971)

Radish 'French Breakfast' was germinated in shallow trays containing moist filter paper. After 36 hours the smaller cotyledons were excised from each seedling. Cotyledons of uniform size were selected and placed in the petri dishes containing the eluate. Incremental diameter growth was recorded after 24 hours.

8. Myrtus rooting bioassay

Cuttings of Myrtus communis were prepared as described for Eucalyptus on pp. 69-72. Twenty-five cuttings were placed for 24 hours in each of the eluates of chromatograms from adult and juvenile Eucalyptus leaf extracts alone and with added known concentrations of ABA. Thereafter the cuttings were pushed into the peat/perlite rooting medium under fine mist propagation at a temperature of 10°C. The numbers of cuttings rooted were counted after two months.

Specific bioassays for each group of growth substances are given in Table 37. Control throughout refers to bioassay response using a chromatogram paper strip of the same width from above the solvent front.

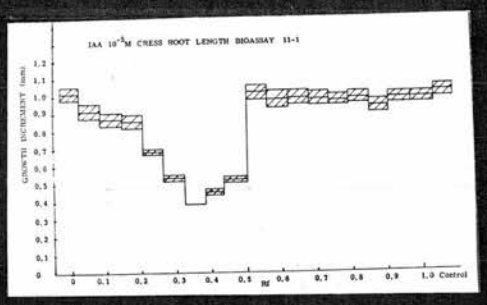
Results and Discussion

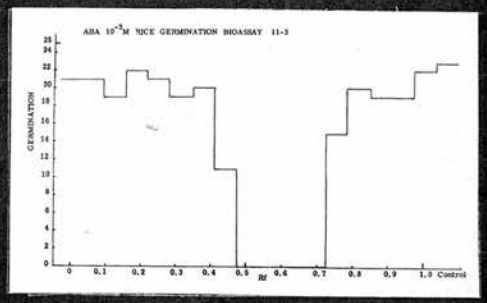
The relative positions of known concentrations of pure IAA, kinetin, GA_3 and ABA found by bioassay are given in Fig. 16. Fig. 17 gives the relative position of combinations of these growth substances where it should be noted that there is spreading and mutual interference at higher concentrations. Whatever the appropriate bioassay used, with increased concentrations of the pure inhibitor, ABA, its inverse peak or "valley" deepened, and widened or spread. This was similarly found when pure ABA was used in

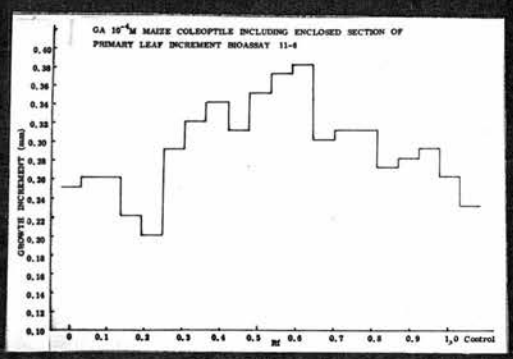
Table 37 Growth Substances and their Specific Bioassays

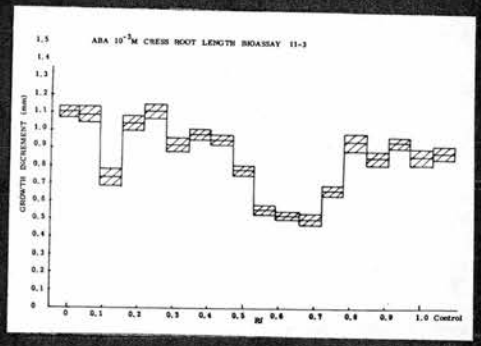
Endogenous Growth Substance	Bioassay Used	Rf Region	Notes	Expt. Ref.
IAA	Cress root length	0.20–0.50	Tested at 10^{-5}M	11–1
GA	Dwarf Maize Coleoptile segment with enclosed section of primary leaf increment	0.45–0.65	Tested at 10^{-4}M	11–6
Kinetin	Radish cotyledon increment	0.20–0.30	Tested at 10^{-6}M	11–11
ABA	Rice germination	0.50–0.75	Tested at 10^{-3}M	11–3
	Cress root length	0.65–0.73	Tested at 10^{-5}M	9–2
	Cress root length	0.50–0.75	Tested at 10^{-3}M	11–3
IAA/ABA	(Cress root length	0.30–0.47	Tested at 10^{-5}M	(11–2
IAA	(Lettuce germination	0.10–0.35	Tested at 10^{-5}M	(11–2
ABA	(Cress root growth	0.70	Tested at 10^{-5}M	(11–2
	(Lettuce germination	0.55–0.80	Tested at 10^{-5}M	(11–2
Kinetin/ABA				
Kinetin	Lettuce germination	0.10–0.33	Tested at 10^{-6}M	15–4
ABA	Lettuce germination	0.40–0.75	Tested at 10^{-3}M	15–4
GA/ABA				
GA	Dwarf Maize Coleoptile segment with enclosed section of primary leaf increment	0.40–0.52	Tested at 10^{-4}M	11–5
ABA	Dwarf Maize Coleoptile segment with enclosed section of primary leaf	0.60	Tested at 10^{-5}M	11–5
GA/Kinetin				
GA	Dwarf Maize Coleoptile segment with enclosed section of primary leaf increment	0.42–0.65	Tested at 10^{-4}M	11–9
Kinetin	Dwarf Maize Coleoptile segment with enclosed section of primary leaf increment	0.20–0.30	Tested at 10^{-6}M	11–5

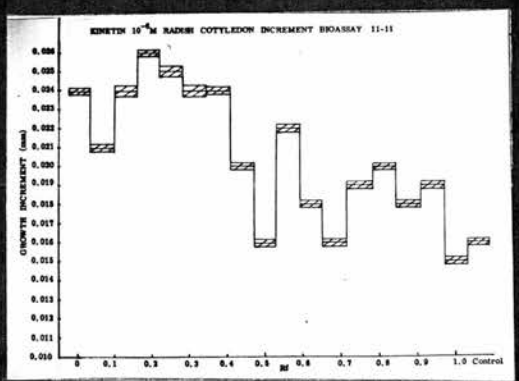
Fig. 16 Similar bioassays of chromatograms of known concentrations of pure growth substances alone.











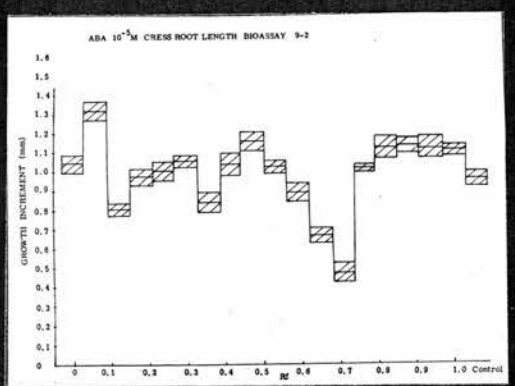
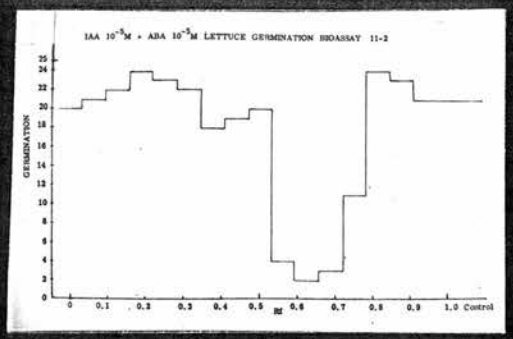
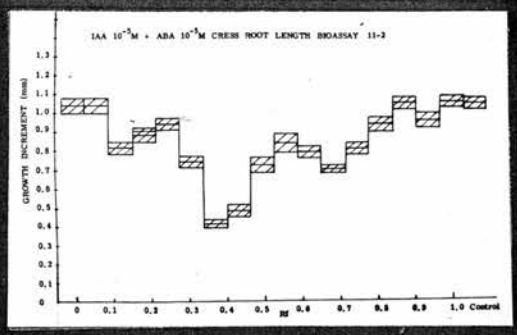
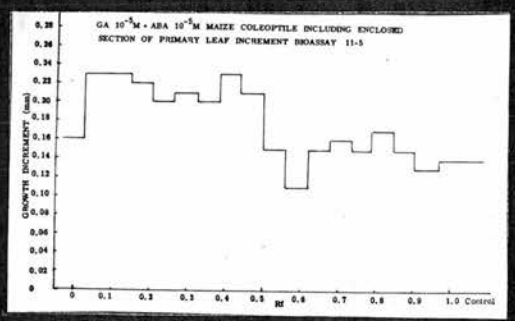
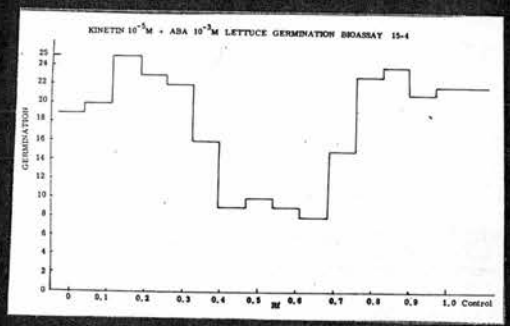


Fig. 17 Bioassays of chromatograms of known concentrations of pure growth substances mixed together.









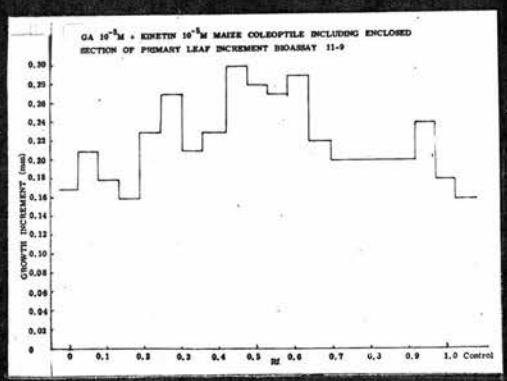
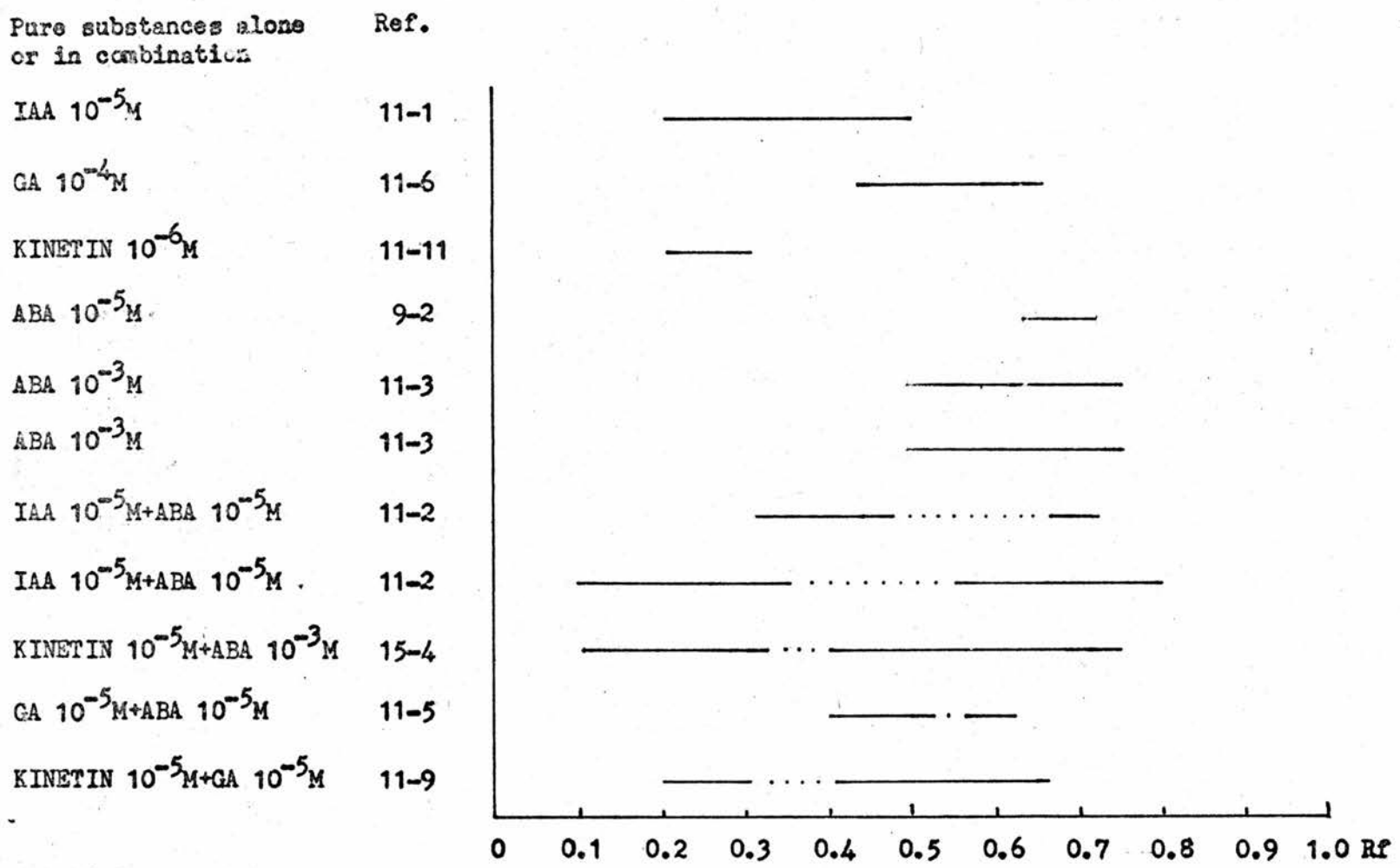
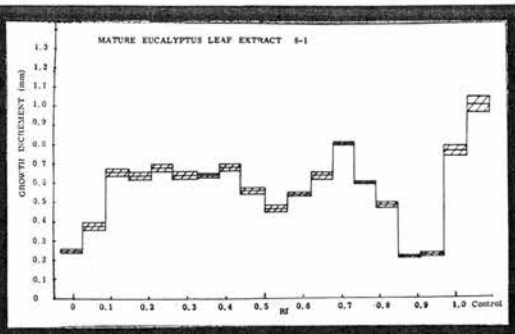


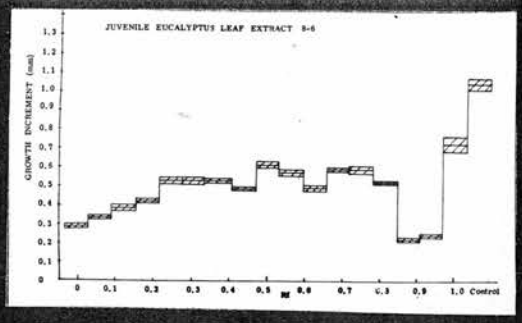
Fig. 18 The Relative Positions of Known Concentrations of Pure Growth Substances

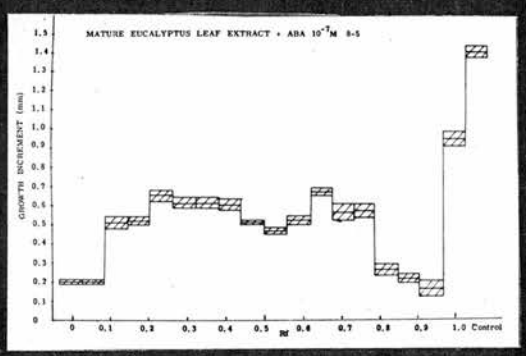


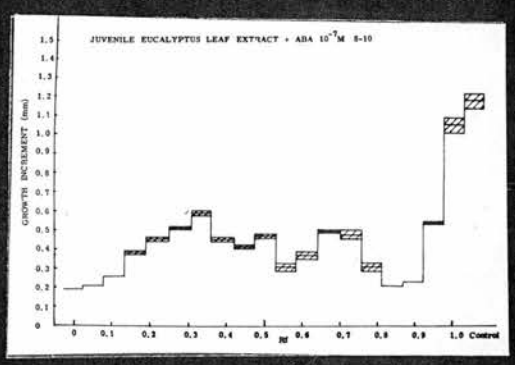
These positions have been obtained by specific bioassays for each growth substance following paper partition chromatography using 80/20 Isopropanol/0.15 N Ammonia as the solvent.

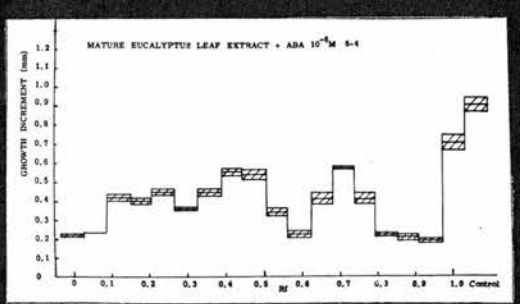
Fig. 19 Bioassays of chromatograms of leaf extracts of adult and juvenile Eucalyptus leaves with added known concentrations of Abscisic acid.

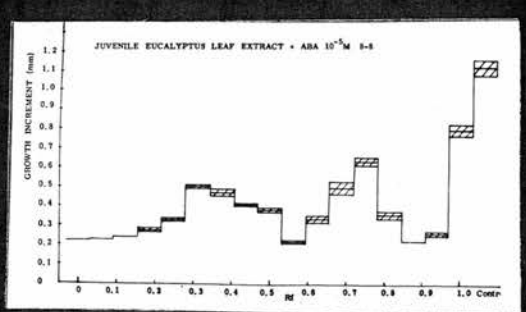












The Cress Root Bioassay was used in all the chromatographic tests

combination with other growth substances viz. IAA, and kinetin. Here there is obvious mutual interference, for the peaks and/or "valleys" are found to be positioned further apart when additional ABA is present.

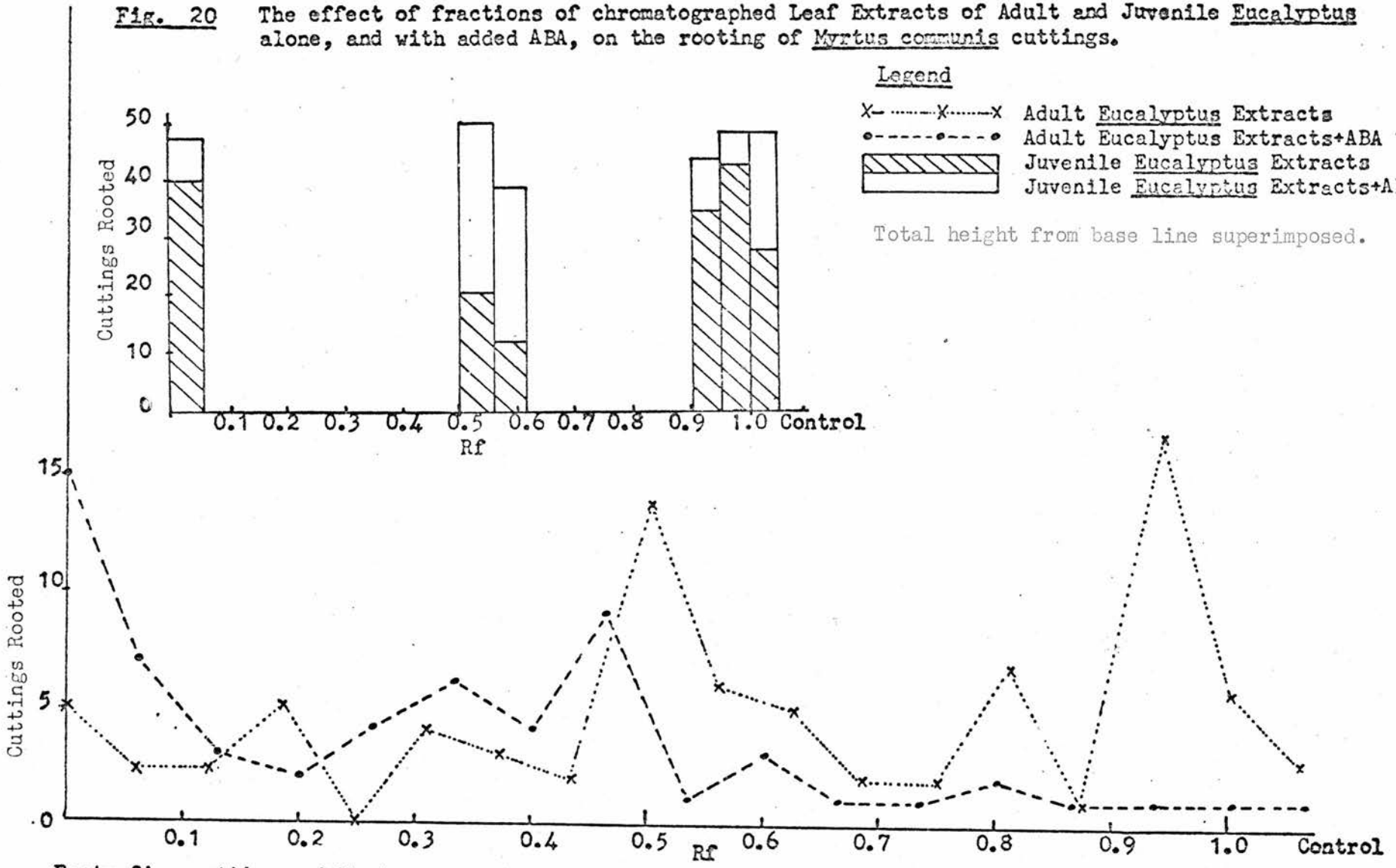
Fig. 18 summarises the relative positions of the known concentrations of growth substances.

Chromatograms of both adult and juvenile Eucalyptus extracts have been compared with the same Eucalyptus extracts with ABA added. The results are given in Fig. 19. Valley and widths of spread are produced at identical positions on the Rf scale but added ABA deepens and widens the "valleys". Thus, detectable traces of substances similar to, and present at the same Rf value as, endogenous ABA are present in leaf in natural adult and juvenile Eucalyptus leaf extracts.

In the Rice Germination Bioassay which has proved singularly sensitive and responsive to a range of ABA concentrations, it should be noted (in Fig. 12) that a much greater concentration of ABA was found in the rooted Eucalyptus extract and a slightly weaker response in unrooted juvenile leaf extracts compared with control. The natural ABA concentrations must be less than 10^{-5}M.

Far from inhibiting rooting of Myrtus communis, the addition of ABA at 10^{-4}M to the ABA containing region of juvenile Eucalyptus extract 0.50-0.60 Rf, stimulated rooting threefold compared with the same Rf region of the juvenile extract alone. There is no significant inhibition in the adult Eucalyptus extracts with or without added ABA as shown in Fig. 20. Hence it therefore seems unlikely that ABA is the prime or major cause of lack of rooting.

Fig. 20 The effect of fractions of chromatographed Leaf Extracts of Adult and Juvenile Eucalyptus alone, and with added ABA, on the rooting of Myrtus communis cuttings.



Forty-five cuttings of Myrtus communis were set in each treatment in August 1972. The cuttings were pushed into a peat/perlite mixture under intermittent mist and a temperature of 10°C. Rooting data were recorded after 8 weeks. Only 6 regions of the juvenile extracts were tested and the results are given in the histogram.

Conclusion

ABA, while present, is not considered to be the cause of the failure of cuttings to root. Rather, inhibition is attributed to those substances present at Rf 0.85 to 0.95.

Rooted Eucalyptus cuttings have identical endogenous inhibitors at concentrations no lower than in those which will not root, and this suggests that a bypass for inhibition exists which is as yet unidentified. However, cuttings from these very rooted cuttings themselves failed to root and were indistinguishable statistically in rooting, in survival, and in inhibitor content upon extraction, chromatography and bioassay, from the ordinary raw material received from Kent. Hence, such a bypass is not a heritable trait. This is an unavoidable conclusion.

These tests suggest that a substance with an Rf 0.85 to 0.95 might be the cause of failure to root in cuttings. Further investigation of this Rf region follows. Means to overcome this inhibition are now also under consideration.

6. The Effect of Four Macerated Leaf Extracts on Rooting of Mung Bean (Phaseolus mungo) Cuttings

Experiments so far conducted on germination and growth of various plants give no information on root (branch) primordial formation. Seedlings were not retained long enough to observe the formation of secondary roots. However, germination of mustard and cress was significantly reduced with higher concentrations of Eucalyptus oil with volatile inhibitors partially effective. There may be other fully effective inhibitors present in whole cuttings too.

It is well known that lack of vegetative growth under certain trees and shrubs can be due to solutes in water drips from the leaves. Bonner (1946) gives an account of this in relation to desert species. Yardini and Evenari (1952) provide a similar account with Eucalyptus species.

Plant growth inhibitors are known to be present in many young plants and these prevent or greatly reduce the capacity of cuttings to form roots.

It is known that seedling juvenile Eucalyptus shoots can be rooted provided that seedling cuttings are taken before reaching the 15th leaf pair. Hence, such shoots cannot contain sufficient endogenous growth retardants to prevent rooting. It is therefore valid to test extracts of later juvenile and mature leaves for differences in contents for possible endogenous growth hormones and inhibitors. Testing for both hormonal stimulants and inhibitors is necessary since their effects may oppose each other. Since separation of the hormonal components is necessary, Eucalyptus shoot extracts were chromatographed and the different fractions

tested separately on cuttings removed from young seedlings of Mung Bean (Phaseolus mungo). Rooting of these cuttings is very sensitive to the presence of such rooting inhibitors.

The underlying hypothesis which is widely assumed is that the presence or absence of distinctive growth-controlling substances in homogenised tissue extracts is sufficient to explain cutting viability and rooting. Their mere presence or absence is sufficient explanation of the cause of the lack of rooting. However, this simple hypothesis does not allow for possible compartmentalisation of growth inhibitors away from sensitive meristems but assumes free access of all tissue contents to all regions of bioassay organs and Eucalyptus cuttings.

Method

Plant Material for Extraction of Growth Substances

Adult and juvenile Eucalyptus shoots were obtained from the same trees as the cuttings used in the previous experiments. On arrival they were placed in water to restore them to full turgidity. Shoots of other Eucalyptus plants which had rooted during the previous experiments, and of Myrtus communis, an easily rooted member of the same family Myrtaceae, were tested.

Extraction, concentration and separation of the four leaf extracts by paper partition chromatography have been described previously.

Assay Method

Each 1 cm. strip from the chromatogram was placed in a small test tube to which 5 ml of distilled water was added to dissolve the fraction which could then be tested. All fractions of the four leaf extracts were kept in the dark for 24 hours before 10 cuttings of

Mung Bean (showing only the cotyledons) were placed in the eluate. Water was added at intervals to keep the levels at the 5 ml mark. Survival, and number of roots for cuttings were recorded after 10 days.

Results and Interpretation

It can be seen from Fig. 21 (No. of Cuttings Alive) that inhibitors present at Rf 0.5 to 0.65 and 0.8 to 0.95 Rf are toxic to Myrtus to the extent of killing all the cuttings. However, growth hormones chromatographing at 0.2 to 0.34; o.45 to 0.50; and 0.6 to 0.7 Rf maintain cutting viability.

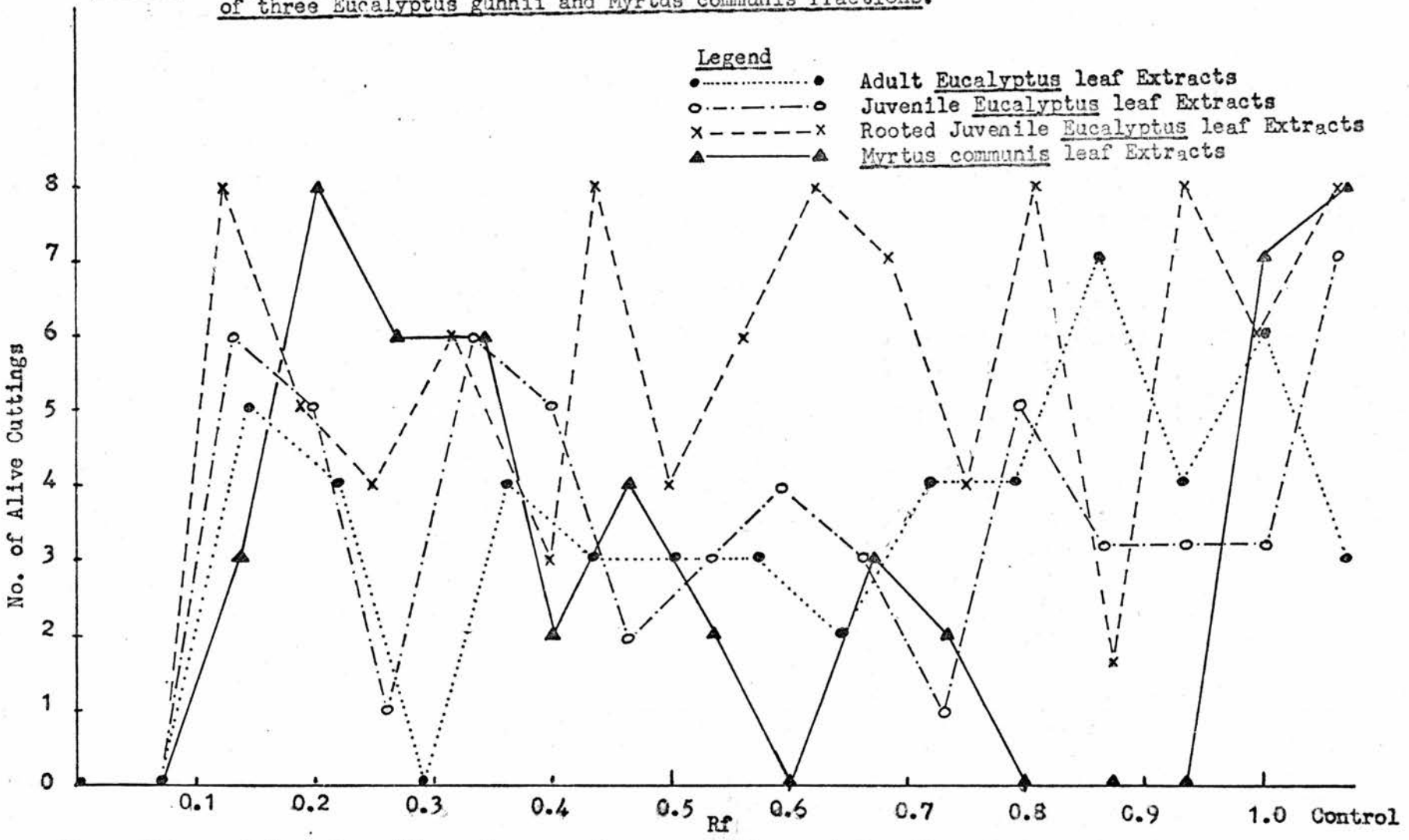
The index used in this work has been the viability of the cuttings since rooting occurred so infrequently. Viability is, however, distinctly related to rooting, and the Roots per Cutting analysis (Fig. 22) exemplifies the hypothesis that survival and rooting are related.

Substances present at Rf 0.25 to 0.32 from adult and juvenile Eucalyptus extracts are toxic and there was little survival of the cuttings. The same substances from rooted juvenile Eucalyptus and Myrtus communis extracts provided a better survival ratio.

If attention is to be focussed on chemical substances which are related crucially to survival, then those present at Rf 0.25 to 0.30 could be important.

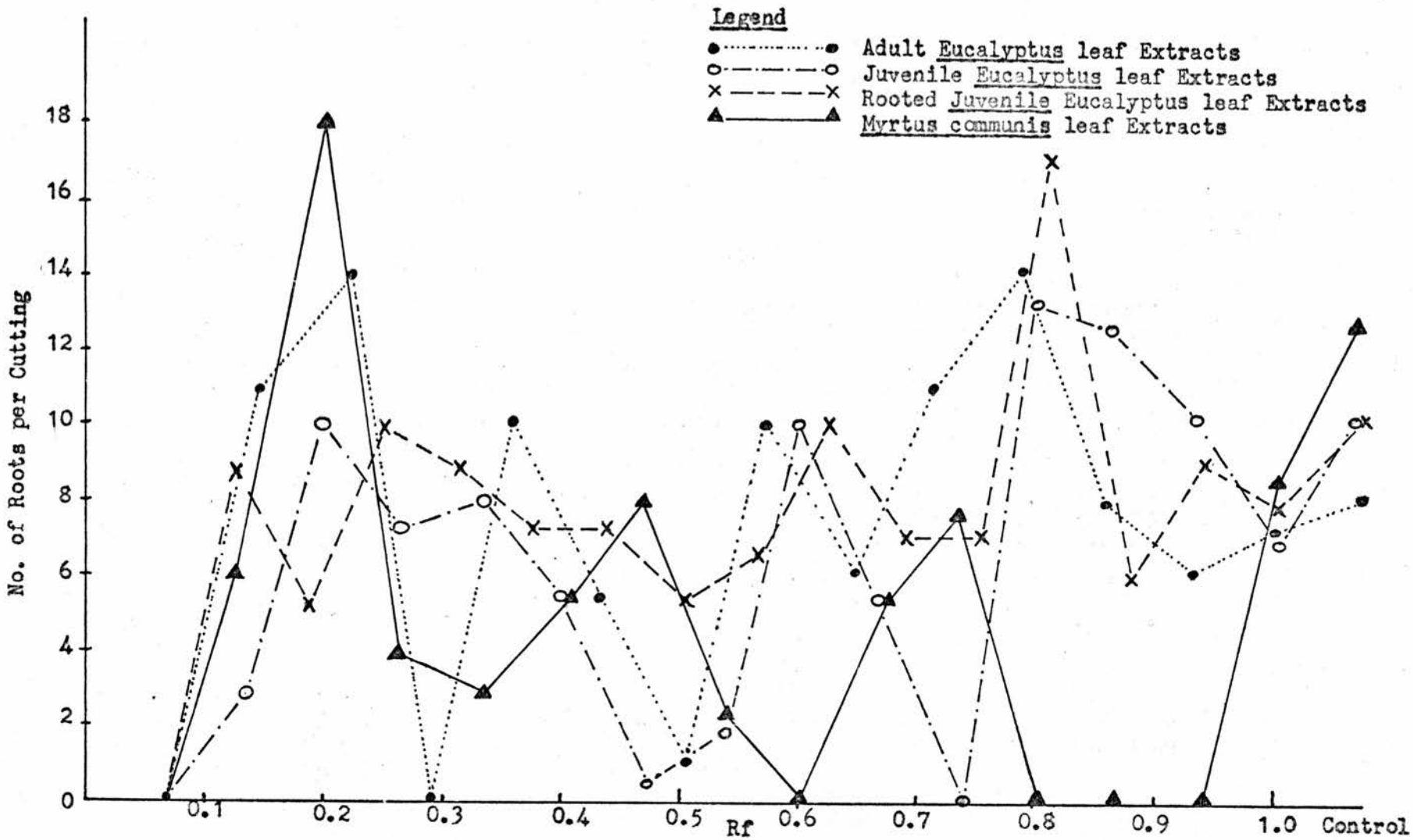
In Fig. 22 Roots per Cutting, inhibitors from all extracts occurred at Rf 0 to 0.05. This is due to baseline phenolics and insoluble substances present at high concentrations. We should therefore ignore this region.

Fig. 21 The Number of Cuttings of Mung Bean remaining alive following treatment with leaf Extracts of three Eucalyptus gunnii and Myrtus communis fractions.



Ten cuttings of Mung Bean (Phaseolus mungo) were used for each fraction of the Adult, rooted and unrooted Juvenile Eucalyptus, and Myrtus communis. Survival was recorded after 10 days.

Fig. 22 The average number of Roots per Cutting following treatment with leaf Extracts of three Eucalyptus and Myrtus communis fractions.



The data was obtained from the total number of roots per treatment and divided by the number of cuttings remaining alive.

From Rf 0.05 to 0.30, growth hormones produced the highest peak with adult Eucalyptus extract and, to a lesser extent, with both rooted and juvenile Eucalyptus.

This has been found to be the position of kinetin. Concentrations of kinetin are very high in Myrtus. Kinetin, however, has nothing to do with rooting since the easily rooted Myrtus and the unrootable adult Eucalyptus both show high endogenous kinetin levels while both juvenile and rooted Eucalyptus extracts show the presence of low endogenous concentrations. Above all, additional exogenous kinetin does not cause rooting.

It could be argued that substances found at Rf 0.3 are present in lesser concentrations in rooted and juvenile Eucalyptus than in adult Eucalyptus and Myrtus communis. IAA is known, from the earlier bioassays, to be present at this point and could account for the inhibitions of rooting in Eucalyptus. It might therefore later be worth testing the hypothesis that the application of exogenous anti-auxins to antagonise excessive endogenous auxin levels may lead to root initiation in Eucalyptus gunnii.

The substances present in the region 0.45 to 0.60 Rf contain the ABA, IAA and GA complex. It is suggested that the position and width of the central peak or valley varies according to relative and absolute concentrations of GA and ABA and perhaps also of IAA at the lower Rf values. This partial superposition of growth substances makes it difficult to interpret the results. However, GA appears to be below ABA on the Rf scale as shown in Fig. 18. Allowing for shifting, Myrtus communis contains a high concentration of ABA. Myrtus, however, roots very easily.

By contrast, all Eucalyptus extracts appear to have relatively low endogenous concentrations at Rf 0.40 to 0.60 and relatively high values at Rf 0.60 to 0.70, suggesting low concentrations of ABA. Hence, as shown with cress, lettuce, rice and maize bioassays, there is good evidence on rooting itself that ABA is not a major rooting inhibitor.

There is a significant difference between Myrtus and Eucalyptus since Eucalyptus extracts at Rf 0.8 to 0.85 alone enhance rooting. It is interesting to note that this growth promoter is only present in Eucalyptus extracts and not in Myrtus: and if present in Myrtus, then it is heavily masked by inhibitors. The peak at Rf 0.80 to 0.85 does not correspond to any known growth substances tested. While this is a distinctive difference between Myrtus and Eucalyptus, it is the very reverse of what one would look for if substances present at Rf 0.80 to 0.85 were to be responsible for lack of rooting in Eucalyptus.

Finally, inhibitors are present at Rf 0.85 to 1.0 in all extracts but again most abundant in Myrtus communis. There was little difference of any significance between the various Eucalyptus extracts. Among other endogenous inhibitors which chromatogram at the 0.85 to 1.0 Rf region, xanthoxin is one recognized to be of increasing importance, (Kundu and Audus, 1974).

Xanthoxin is produced from violoxanthin or lutein and correlates with leafy tissue. A comparison of light (pretreated) and dark (pretreated) cuttings was made, but it was not realised at the time that preparation of extracts in the light could have generated xanthoxin in dark pretreated cuttings. Reassessment of this test requires extraction of leafy shoots under dim red light.

Conclusion

Myrtus roots freely while Eucalyptus does not root at all. Myrtus contains high concentrations of root inhibitor(s) - much higher concentrations than in Eucalyptus.

The basic simple hypothesis that distinctive inhibitors in extracts of homogenised tissue are sufficient and adequate explanation of growth inhibition observed in whole living cuttings is thus invalid. Mere correlation between inhibitor content and failure to root, in other species, is not necessarily causal.

These results for Myrtus can best be explained on the assumption that the abundant inhibitors at Rf 0.85 to 0.95 are compartmented away innocuously in vivo, but on maceration become available to the root primordia as an effective inhibitor. It may be that one difference between Myrtus communis and Eucalyptus lies in the extent to which growth inhibitors are compartmentalised away from sensitive meristems.

7. Access of Endogenous Inhibitors to Sites of Root Initiation

The Response of Myrtus communis Cuttings to Macerated Leaf Extracts from the Parent Plant

I have found inhibitors of rooting at Rf 0.85 to 0.95 in both adult and juvenile Eucalyptus leaf extracts. Paton et al (1970) have independently isolated from adult leaves of Eucalyptus grandis three inhibitors of growth and rooting. These have subsequently been analysed by Nicholls, Crow and Paton (1970, 1972) and found to have close similarities to leptospermone - a β- triketone, but no definite identifications have been made. These inhibitors were relatively absent in Eucalyptus deglupta, the only species known to root easily, and Paton (1970) has suggested that there is "a direct and quantitative association between decreased rooting ability of stem cuttings and increased levels of a rooting inhibitor ...". He is therefore suggesting that the presence of inhibitors is sufficient reason to conclude that these endogenous inhibitors explain why Eucalyptus cuttings will not root.

An inhibitor with the 0.85 to 0.95 Rf value is present in Myrtus communis which roots very easily. Myrtus is a genus related to Eucalyptus being a member of the same family Myrtaceae, and the genera overlap in general distribution. Since Myrtus communis roots very freely I have therefore used it as a test organism due to its relationship with Eucalyptus gunnii.

The fact that Myrtus communis will root in the presence of its own endogenous inhibitors (Rf 0.85 to 0.95) leads me to consider that lack of rooting due to the presence of inhibitors is an inadequate explanation. Rather it is part of a larger phenomenon which demands our attention.

It is evidently not safe to conclude from the analysis of macerated tissue that the presence of an inhibitor "explains" an inhibition which may or may not occur in whole organs in vivo. Internal compartmentalisation of rooting inhibitors which appears to be efficient in Myrtus communis but inefficient, or absent, in Eucalyptus gunnii and E. grandis should therefore be investigated.

A simple direct test of this new hypothesis is therefore to test whether the endogenous rooting inhibitors of Myrtus do in fact prevent rooting in Myrtus when allowed free access to the untreated cutting. Another method not tested in this work, but one which demands early attention, is the effect of Eucalyptus diffusate from whole living Eucalyptus shoots into Agar gel, and its subsequent effect on rooting: the gel blocks will absorb only the non-compartmentalised inhibitors and promoters.

Method

Myrtus communis leaves were macerated, concentrated and separated by paper partition chromatography by the methods already described.

The chromatograms so produced were cut into 1 cm. strips. To concentrate the eluates, 4 comparable strips from the 0.83 to 1.00 Rf region were used together. The paper strips were soaked for 24 hours in 25 ml of distilled water in small beakers to extract growth substances; 25 cuttings of Myrtus communis were placed in each for 48 hours. These cuttings were then immediately placed in the propagation medium of peat and perlite. The temperature minimum was set at 15°C. The number of roots per cutting were counted after 45 days. Control means the 1 cm strip above the solvent front.

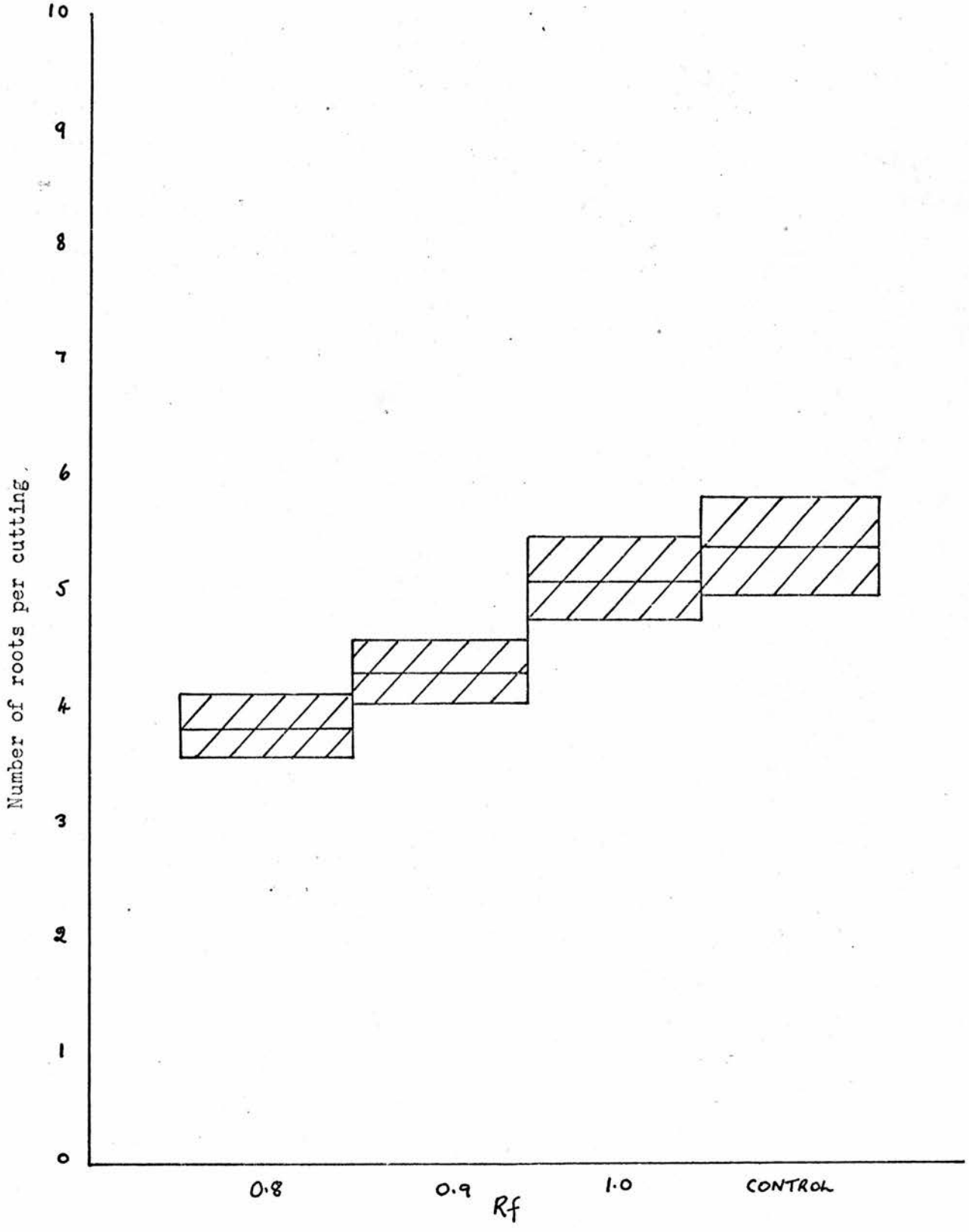
Results

The results are given as roots per cutting in Fig. 23 where it can be seen that there is a significant difference between rooting with the Rf fractions 0.83 to 0.93 and both the 0.93 to 1.0 fraction and the control. Significance was tested at the 5% level. The control sample produced 5.38 $\pm$ 0.46 roots per cutting while the inhibitor fractions produced 3.78 $\pm$ 0.26 and 4.28 $\pm$ 0.28 respectively.

Discussion

The numbers of new roots initiated per cutting are the biological criteria by which the hypothesis of inhibitor compartmentalization is best tested. Root initiation is also one of the first relevant responses to occur after cuttings are taken. Use of this criterion would also minimise any masking effects of decline with time in inhibitor content of the extracts of macerated tissue. Elongation growth of the newly formed roots would be an inappropriate criterion since it is a later consequence following initiation, and would produce less response if absorbed inhibitor content diminished with time. Myrtus communis cuttings root readily despite their high endogenous content of a rooting inhibitor. Eucalyptus gunnii cuttings with a similar or lower content of an indistinguishable rooting inhibitor, will not root. This experiment establishes that the rooting inhibitor of Myrtus inhibits its own rooting if applied exogenously. This shows that exogenously applied inhibitor gains access to sites of root initiation in Myrtus cuttings; by the same argument it follows that the same inhibitor present endogenously

Fig. 23 The effect of inhibitor and control fractions of chromatographed leaf extracts of Myrtus communis on the rooting of Myrtus communis cuttings.



Twenty five cuttings of Myrtus communis were set in each treatment in July 1974. Rooting data was recorded after 45 days.

within the fresh Myrtus cuttings has no such access - it is therefore safely compartmentalized away from initiation sites. It could follow then that Eucalyptus gunnii differs from Myrtus communis in that the endogenous inhibitor of Eucalyptus has access to potential initiation sites and is not effectively compartmentalized away from them. Since Eucalyptus gunnii cuttings do not root significantly under any circumstances, this "inhibitor and compartmentalization" hypothesis may not be the sole or true cause of failure to root; an inhibition at this or any other stage in the morphogenetic sequence leading to rooting may be overriding. Nevertheless, we have shown at least for Myrtus communis that it is altogether too simplistic to assume that mere presence of an endogenous rooting inhibitor is an adequate explanation of failure of cuttings to root; its natural accessibility to sites of root initiation is an essential aspect of successful rooting in Myrtus communis.

Conclusion

Mere presence of extractable rooting inhibitors in cuttings of both Myrtus communis and Eucalyptus gunnii cannot explain rooting of the former but not of the latter. The extracted inhibitor from Myrtus inhibits its own rooting and that of other species, indicating that the inhibitor is kept away from sites of root initiation in the intact cutting. The similar inhibitor in Eucalyptus gunnii may therefore not be compartmentalized away from sites of root initiation, thus explaining lack of rooting in Eucalyptus. Alternatively an overriding inhibition of rooting in Eucalyptus may lie elsewhere in this developmental sequence. It has nevertheless been established that compartmentalization of endogenous growth factors is as important as their mere presence.

CHAPTER V

CONCLUSIONS

V CONCLUSIONS

The Eucalyptus gunnii cuttings as received had a low water content and therefore received a preliminary treatment by placing their bases in water overnight to restore them to full turgidity.

Rooting of cuttings of Eucalyptus gunnii was very low - 0.4%. Possible causes and means of circumventing these were therefore tested.

Another environmental requirement necessary in propagation was fine misting techniques which better maintained relative water content of the cuttings and increased the chances of survival and subsequent rooting.

Antidesiccant treatment of the cuttings prior to setting was found to be important, as it reduced the actual water loss from the cuttings. Together with fine mist propagation this method is suggested for all future work with Eucalyptus.

Long day treatments together with the full spectrum of visible daylight maintained the survival of cuttings better than short day or darkness; additional red and far red light were both deleterious. A cool temperature regime of 10-15^{o}C proved to be more beneficial to survival and rooting than a warmer one which was associated with a higher frequency of micro-organismal rotting.

Cuttings taken during spring and early autumn consistently produced better results. Summer cuttings were "soft", and wilted and rotted very quickly, compared with those taken during spring and autumn. There was, however, a correlation between growth and rooting, since there was a poor survival rate with winter-set cuttings.

Essential cultural requirements in propagation include an optimal rooting medium and freedom from fungal attack. During the work for this thesis, the most suitable rooting medium was found to be an Irish moss peat/perlite mixture, although it is not suggested that this would be the case in other places. The local environmental conditions and the kind of peat used in the mixture provided better balance of aeration and moisture supply than any sand/peat mixture available.

Microbial attack was frequently associated with death of cuttings. Hence, a variety of fungicidal treatments were applied as a basic systemic pretreatment and as a regular overhead weekly drench of the cuttings and the rooting medium. These significantly reduced infection and increased the length of survival. Micro-organisms were therefore isolated from the interior of fresh and dying cuttings before and after fungicidal treatment in a search for possible pathogens. The micro-organisms were identified. While microbial organisms were absent from internal tissue of cuttings after pretreatment with systemic fungicides and overhead drenches, there was no significant change in ultimate survival and rooting rate of the cuttings. Pathogenic infection could not explain failure to root. Hence it is suggested that the cuttings first became physiologically senescent and subsequently rotted. Physiological causes were therefore examined.

Exogenous application of known plant growth substances has produced variable results. Auxin alone and in all combinations with other substances, particularly when applied at higher concentrations, significantly decreased viability and rooting. Gibberellic acid,

kinetin, chlorogenic acid, vitamins, sugars and organic nitrogenous substances, alone and in various combinations and concentrations, were known to enhance rooting in other genera when applied at various concentrations and by various methods. These did not produce any significant improvement in the rooting or survival of Eucalyptus gunnii and in several cases decreased rooting and survival. Sugars, however, produced a more favourable, although non-significant, effect and could be worth further study.

A deficiency of endogenous growth promoters cannot explain lack of growth and rooting since the exogenous application of growth promoting substances alone and in various combinations did not cause significant growth or rooting. Even after the application of shoot growth retardants to prevent shoot growth monopolizing the growth promoters at the expense of root growth, no rooting occurred and all the cuttings eventually died.

It was concluded that some internal physiological control positively inhibited rooting. Therefore the absolute concentrations and relative proportions of endogenous growth-controlling substances, promoters and inhibitors, were estimated. The physiological effect of endogenous inhibitors, especially ABA and/or other related terpenoids, is suggested as one of the reasons for the poor rooting and high mortality in cuttings. A means of eliminating or antagonising the endogenous inhibitor(s) was not found.

Inhibitors of germination were found in whole Eucalyptus oil when seeds of Mustard and Cress were placed in various concentrations of oil on wet filter paper, the higher concentrations producing the greatest inhibition. However, by heating the Eucalyptus oil to 60° and to 90°C almost full germination was restored. Comparable results were obtained with similar concentrations of medicinal liquid Paraffin oil. This suggests that while some volatile or heat-labile substances present in whole Eucalyptus and Paraffin oils are partial inhibitors of growth, those in Eucalyptus may not be the main natural cause of inhibition of rooting of cuttings.

Extracts of leaves of adult, unrooted and rooted juvenile Eucalyptus and of the easily rooted Myrtus communis were therefore fractionated by paper partition chromatography to isolate endogenous inhibitors and promoters of growth and rooting. These were bioassayed and from the resultant chromatograms of the four leaf extracts differences in relative content of inhibitors and promoters were detected.

Infrared Spectroscopy of chromatographic fractions failed to identify any specific substance since the IR spectra produced an indistinct pattern due to mutual cancellation of the absorption and transmission regions of the various components. More elaborate and effective means of separating the endogenous components are therefore necessary before using IR Spectroscopy for future identification purposes.

Both growth inhibitors and growth promoting substances were found in all leaf extracts by specific bioassay methods at levels comparable with those reported naturally in other plants. Thus again lack of rooting may not be due to a deficiency of natural growth promoting substances. The alternative remains that endogenous inhibitors are a major cause of lack of rooting.

Growth inhibitors were found on the paper chromatograms at Rf 0 to 0.1; 0.5 to 0.7; and at 0.85 to 0.95. Inhibition at Rf 0 to 0.1 is attributed to concentrations of a mixture of insolubles and includes some phenolics left at the base line due to extraction methods. This area was discounted. From specific bioassay tests, ABA is known to be present at about Rf 0.5 to 0.7 and is not considered to be the cause of the failure of cuttings to root since, firstly, cuttings of Myrtus communis, which were found to contain even higher concentrations of ABA, rooted freely. Secondly, added exogenous ABA did not inhibit Myrtus rooting. If the Rf 0.5 to 0.7 inhibitors do prevent Eucalyptus rooting, then Eucalyptus must be far more sensitive to ABA than is Myrtus. Inhibition could rather better be attributed to those substances present at Rf 0.85 to 0.95. However, rooted juvenile Eucalyptus cuttings and Myrtus communis cuttings also contain abundant inhibitors at this Rf region. This would suggest that a rare bypass for the inhibition exists occasionally even in older juvenile Eucalyptus. This bypass could be either a very low inhibitor content in the rare cutting, by chance, before rooting; or that these rare rooted cuttings had

effectively compartmentalized inhibitors away from sensitive meristems. The latter is by far the more probable explanation for Myrtus communis, which both roots freely and yet is rich in inhibitors.

vi Summary of Conclusions

One outcome of my six year's work has been the rooting of 98 cuttings out of 24,000 initially set; the 98 successfully rooted being so distributed among 58 treatments applied as to show that no treatment had any significant positive effect. Rooting was a rare event not significantly affected by the cultural or hormonal treatments used; however reasonable the attempts may have appeared on theoretical grounds, there was no significant success. Hence it follows that success most probably depends on eliminating or antagonising endogenous inhibitors. So far there are no known methods for doing this. Since even rooted cuttings were rich in inhibitors and cuttings taken from these would not root, ability to root was not a genetically heritable trait.

It was clear that this rooting inhibitor was not ABA (ABA forms as a result of stress, cutting, etc.). It is quite probable that the endogenous rooting inhibitor was present before, as well as after, the taking of cuttings. Evidence for this includes

(a) that air layering (while the shoot is still attached to the parent plant) is no more successful than the rooting of cuttings, and

(b) that inhibitors are present in effective concentrations in the adult but are deficient in early juvenile tissue even after detaching cuttings.

Eucalyptus oil was an inhibitor of root growth since it stopped germination, but when volatile components were removed by heating, it no longer inhibited. Since medicinal Paraffin oil had the same effects, Eucalyptus oil may not be a major natural rooting inhibitor.

Strong inhibition of rooting was caused by a fraction of adult Eucalyptus leaf extract chromatographing at Rf 0.85 to 0.95. The same content of inhibitors was present in the rare rooted and the unrooted juvenile Eucalyptus leaf extracts. Myrtus communis also contained high concentrations of inhibitors at this same Rf region. Myrtus and Eucalyptus belong to the same family - Myrtaceae - and are therefore taxonomically related genera. Species of both genera overlap in their general distribution although Myrtus communis is a native of Southern Europe.

Myrtus species in general root freely, thus the simple and popular idea that the mere presence of endogenous inhibitors adequately explains inhibition is clearly not correct for Myrtus. This suggests the idea that Myrtus might be inhibited by its own endogenous inhibitors if these had access (e.g. by external application) to potential root meristems in cuttings. It is possible that there is differential sensitivity, or that the inhibitors present in unmacerated Myrtus shoots are compartmentalized away from these sensitive root meristems in Myrtus but not in the non-rooting species of Eucalyptus. The fact that inhibitors are present in Eucalyptus thus may not be the complete or simple cause of lack of rooting.

It is clear that the following treatments promote survival, and this in turn may increase the chances of delayed rooting;

restoration of turgor;

sterilization with fungicide, systemic and external;

sealing with antidesiccant film;

high misting rate with a fine droplet size;

a peat/perlite rooting medium in a temperature of 10°C, and

a long photoperiod, using spring or autumn cuttings.

vii. Appendix I

Typical Statistical Analysis of Data

Ninety cuttings were set for each treatment and set out randomly in three parallel bands, each such set containing 3 subgroups each of five or ten cuttings in two or three parallel rows.

Due to the high initial mortality rate the figures for the first month were ignored since the high death rate was presumed to be due to initial infection, damage or physiologically weak cuttings etc. Thus the survivors at month 1 were taken as the 'starting frequency' and used as a reference level for the statistical analysis of the data for months 2 to 7.

Method of Statistical Analysis

Both χ^2 and co-variance analysis were applied. As the example of my working, the data presented on Tables 17 and 18 respectively (pp. 116 and 119) are here analysed in full. Analyses are given in Tables

Interpretation

(a) The χ^2 test was conducted on the monthly death rate of cuttings and was designed to show up any significant differences in the pattern of treatment effect. In the case of Table 17, with 10 d.f., $\chi^2 = 10.51$ giving $p = < 0.80, > 0.50$, i.e. there was no significant difference in the effects of different treatments.

The χ^2 test of the data in Table 18 with 12 d.f., $\chi^2 = 29.27$ giving $p = < 0.01, > 0.001$, a significant value. Since the value for χ^2 is mostly weighted by three figures involving two persistent auxins, singly and in combination, giving a higher death rate

than the control, the commonly held hypothesis that auxins have a beneficial effect on rooting is certainly not justified in the case of Eucalyptus gunnii.

(b) In the Analysis of Co-variance which is based on the frequency of survival of cuttings over the same 2 to 7 month period, for Table 17, we find that $F = 3.04$ for $n_1 = 5$ and $n_2 = 11$ giving p almost down to 0.05. The error component accounts for the greater part of the variance, and therefore the treatment effects are just not significant at the 5% level; i.e. indoleacetate effects on cuttings are almost indistinguishable from non-auxin controls.

For Table 18 we find $F = 1.78$ for $n_1 = 6$, $n_2 = 13$ giving $p = < 0.20, > 0.05$. The error component is almost equal to the treatment component and therefore there is no significant difference from this analysis for survival differences for the longer lasting auxins.

(a) χ^2 ANALYSIS.

BASED ON DEATH RATE PER MONTH FROM THE DATA IN TABLE 17 (p 116)

TREATMENT	REF NO.	OBSERVED (O) EXPECTED (E)	FREQUENCIES OF DEATHS PER MONTH 2	3	4–7	Σ 2–7
CONTROL P. NO AUXIN	6–1	O	7	7	14	28
		E	8·93	6·36	12·71	
CONTROL L. NO AUXIN	7–1	O	6	8	20	34
		E	10·84	7·72	15·44	
AUXIN P. IAA 0·2% w/w	6–2	O	7	5	13	25
		E	7·97	5·68	11·35	
AUXIN L. IAA 2000 ppm	7–2	O	15	9	16	40
		E	12·76	9·08	18·16	
AUXIN P. IAA 2·0% w/w	6–3	O	9	6	8	23
		E	7·34	5·22	10·44	
AUXIN L. IAA 4000 ppm	7–3	O	15	7	13	35
		E	11·16	7·97	15·89	
P = POWDER L. = LIQUID		TOTAL DEATHS	59	42	84	185

$$\chi^2 = \sum \frac{(O-E)^2}{E} = 8.24$$

0·42
2·16
0·11
0·39
0·38
1·32
0·06
0·01
0·08
0·00
0·12
0·11
0·13
1·35
0·84
0·26
0·57
0·53

For 10 degrees of freedom.

$P = <0.80, >0.50$

A. NON SIGNIFICANT DIFFERENCE.

(b) ANALYSIS OF COVARIANCE OF FREQUENCIES OF SURVIVAL OF CUTTINGS PER MONTH AND OF SUBSAMPLES.

TREATMENT	REF NO.		SUBSAMPLES 1	2	3	TREATMENT SUMS
CONTROL P. NO AUXIN	6–1	x	11	13	7	31
		y	21	20	14	55
CONTROL L. NO AUXIN	7–1	x	18	10	11	39
		y	44	13	29	86
AUXIN P IAA 0·2% w/w	6–2	x	10	9	7	26
		y	8	14	15	37
AUXIN L IAA 2000 ppm	7–2	x	15	15	11	41
		y	19	19	10	48
AUXIN P. IAA 2·0% w/w	6–3	x	6	9	10	25
		y	7	16	9	34
AUXIN L. IAA 4000 ppm	7–3	x	19	8	8	35
		y	21	7	7	35
P = POWDER L = LIQUID	SUBSAMPLE SUMS	X	79	64	54	197
		Y	122	89	84	295.

$SX^2 = 2391 \qquad SXY = 3620 \qquad SY^2 = 6247$

$\frac{(SX)^2}{18} = 2156.06 \qquad \frac{(SX)(SY)}{18} = 3228.61 \qquad \frac{(SY)^2}{18} = 4834.72$

$S_x^2 = 234.94 \qquad S_{xy} = 391.39 \qquad S_y^2 = 1412.28$

$E_{xy} = \frac{(31)(55) + \cdots + (35)(35)}{3} - 3228.61 = 226.06$

SOURCE OF VARIATION	DEGREES OF FREEDOM	ORIGINAL SUMS S_y^2	S_{xy}	S_x^2	ADJUSTED RESULTS. SS	d.f.	MS
TOTAL	19	1412·28	391·39	234·94			
BLOCKS	5	656·95	126·06	73·61			
ERROR	12	755·33	265·33	161·33	318·96	11	28·995
(ERROR)'	17	1412·28	391·39	234·94	760·26	16	
ADJUSTED TREATMENTS					441·30	5	88·26

$F = \frac{88.26}{28.995} = 3.04.$ For $p = 0.05$, $F = 3.2$. P IS THEREFORE ALMOST DOWN TO 0.05.

(a)

χ^2 ANALYSIS BASED ON DEATH RATE PER MONTH FROM THE DATA IN TABLE 18 (p 119).

TREATMENT	REF. NO.	OBSERVED / EXPECTED	FREQUENCIES OF DEATHS PER MONTH 2	3	4–7	Σ 2–7	
CONTROL NO AUXIN	7-1	O	6	8	20	34	1·36
		E	9·62	8·24	16·14		0·57
IAA	7-14	O	11	3	17	31	0·61
		E	8·77	7·52	14·72		1·03
IBA	7-13	O	5	11	9	25	0·06
		E	7·07	6·06	11·87		9·73
NAA	7-15	O	6	8	18	32	0·47
		E	9·05	7·76	15·19		0·01
IAA+IBA	7-16	O	8	7	11	26	8·72
		E	7·35	6·30	12·34		4·03
IBA + NAA	7-17	O	14	5	3	22	0·01
		E	6·22	5·33	10·44		0·08
IAA+NAA	7-18	O	6	6	16	28	0·02
		E	7·92	6·79	13·29		0·09
TOTAL DEATHS.			56	48	94	198	0·92
							0·35
							0·69
							0·52
							0·15
							5·30
							0·55

$\chi^2 = \sum \frac{(O-E)^2}{E} = 29.27$

12 d.f.

$P = <0.01, > 0.001$

A SIGNIFICANT EFFECT.

(b)

ANALYSIS OF COVARIANCE OF FREQUENCIES OF SURVIVAL OF CUTTINGS PER MONTH AND OF SUBSAMPLES

TREATMENT	REF. No.		SUBSAMPLES 1	2	3	TREATMENT SUMS
CONTROL NO AUXIN	7-1	x	18	10	11	39
		y	44	13	29	86
IAA	7-14	x	9	9	14	32
		y	15	14	17	46
IBA	7-13	x	11	8	12	31
		y	14	29	23	66
NAA	7-15	x	10	11	11	32
		y	15	17	12	44
IAA/IBA	7-16	x	11	6	9	26
		y	13	7	10	30
IBA/NAA	7-17	x	5	10	9	24
		y	7	10	12	23
IAA/NAA	7-18	x	6	12	10	28
		y	8	16	14	38
SUBSAMPLE SUMS		X	70	66	76	212
		Y	110	106	117	333

$SX^2 = 2298$ $\quad SXY = 3740$ $\quad SY^2 = 6959$

$\frac{(SX)^2}{21} = 2140.19$ $\quad \frac{(SX)(SY)}{21} = 3361.71$ $\quad \frac{(SY)^2}{21} = 5280.43$

$Sx^2 = 157.81$ $\quad Sxy = 378.29$ $\quad Sy^2 = 1678.57$

$Exy = \frac{(39)(86) + \cdots + (28)(38)}{3} - 3361.71 = 184.00$

SOURCE OF VARIATION	DEGREES OF FREEDOM	ORIGINAL SUMS Sy^2	Sxy	Sx^2	ADJUSTED RESULTS SS	d.f.	M.S.
TOTAL	20	1678·57	378·29	157·81			
BLOCKS	6	945·24	194·29	48·48			
ERROR	14	733·33	184·00	109·33	423·66	13	32·59
(ERROR)′	20	1678·57	378·29	157·81	771·76	19	
ADJUSTED TREATMENTS					348·10	6	58·02

$F = \frac{58.02}{32.59} = 1.78$ $\quad$ For $p = 0.20$, $F = 1.7$

THUS p = ALMOST 0.20.

A NON SIGNIFICANT DIFFERENCE

Names, Abbreviations and Sources of Chemicals used in this Work

Abscisic acid	ABA	Sigma, London
Ammonia		British Drug Houses, Poole
Aneurine hydrochloride	Vitamin B	B.D.H., Poole
Ascorbic acid	Vitamin C	B.D.H., Poole
Benomyl		Duphar Midox, Kent
Captan		Murphy Chemicals, St. Albans
Casein hydrolysate		B.D.H., Poole
Chlormequat	Cycocel	Growers Requisites, Littlehampton
2-chloroethyl-phosphonic acid	Ethrel	A.H. Marks, Bradford
Chlorogenic acid		Sigma, London
Chloros		Boots Farm Sales, Nottingham
N-Dimethylaminosuccinamic acid	B-9	Growers Requisites, Littlehampton
Dimethylsulphoxide	DMSO	B.D.H., Poole
Dithiocarbanate	Thiran	Murphy Chemicals, St. Albans
Eucalyptus oil		Boots, Chemist, St. Andrews
6-furfurylaminopurine	Kinetin	Sigma, London
Gibberellic Acid A_3	GA	B.D.H., Poole
Indole-3-yl-acetic acid	IAA	B.D.H., Poole
Indole-3-butyric acid	IBA	B.D.H. and May & Baker
Isopropanol		B.D.H., Poole
Naphthalene-acetic acid	NAA	B.D.H. and May & Baker
Paraffin oil (medical)		Boots, Chemist, St. Andrews
Phosphon		Growers Requisites, Littlehampton
Sucrose	Sugar	Sigma, London
S600 Antidessicant	S.600	Synchemicals, London
Zinc bacitracin + Neomycin sulphate	Cicatrin	Calmic Ltd., Crewe

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